Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Agrochemicals Unit

Activities Report 2006

Executive Summary

The objectives of the Agrochemicals Unit are to provide assistance and support to developing countries in their efforts to ensure the safety and quality of food and agricultural commodities, thereby safeguarding the health of consumers and facilitating international trade. The Unit's work focuses on food and environmental contaminants such as residues of pesticides and veterinary drugs, and mycotoxins. The main areas of activity in pursuit of the objectives are: applied research and development, technology transfer, training, and support for the development of international guidelines. These activities were the focus of two evaluations in 2005-2006. The Unit, as part of the Food and Environmental Protection Sub-programme, underwent a FAO Autoevaluation, and also participated in an OIOS evaluation of the TC Food Safety activities of the Agency. This report summarises the main activities undertaken by the Unit in 2006.

Several analytical methods were developed or adapted and validated for transfer to Member States for application in regulatory and research laboratories. Emphasis was placed on simple, multi-residue methods to improve cost-effectiveness and applicability, whilst meeting the performance requirements necessary for use as regulatory methods for international trade. Radiolabelled compounds, when available, provided a comparative advantage as a quality control tool during method development.

In response to a need for an easily applied multiresidue pesticide method for wheat flour and similar matrices, which are difficult to analyse, a method previously developed in the Unit for the determination of a range of pesticides using GC ECD/NPD was further developed to improve the extraction of pesticides from these matrices. The method was validated in Seibersdorf by a Fellow from Tanzania and has now been transferred for application in the Government Chemical Laboratory Agency in Dar Es Salaam. A paper describing the method was presented at an international conference and the method will be included in future training activities.

A multiresidue HPLC method for the analysis of residues of the tetracycline class of antibiotics in animal tissue was adapted from the literature and a preliminary validation performed. A multiresidue confirmatory method for residues of a range of sulphonamide veterinary antibiotics by LC-MSMS was developed, based on the simple and cheap extraction method used in an HPLC method previously validated in the Unit. Both methods were used to train Fellows and will form the basis of practical exercises in a training course in 2007. Different approaches were evaluated for the estimation of uncertainty of analytical results and a simplified approach suggested for application in laborties wishing to meet the requirements for uncertainty estimation for ISO17025 accreditation.

A collaborative project with the Austrian Research Centre on the influence of climate change on the environmental behaviour of a herbicide was completed.

Internal quality control procedures were elaborated for fumonisin B1 analysis. The procedures are suitable for application in Member State laboratories for a wide range of analytical techniques. The results of this study were presented at an international conference and the procedures developed will be used in future training activities by the Unit.

The final research meeting for a coordinated research project (CRP) on veterinary drug residues was held and the project successfully completed. A new CRP on integrated analytical approaches to assess indicators of pesticide management practices was commenced; a consultants' meeting was held to elaborate the work plan and contracts and agreements were issued. Agrochemicals Unit staff also provided direct support as technical officers for 10 national TC projects, and *ad hoc* technical support as requested for a number of other projects.

Training activities at Seibersdorf included a 4-week inter-regional training workshop on QA/QC in pesticide residue analysis, which had 19 participants from 18 developing countries, training of 2 IAEA TC Fellows and 2 FAO TC trainees, and the development of distance-learning materials for the FEP-ACU eLearning system. Agrochemicals Unit staff also attended various technical training programmes and seminars at the laboratory and in Vienna. Extra-budgetary financial support was secured to hold Food Safety Summits for the Asia/Pacific region in Singapore and Thailand. Technical training on pesticide analysis and radiotracer technique was provided by staff members in Costa Rica and Argentina.

Support was provided for the development of international guidelines through interaction with the committees of the Codex Alimentarius Commission. Unit staff was involved in several working groups associated with the Codex Committee on Residues of Veterinary Drugs in Food. Draft Guidelines on the estimation of uncertainty of results, originally drafted at a meeting organised by the Unit, were revised by the Codex Committee on Pesticide Residues and forwarded for adoption by the Commission. A sampling manual for mycotoxins was completed by two consultants in 2006, including analytical data produced in the Unit in 2005. The manual is undergoing final editing by Agrochemicals Unit staff before publication.

