FINAL REPORT

Project RMIS No. 4848

Nitrofurans: Measuring Tissue-Bound Residues in Meat

Ashtown Food Research Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 83

NITROFURANS: MEASURING TISSUE-BOUND RESIDUES IN MEAT

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SUMMARY

The aims of this project were to (a) develop a range of screening and confirmatory test methods that might be applied to effectively test for the illicit use of the prohibited nitrofuran antimicrobials, (b) study the persistence of nitrofuran antimicrobials as bound metabolite residues in edible tissues and (c) undertake a pan-European survey of the incidence of nitrofurans in retail pork.

Suitable enzymeimmunoassay (ELISA) screening methods were developed for metabolites of the two principal nitrofurans (furazolidone and furaltadone) and these ELISAs are marketed as commercial kit methods. A mass spectrometry (LC-MS/MS) confirmatory method was developed for the range of nitrofuran metabolites and this method has been introduced to laboratories in EU member states and in many non-EU countries. Studies on the persistence of nitrofurans in animals post-treatment confirmed that the protein-bound metabolites are the appropriate target analytes for detection of illicit use of these chemicals in animal production. The pan-European survey of retail pork identified a problem in at least one EU member state and demonstrated the effectiveness of the developed methods in identifying abuse of nitrofuran antimicrobials.

The project made a major contribution to dealing with the food safety issue posed by nitrofuran antimicrobials by (a) providing testing laboratories with the analytical tools required for residue testing and (b) providing research laboratories, regulatory agencies and the food industry with knowledge on the persistence of these drugs in edible tissues and on their occurrence in meat.

INTRODUCTION

Use of the nitrofuran antibiotics in food-producing animals was prohibited within the European Union (Commission Regulation 1442/95) because of their potentially carcinogenic and mutagenic effects on human health. Previously, nitrofurans had been widely and effectively used for the prevention and treatment of gastrointestinal infections caused by *Escherichia coli, Salmonella* spp., *Mycoplasma* spp., *Coccidia* spp., coliforms and some other protozoa and as growth promoters in livestock.

The four main nitrofuran antibiotics are furazolidone, furaltadone, nitrofurantoin and nitrofurazone. Various studies have demonstrated that the nitrofurans are rapidly metabolised by animals *in vivo* but that persistent, tissue-bound metabolites are formed which may be released by acid hydrolysis in the stomach, giving rise to potentially toxic chemicals.

Most research on the accumulation of parent nitrofurans in food for human consumption and depletion following withdrawal of medicated feed has focused on furazolidone, largely because it was the most widely used and the last of the four major nitrofurans to be banned. A summary of the research on furazolidone is that the parent compound may only be detected in tissues for a very short period after withdrawal of treatment but that the tissue-bound metabolite, AOZ (3-amino-2-oxazolidone), persists for considerable periods (*e.g.* at least six weeks) post-withdrawal of furazolidone. This instability of furazolidone in animal tissues prompted the development of analytical methods for its metabolite AOZ. However, the decision to monitor metabolites of furaltadone (3-amino-5-morpholino-methyl-2-oxazolidinone, AMOZ), nitrofurantoin (1-aminohydantoin, AHD) and nitrofurazone (semicarbazide, SEM) rather than their parent compounds was based on limited data suggesting that the parents were as unstable *in vivo* as was furazolidone.

Under the European Commission's Fifth Framework Programme, a project

entitled “Bound Residues and Nitrofuran Detection” (FoodBRAND 2000-2004), was established to work on these issues. The project, with seven partners from the UK (co-ordinator), The Netherlands, Germany, Ireland, Belgium/Spain, Czech Republic and Hungary undertook the following main areas of research:

- Developing immunoassay kits and chromatographic (HPLC) methods as screening tests for bound residues of nitrofurans;
- Developing a mass spectrometry reference (confirmatory) method for bound residues of nitrofurans;
- Validating the developed methods according to the standards required by the European Commission;
- Studying the persistence of nitrofurans as bound residues in edible tissues;
- Surveying the occurrence of nitrofuran residues in retail pork;
- Providing the validated methods to testing laboratories.

This report describes the main results for this project and highlights its important contribution to food safety.

IMMUNOASSAY SCREENING KITS FOR NITROFURANS

Antibody production

Production of suitable antibodies is the key to developing an efficient enzymeimmunoassay screening method. Antibodies capable of binding nitrofuran metabolites may be produced by experimental animals (such as mice and rabbits) when the animals are exposed to the nitrofuran metabolites. Because the nitrofuran metabolites are relatively small molecules (Figure 1), they must be linked in an appropriate way to a protein foreign to the experimental animal; for example, the nitrofuran metabolite of furazolidone,

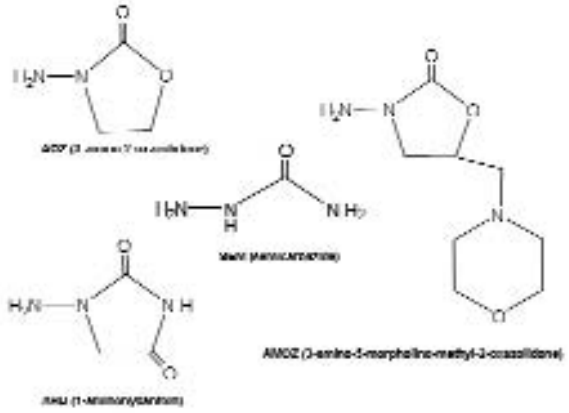

Figure 1. Structures of nitrofuran metabolites

AOZ, might be linked to human serum albumin (HSA). When this preparation (the “immunogen”) is injected into the experimental animal, an “immune response” is triggered and antibodies are produced; ideally some of these antibodies will be highly selective for binding the nitrofuran metabolite. This binding, or cross-reactivity, is the basis for an enzymeimmunoassay intended to measure the presence of the nitrofuran metabolite in test samples.

Various approaches to producing antibodies with cross-reactivity to the nitrofuran metabolites were investigated by the two partners charged with antibody production, Queen’s University Belfast (polyclonal antibodies) and Veterinary Research Institute, Brno (monoclonal antibodies). For example, antibodies might be produced with cross-reactivity to the free nitrofuran metabolites, AOZ, AMOZ, AHD, SEM, or with cross-reactivity to their nitrophenyl derivatives NPAOZ, NPAMOZ, NPAHD, NPSEM. Because the nitrophenyl derivatives are larger than the free nitrofuran metabolites and, therefore are likely to be better immunogens, and because formation of nitrophenyl derivatives was a critical step in methods for release and determination of protein-bound nitrofuran metabolites, antibody production

concentrated on development of immunogens that would have cross-reactivity to the nitrophenyl derivatives.

Immunoassay kits

After extensive research, also involving Ashtown Food Research Centre in antibody evaluation, suitable polyclonal antibodies were developed at Queen's University Belfast with sensitive and specific cross-reactivity to the nitrophenyl derivatives of furazolidone (NPAOZ) and furaltadone (NPAMOZ). The commercial partner in the project, R-Biopharm, used these antibodies to develop two enzymeimmunoassay kits for determination of AOZ and AMOZ in a broad range of foods including meat, liver, fish, shellfish, egg and milk. These commercial kits provide testing laboratories and

Table 1. Performance of RIDASCREEN® ELISA kits for nitrofuran metabolites

Test Kits:	AOZ (Art. No. R3701) AMOZ (Art. No. R3711)
Time required for assay of 10 samples:	1 working day (plus overnight incubation)
Cross-reaction with other nitrofurans:	< 0.05%
Recovery of residue from test samples:	70 - 90 %
Detection limit:	0.2 ppb

the food industry with the capacity to carry out screening assays on food products for some of the principal nitrofurans, furazolidone and furaltadone (Table 1).