Work commenced on a complete revision of the quality assurance system in place in the Unit. The new system will be compliant with the ISO 17025 standard, and will provide the basis for possible future accreditation of the Unit.

Feedback from trainees and counterparts indicates that the training and methodologies provided by the Unit are being implemented in many countries. The "train the trainers" approach is successful, with follow–up courses being held by former trainees in several countries. Capacity building has resulted in the implementation of residues testing in several countries and accreditation of several laboratories, and has assisted in maintaining trade channels in agricultural food commodities. Networking has also been successful, with informal technology transfer agreements between the Agrochemicals Unit, Brazil, South Africa, Sri Lanka and Germany being examples of international cooperation fostered by the ACU activities.

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1. Introduction

1.1. Sub-programme and Unit objectives

The Food and Environmental Protection (FEP) Sub-programme provides assistance to Member States in their efforts to ensure the safety and quality of food and agricultural commodities, both to protect the health of the domestic consumer and to facilitate international trade. The objectives of the Sub-programme are to strengthen the ability of Member States to apply international standards on irradiation for food preservation and phytosanitary treatments, and to use nuclear and related analytical techniques to build capacity for the management of food and environmental hazards. The Agrochemicals Unit, as an integral part of the FEP Sub-programme, focuses its activities on the latter objective (Project E3.02). The principal methods used to pursue the Sub-programme's objectives are: coordinating and supporting research; providing technical and advisory services; providing laboratory support and training in nuclear and related techniques; and collecting, analysing and disseminating information.

Many countries have recognised that effective food control systems must be based on a coordinated approach that integrates control of the production of agricultural commodities from "farm to fork", and this has also been acknowledged in the FAO Medium Term Plan for 2002-2007. Recognition of this need is a response to increasing concerns about food safety related to both domestic and internationally traded foodstuffs. These concerns have been brought to the fore in recent years by the enforcement of international standards under the World Trade Organisation Agreements on the Application of Sanitary and Phytosanitary Measures and on Technical Barriers to Trade.

There is an increasing demand from FAO and IAEA Member States for support and technical advice in implementing the "farm to fork" concept, including the integration of analytical laboratory services along with good agricultural and production practices and systems such as the Hazard Analysis Critical Control Point (HACCP) to protect the public from safety hazards throughout the food production chain. With this shift in emphasis away from end-point testing of agricultural commodities, the analytical laboratory is assuming an extended role, not only providing testing of commodities for compliance with export or import requirements, but also to provide feedback to producers and regulators on the efficacy of the production practices in place with regard to food safety.

The Agrochemicals Unit aims to assist in improving the services provided by national food safety and regulatory institutions in support of consumer and environmental protection through improved analytical methods and capacities to assess and manage the risks associated with pesticide and veterinary drug residues and mycotoxins. The Unit also provides technical support for the negotiation and development of international guidelines related to pesticides and veterinary drugs through the Joint FAO/WHO Codex Alimentarius Commission.

During 2005-06, the FEP Sub-programme, as part of the Joint FAO/IAEA Programme, underwent an FAO Autoevaluation, which was carried out by an external consultant. One of the findings relevant to the Agrochemicals Unit in the Evaluation Report was:

"The application of HACCP and GAP to reduce contamination and improve the quality of agricultural commodities will require the analysis of residues of pesticides, mycotoxins, veterinary drugs and other food contaminants (including on occasion radionuclides) at earlier, additional points in the food chain and not just on end products. Thus there will be an increased need for the verification of contaminants in food and the environment so the requirement to build capacity in analytical methodologies and risk assessment will remain and probably expand. Help for laboratories to comply with ISO standards and/or GLP to ensure the credibility of analytical results will be

necessary for the foreseeable future." (paragraph 1.2.4., FAO Autoevaluation of Programme Entity 215P1)