Research at the Veterinary Research Institute, Brno on developing antibodies with cross-reactivity to the free nitrofuran metabolites did not produce

suitable antibodies within the timescale of the project. Subsequently, using immunogens developed in Belfast, the Brno laboratory successfully prepared a monoclonal antibody with cross-reactivity to derivatised AOZ. An immunoassay was developed using this antibody that gave a detection capability of 0.4 µg/kg in a range of food types.

EXTRACTION METHOD FOR NITROFURANS

Ashtown Food Research Centre undertook to develop a suitable extraction method for determination of nitrofuran metabolites by enzymeimmunoassay. The traditional approach to analysis for tissue-bound residues of nitrofurans, previously developed at Ashtown Food Research Centre (E. Horne, A Cadogan, M. O'Keeffe and L.A.P. Hoogenboom, *Analyst, (*1996), 121, 1463), involved extensive pre-washing of the tissue sample, acid hydrolysis and derivatisation with nitrobenzaldehyde, solvent extraction of the derivatised metabolites, clean-up by solvent washing and determination by high performance liquid chromatography. The aim in this project was to develop a much simpler procedure that might be automated and would be suitable for rapid sample screening by immunoassay.

The approach adopted was direct acid hydrolysis/derivatisation followed by solid phase extraction (SPE) to isolate the nitrophenyl derivatives both from the tissue matrix and from interfering nitrobenzaldehyde derivatising agent. Following acid hydrolysis/derivatisation, centrifugation and filtration were used to prepare the samples for SPE but this procedure was time-consuming and gave low recovery of the nitrofuran metabolite. An alternative protease digestion step was used prior to acid hydrolysis/derivatisation and this allowed for direct application of the

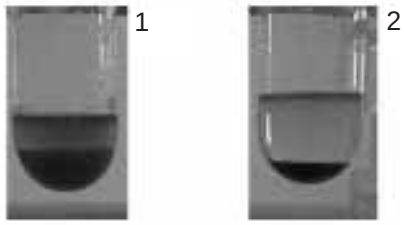

Figure 2. Effect of protease digestion on sample clarification
Sample 1 – no protease digestion
Sample 2 – protease digestion

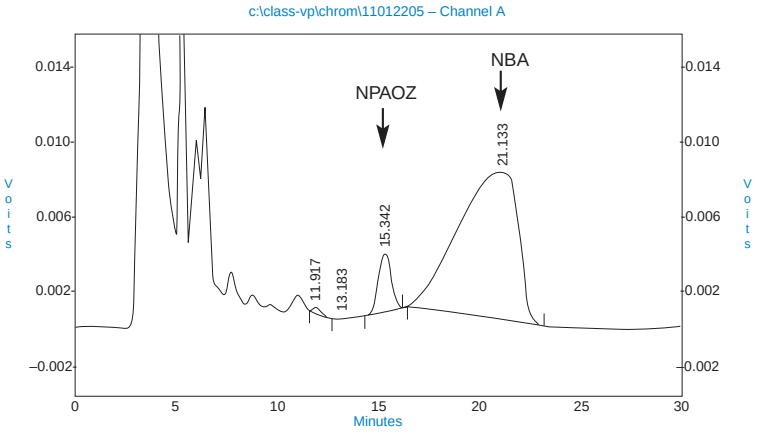

Figure 3 (a). Chromatogram of tissue extract containing NPAOZ after Oasis™ MAX clean-up

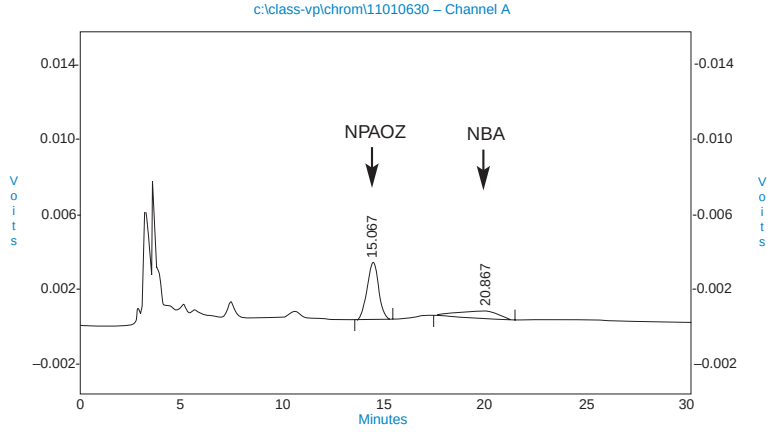

Figure 3 (b). Chromatogram of tissue extract containing NPAOZ after Oasis™ MAX plus Oasis™ HLB clean-up