The consultant also commented that "Distance learning will become increasingly important to ensure the best use of FEP resources" (1.2.8). This supports the Agrochemicals Unit's continued efforts to contribute to the eLearning system developed in the FEP Sub-programme, including through the provision of the training materials developed for training courses in electronic format. An important recommendation in the report is "The overall aims of the Entity as set out above should be maintained as they exploit the comparative advantages of expertise in irradiation technologies and laboratory procedures, where the latter will make an important contribution to the implementation of HACCP and GAP."

To achieve its objectives, the Unit works in harmony with the Food and Environmental Section of NAFA and also collaborates very closely with other Units and Sections, for example working closely with the Animal Production and Health Section in the field of veterinary drug residues.

1.2. Staff

In addition to the regular staff listed below, two consultants worked in the Agrochemicals Unit during 2006. Ms. Gesa Schad worked in the Unit from November 2005 to February 2006 on the adaptation and validation of a method for the quality control of trypanocidal drugs as part of joint project instigated by FAO and the International Federation for Animal Health. Mr. Bruno Carniero, a cost-free expert from Microbioticos Laboratories in Brazil, joined the Unit in December 2005 to provide expertise on methodologies for veterinary drug residue monitoring and complete robustness testing of a method for sulphonamide analysis validated in the Unit. He completed his work with the Unit in February 2006.

Mr. Elmer Kaltenbrunner, a student of the Fachhochschule Wels, also worked in the Unit between January and May 2006 on a collaborative project between the Agrochemicals Unit and the Department of Agricultural Research of the Seibersdorf Austrian Research Center (ARC) entitled 'Influence of climate change on the environmental behaviour of s-metolachlor in a soil-plant-water system' (see section 2.5).

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Consultants	**From**	**To**	
Gesa Schad	14 Nov 2005	21 Feb 2006	
Bruno Magalhaes Carniero	1 Dec 2005	28 Feb 2006	

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2. APPLIED RESEARCH AND DEVELOPMENT

2.1. Adaptation of the IAEA-ethyl acetate multi residue method to determine pesticide residues in wheat flour

Analytical methods must be available to determine pesticide residues in crops, feeds, and food commodities for a variety of purposes, which include regulatory monitoring and enforcement, import/export certification, risk assessment, field-application trials, organic food verification, and marketing to consumers. For all of these purposes, the methods should be robust, give accurate results, meet detection limit needs, and cover the desired scope of matrices and analytes. The most common methods in current use for pesticide residue monitoring stem from methods developed in the 1960s and 1970s. However, due to the increasing cost of labor, solvents, equipment, and laboratory space, there is an urgent need for residue chemists to develop and use more cost-effective procedures.

Raw agricultural commodities such as fruits, vegetables and grain are the most commonly analysed foods for pesticide residues. A method was previously developed in the Agrochemicals Unit for the determination of pesticide residues in fruits and vegetables. This IAEA–ethyl acetate multi residue method[1] was an adaptation of the QuEChERS method[2], which originally employed acetonitrile as the extraction solvent and gas chromatography-mass spectrometry (GC-MS) for analysis. The IAEA method used ethyl acetate for sample extraction to permit analysis by gas chromatography with conventional detectors - electron capture detector (ECD) and nitrogen-phosphorous detector (NPD), as well as mass spectrometric detectors, because the acetonitrile extract of the original QuEChERS method is not compatible with conventional detectors. This increased the scope of applicability of the method to the analysis of GC-amenable pesticides in fruits and vegetables in laboratories where mass spectrometry is not available.

In response to a request from Tanzania for a multiresidue pesticide method applicable to wheat flour and similar products, application of either of the above methods resulted in low recovery of the target pesticides.