filtrate to the SPE cartridge (Figure 2). For the SPE purification, a combination of two cartridges were used - (a) a mixed-mode polymeric sorbent with reversed phase and anion exchange properties (Oasis™ MAX) was used to trap over 90% of the surplus nitrobenzaldehyde while selectively recovering the derivatised nitrofuran metabolites, and (b) a further reversed phase sorbent (Oasis™ HLB) was used, with modification of pH, to remove in excess of 99% of the nitrobenzaldehyde and matrix interferences. The

effective purification of the tissue extract using this dual-SPE approach is shown in Figures 3(a) and 3(b) while results obtained for AOZ determination are shown in Table 2.

Table 2. Data on performance of dual-SPE method for Furazolidone

Sample	AOZ determined (mean ± SD); n = 9	
	AOZ (µg/kg)	Recovery (%)
Pig liver, fortified (400 mg/kg)	316 ± 119	79 ± 29.8
Pig liver, incurred	223 ± 24.9	

CHROMATOGRAPHIC METHODS FOR NITROFURANS

High performance liquid chromatography (HPLC)

Based on the method for determination of nitrofuran metabolites by enzyme immunoassay, a method suitable for determination by HPLC was developed at Ashtown Food Research Centre. Because HPLC methods are generally not as sensitive as immunoassays, a larger sample size of 5g was used for the HPLC assay (instead of the 1g sample size used for the immunoassays). The

Figure 4. Extraction and dual-SPE procedure, using Oasis™ MAX and HLB cartridges, for HPLC determination of nitrofuran metabolites

Table 3. Performance of HPLC method for Furazolidone in porcine liver

(a) Validation of the HPLC method

Sample	n	AOZ determined (µg/kg)		Mean recovery (%)
		Mean ± SD		RSD (%)
Control	6	None detected		
Control + 5 µg/kg	6	4.2 ± 0.80	19.2	83.5
Control + 25 µg/kg	5	22.5 ± 3.67	16.3	89.8
Incurred sample	5	170 ± 30.4	17.9	

(b) Comparison between HPLC and LC-MS/MS determination of AOZ

Sample	AOZ (µg/kg)	
	HPLC	LC-MS/MS
Incurred pig liver, No. 1	217	203
Incurred pig liver, No. 2	750	684
Incurred pig liver, No. 3	990	1084

traditional method, involving pre-washing of the tissue sample, acid hydrolysis/derivatisation and solvent extraction, was used together with the dual-SPE clean-up described previously (Figure 4).

This method was developed based on the furazolidone metabolite, AOZ, and validated with fortified and incurred samples (Table 3). In addition, an inter-laboratory comparison between the HPLC method at Ashtown Food Research Centre and an LC-MS/MS method at the RIKILT laboratory demonstrated agreement within 10% for three different liver samples. The liver samples were from pigs (15 kg) treated with furazolidone (300 mg/day) for 5 days and following withdrawal periods of 10 days (pig 1), 3 days (pig 2) and 0 days (pig 3).

The applicability of the developed method as a multi-residue method for nitrofuran metabolites was tested. While recovery of AOZ and SEM was 90%, recovery of AMOZ and AHD was approximately 50%, indicating the need for modification of the SPE procedure for a multi-residue nitrofuran metabolite method. Further development of this method was not undertaken because the LC-MS/MS method developed in the project (*see below*) has become the method of choice for chromatographic tests for nitrofurans.

Figure 5. Analytical procedure based on LC-MS/MS for tissue-bound nitrofuran metabolites

Mass spectrometry (LC-MS/MS) method

The RIKILT laboratory was primarily responsible for development of an LC-MS/MS method that would be suitable as a reference (confirmatory) method for nitrofuran metabolites. This method was developed (Figure 5) and validated (Table 4) and made available, through training courses, to the Community and National Reference Laboratories within the EU and to Third Countries. It was used also for the pan-European Retail Pork Survey in the project (*see below*).

PERSISTENCE OF NITROFURANS IN TISSUES

Since the methods developed in the project are all directed at determination of bound nitrofuran metabolites, it was important to demonstrate that these marker residues are appropriate for checking for abuse of nitrofurans. The Queen's University Belfast, together with partners at RIKILT, Wageningen and the National Food Investigation Institute, Budapest, studied the persistence of the four nitrofurans in edible tissues (muscle, liver, kidney) of pigs for a period

Table 4. Performance characteristics for LC-MS/MS method for nitrofurans (tissue samples fortified with nitrofuran metabolites at 1 mg/kg)

Parameter	AOZ	AMOZ	AHD	SEM
(a) Pig muscle				
Accuracy (%)	92	94	103	97
Repeatability	0.21	0.21	0.24	0.18
Within-laboratory Reproducibility	0.24	0.22	0.24	0.22
Limit of Detection (µg/kg)	0.15	0.20	0.10	0.10
Limit of Quantitation (µg/kg)	0.19	0.70	0.50	0.30
(a) Pig liver				
Accuracy (%)	102	105	105	108
Repeatability	0.15	0.28	0.28	0.19
Within-laboratory Reproducibility	0.21	0.27	0.30	0.22
Limit of Detection (µg/kg)	0.20	0.10	0.10	0.30
Limit of Quantitation (µg/kg)	0.60	0.30	0.60	0.60

of six weeks after treatment. Four groups of 18 pigs each received therapeutic doses of the four nitrofuran drugs for 10 days and then 3 animals from each treatment were slaughtered at weekly intervals during the subsequent period.

At zero withdrawal time, no residues of nitrofurantoin parent drug were determined in any tissue and low levels (< 1µg/kg) of furazolidone and furaltadone parent drugs were determined in only one of three samples of muscle and liver respectively. Residues of nitrofurazone parent drug (4-22 µg/kg) were determined in all three muscle tissue samples at zero withdrawal

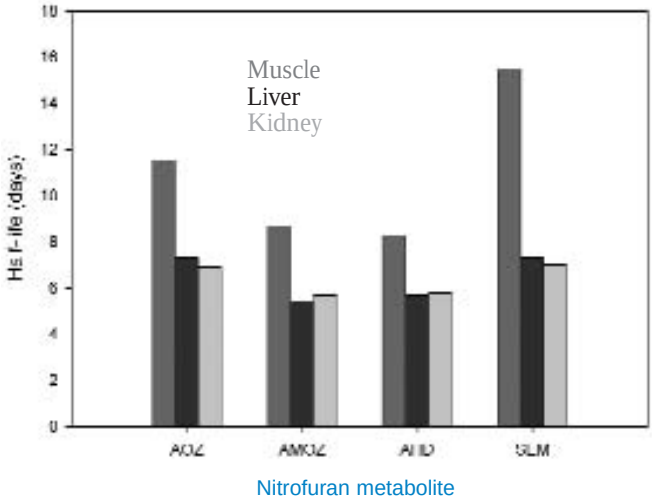

Figure 6. Depletion half-lives for tissue-bound nitrofuran metabolites in pigs fed nitrofuran-medicated feed (400 mg/kg) for 10 days followed by a six-week withdrawal period.

time but not in liver or kidney. After one week withdrawal, no residues of the nitrofuran parent drugs were determined in any tissue samples. These results confirmed the instability of the nitrofuran parent drugs and their unsuitability as marker residues for abuse of nitrofurans in animal production.

In contrast to the nitrofuran parent drugs, residues of the four tissue-bound metabolites, AOZ (furazolidone), AMOZ (furaltadone), AHD (nitrofurantoin) and SEM (nitrofurazone), occurred at high concentrations in all tissues at zero withdrawal time; 900-5000 µg/kg in liver, 700-4000 µg/kg in kidney and 250-2000 µg/kg in muscle. Residues of these metabolites were measurable for a period of six weeks post-treatment in all tissues and residue concentrations in the three tissues after six weeks withdrawal were 10-40 µg/kg AOZ, 10-60 µg/kg AMOZ, 4-7 µg/kg AHD and 40-250 µg/kg SEM. Figure 6 gives data for the depletion half-lives for the tissue-bound nitrofuran metabolites; the depletion half-life for a chemical is the time taken for the residue concentration in tissue to decrease by half.

RETAIL PORK SURVEY FOR NITROFURANS

A major component of the research project was to undertake a comprehensive multi-country survey on the incidence of nitrofurans in meat. Because previous usage of nitrofurans had been associated with intensive animal production systems, such as that used for pigs, a pan-European survey of pork was planned. Using pork purchased through retail outlets would provide a profile of the potential exposure of the European citizen to these prohibited substances.

Retail sampling

Retail sampling was designed and co-ordinated by Verbruikers Unie Test-Aankoop S.V. using consumer associations in 12 countries of the EU – Belgium (including Luxembourg), France, Austria, Germany, The Netherlands, Spain, Portugal, Italy, Sweden, Denmark, Greece and Ireland. Samples were also received from the United Kingdom and the Czech Republic (using retail purchase of samples) and from Hungary, through official sampling at pig slaughter houses.

Sampling was undertaken over the period April to September 2002 with sampling in each country normally occurring over a four-week period. Retail samples, 100 samples from each country, were purchased from a variety of outlets such as supermarkets, independent pork butchers, chains of butcher outlets and public markets and included organic meat where available. Sampling was required to take account of geographical distribution and market share for each type of outlet.

Each sample consisted of a 100g portion of pork chop or pork fillet, free of bone. The sample was sub-divided into two equivalent samples of approximately 50 g each (“A” and “B”) which were stored in separately labelled plastic bags. The samples were frozen at -18°C for at least 24 h prior to transport to Ashtown Food Research Centre.

Sample analysis

The "A" samples were individually assigned random numbers, removed from the original bag and repackaged in bags labeled only with the random numbers; the "B" samples were retained in their original packaging in frozen storage. One-third (500) of the "A" samples were provided to each of three laboratories for residue extraction; National Food Investigation Institute, Budapest, Queen's University Belfast, Ashtown Food Research Centre, Dublin. A common procedure was used at each laboratory to develop composite samples (3 to 1) for analysis. The composite samples were extracted in each laboratory according to a common procedure for LC-MS/MS analysis based on the procedure outlined in Figure 5. The dried sample extracts were shipped on ice overnight to RIKILT, Wageningen for residue determination by LC-MS/MS.

Assessment of laboratory performance

Prior to undertaking the sample preparation for the retail survey samples, the three laboratories carried out pre-testing of their procedures. Using a common source of residue-free pork muscle, each laboratory prepared a series of 15 samples fortified with nitrofurans and these were used to determine individual laboratory performance. Table 5 gives data on limits of detection and quantification and on accuracy for each of the three laboratories. These results indicated that the three laboratories might undertake sample preparation for LC-MS/MS analysis with satisfactory and broadly comparable performance in terms of LOD, LOQ and accuracy. Limits of quantification for the assay were 0.1 µg/kg AOZ and AMOZ, 0.2 µg/kg SEM and 0.5 µg/kg AHD.

Results of retail survey

No residues of nitrofuran metabolites were detected in 485 composite samples, representing 1455 individual samples. Positive responses for one or more of the nitrofuran metabolites were determined in 15 composite samples.

Table 5. Performance characteristics (limits of detection, limits of quantification, accuracy) calculated for samples prepared by three laboratories.

Nitrofuran metabolite	*Limit of detection (µg/kg)*			*Limit of quantification (µg/kg)*		
	Laboratory Number			Laboratory Number		
	1	2	3	1	2	3
AOZ	0.03	0.04	0.03	0.11	0.08	0.06
AMOZ	0.03	0.03	0.02	0.07	0.06	0.04
AHD	0.07	0.04	0.04	0.49	0.43	0.37
SEM	0.09	0.07	0.06	0.18	0.15	0.16

	Accuracy (Mean, µg/kg ± CV, %) [based on extraction of nitrofuran metabolites from pork samples fortified at 0.5 µg/kg (n = 6)]		
	Laboratory Number		
	1	2	3
AOZ	0.56 ± 12.6	0.50 ± 3.7	0.51 ± 16.3
AMOZ	0.49 ± 2.8	0.48 ± 6.6	0.47 ± 20.0
AHD	0.47 ± 5.1	0.53 ± 8.8	0.55 ± 15.4
SEM	0.48 ± 7.7	0.48 ± 8.3	0.55 ± 21.8

The individual "A" samples (n = 45) from which the composite samples showing positive results were prepared were extracted by the three laboratories and analysed by LC-MS/MS. In each case, one individual sample (total 15) from each composite gave a positive response for a nitrofuran metabolite. The "B" samples corresponding to the 15 positive "A"samples, together with five other randomly-chosen "B" samples to act as negative controls, were identified and analysed in duplicate, with all steps of sample preparation and LC-MS/MS determination being performed by the RIKILT laboratory. As had been decided prior to the initiation of the testing programme, the results for the "B" samples were taken as the confirmed results

for the presence of nitrofuran metabolites in retail samples of pork. From analysis of the "B" samples, 12 samples of pork were determined to contain measurable levels of nitrofuran metabolites (Table 6). 10 samples contained AMOZ at concentrations between 0.2 and 1.0 µg/kg; nine of these pork samples were purchased in Portugal and one sample was purchased in Italy. Two samples contained AOZ at concentrations of 0.3 and 3.0 µg/kg; these pork samples were purchased in Portugal and Greece respectively.

The results for the survey show a confirmed incidence for measurable nitrofuran metabolites in 12 of 1500 samples of pork purchased in retail outlets in 15 European countries. This represents an incidence of 0.8% overall. However, the incidence varied between 1% and 10% for the three countries in which the nitrofuran-positive pork was purchased. The occurrence of a high incidence of nitrofuran-positive pork, primarily furaltadone (AMOZ), in Portugal was not surprising given the reported occurrence of furaltadone in chicken in Portugal in 2003 [European Union website (http://europa.eu.int/comm/food/fs/rc/scfcah/ah_aw/rap09_en.pdf)]. Following on the notification of the results of this pork survey to the Portuguese authorities in March 2003, additional steps to eliminate the use of nitrofurans in animal production in Portugal have been taken. The results of the retail survey demonstrate the usefulness of the FoodBRAND project in identifying problems with nitrofurans in meat and in supporting EU member states and non-EU countries with effective analytical methodology to monitor the illicit use of nitrofurans in meat production.

CONCLUSIONS

- A range of screening (enzymeimmunoassays) and confirmatory (mass spectrometry) methods have been developed and made available to test for nitrofurans in food samples. Testing laboratories in Europe and in Third Countries were assisted with development of state-of-the-art testing capacity for nitrofurans from the FoodBRAND project. These developments were very timely, coinciding with the emergence of a global

Table 6. Results for testing of composite and individual A samples and individual B samples of pork from the European Retail Survey.

Samples						
Nitrofuran metabolite	Composite A[a] (µg/kg)	Individual A (µg/kg)	Individual B Sample Code[b]	(µg/kg)[c]	Confirmed result	Country of purchase
AMOZ	0.20	0.25	RA01	0.28	Positive	Portugal
AHD	0.15	27.2	RA02	ND	Negative	
AOZ	0.90	2.6	RA03	3.0	Positive	Greece
AMOZ	0.10	0.33	RA04	0.40	Positive	Portugal
			RA05	ND	(Control)	
AMOZ	0.45	0.45	RA06	0.55	Positive	Portugal
AMOZ	0.10	0.25	RA07	0.30	Positive	Portugal
AMOZ	0.20	0.25	RA08	0.25	Positive	Portugal
AOZ	0.10	0.35	RA09	ND	Negative	
AMOZ	0.10	0.30	RA10	0.35	Positive	Portugal
			RA11	ND	(Control)	
AMOZ	0.10	0.25	RA12	0.20	Positive	Portugal
AMOZ	0.10	0.30	RA13	0.25	Positive	Portugal
AMOZ	0.10	0.35	RA14	0.30	Positive	Portugal
			RA15	ND	(Control)	
AHD	-[d]	0.40	RA16	ND	Negative	
AOZ	0.15	0.30	RA17	0.33	Positive	Portugal
			RA18	ND	(Control)	
			RA19	ND	(Control)	
AMOZ	0.25	0.90	RA20	1.05	Positive	Italy

[a] Total of 500 samples, representing 1500 "A" samples; 485 samples showing no residues of nitrofurans

[b] A random numbering system, RA01-RA20, was applied to the 15 "B" samples for investigation and to 5 additional samples used as negative controls (RA05, RA11, RA15, RA18, RA19).

[c] ND - no detectable residue of nitrofuran metabolites.

[d] The composite sample showed an apparent positive result for AOZ, but only a low concentration of AHD was found on subsequent analysis in one of the individual "A" samples.

problem relating to widespread nitrofuran use in 2002-03. Nitrofuran metabolites were determined in poultry and aquaculture products from many countries, including Thailand, Vietnam, Brazil and Portugal. In the two years since 2003, over 150 Rapid Alerts (RASFF) were issued by the European Commission concerning findings of nitrofuran metabolites in food products from 29 countries. Trade restrictions were implemented and positive clearance of shipments of imported food were required. The test methods developed within the FoodBRAND project provided the necessary analytical capability. In 2004 alone, over 2500 immunoassay test kits developed in the project were used for commercial testing. Ashtown Food Research Centre established the LC-MS/MS method and have applied it for testing samples taken in the National Residue Testing Plan (DAF).

- The information on persistence of nitrofurans in edible tissues as tissue-bound nitrofuran metabolites confirmed (a) the potential presence of these drugs for extended periods after use on animals and (b) that tissue-bound metabolites are the appropriate target residues to check for abuse of these drugs in animal production.

- The pan-European survey on retail pork identified a significant abuse situation in one member state (Portugal) and potential abuse in some other countries. This served to alert the EU to a food safety problem; subsequently, there have been incidences of nitrofuran abuse identified in a number of other member states. The pan-European survey on retail pork also served as an excellent model for a large-scale multi-national food safety investigation, including the logistics for sampling, laboratory assessment and multi-laboratory performance of testing to an appropriately high standard.

RECOMMENDATIONS TO INDUSTRY

This research investigated a particular food safety problem relating to residues of the prohibited antimicrobials, nitrofurans, in meat and fish products. The

project developed test kits for two of the major nitrofurans, furazolidone and furaltadone, and confirmatory test methods by mass spectrometry for the range of nitrofurans. The project also confirmed that the most suitable target analytes for residue testing are tissue-bound nitrofuran metabolites and identified a European and world-wide problem with abuse of nitrofuran drugs in animal production.

Industry is required, both under the self-monitoring provisions in EC Directive 96/23 and under HACCP schemes, to ensure that animals for slaughter and animal tissues and fish to be used in food production do not contain any trace of prohibited substances, such as nitrofurans. As part of such due diligence, testing of foods for residues may be required. The commercial test kits and the confirmatory methods developed in this project provide a range of residue testing procedures that may be used by the food industry and by regulatory agencies to ensure that food products are free of nitrofuran residues.

PUBLICATIONS FROM THE PROJECT

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