The aim of this study was to provide a simple, rapid and inexpensive multi-residue method for pesticides in wheat flour and similar matrices that provides accurate and precise results whilst using few reagents in small quantities. The extraction step of the IAEA–ethyl acetate method was modified for application to wheat flour-type matrices based on the extraction step from the method of Ambrus et al.[3], which uses ethyl acetate for the extraction of pesticides from cereal grains. The method was validated by analysing wheat flour samples spiked with 24 representative pesticides at levels between 0.03 and 3 mg/kg.

[1] Aysal, P., Ambrus, Á., Lehotay, S.J. and Cannavan, A. (2007). Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. Journal of Environmental Science and Health B, in press (Vol.B42, No.5)

[2] Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D. Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International 86, 412-431

[3] Ambrus, Á.; Füzesi, I.; Susán, M.; Dobi, D.; Lantos, J.; Zakar, F.; Korsós, I.; Oláh, J.; Beke B. B.; Katavics, L. (2005). A cost-effective screening method for pesticide residue analysis in fruits, vegetables, and cereal grains. Journal of Environmental Science and Health, Part B, 40, 297-339.

Experimental

A radiolabelled pesticide, ^{14}C-chlorpyrifos, was used in preliminary studies to evaluate the efficacy of different combinations of extraction solvents and conditions and optimize the extraction procedure. Samples were spiked with a known amount of ^{14}C-chlorpyrifos with a specified activity. After each extraction regime, portions of the sample extract were added to scintillation cocktail and the activity measured on a scintillation counter to estimate the extraction efficiency.

The modified method, optimized using the above procedure, is outlined in Figure 1. A sample of 20 g flour was vigorously mixed with 20 ml water and 10 g sodium hydrogen carbonate ($NaHCO_3$), and then 40 ml ethyl acetate was added. The mixture was warmed to 35^{o}C for 5 minutes in a water bath, with stirring; then 20 g anhydrous sodium sulphate (Na_2SO_4) was added and the mixture was immediately homogenized using an Ultra Turrax blender. After centrifugation, removal of residual water and clean-up were performed simultaneously by dispersive solid-phase extraction of 10 ml of the ethyl acetate extract with 1 g anhydrous magnesium sulphate ($MgSO_{4)}$ and 0.17 g primary secondary amine (PSA) sorbent. Samples were analysed by GC-ECD and GC-NPD.

Results

Efficiencies of some extraction procedures in wheat flour by using ^{14}C-Chlorpyrifos

In preliminary studies, different combinations of the extraction procedures based on two methods were tested using ^{14}C-chlorpyrifos. Table 1 summarizes the ^{14}C-chlorpyrifos recoveries for the various extraction conditions tested.

Table 1. ^{14}C-chlorpyrifos recoveries of various extraction procedures

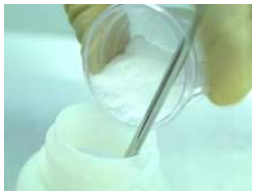
Method	Brief method description	Extraction Recovery (%)	PSA Clean-up Recovery (%)	Overall recovery (%)
[1] QuEChERS-EtOAc (IAEA)	30g flour + 30ml water + 5g $NaHCO_3$ 60ml EtOAc + 30g Na_2SO_4	63	97	**61**
[2]. IAEA + NaCl	30g flour + 30ml water + 5g $NaHCO_3$ + 3g NaCl 60ml EtOAc + 30g Na_2SO_4	60	99	**59**
[3]. IAEA (1:3)*	20g flour + 20ml water + 10g $NaHCO_3$ 60ml EtOAc + 20g Na_2SO_4	66	97	**64**
[4]. IAEA (1:5)*	20g flour + 20ml water + 10g $NaHCO_3$ 100ml EtOAc + 20g Na_2SO_4 Heat	57-96	103	**59-99**
[5]. IAEA (1:2)*	20g flour + 20ml water + 10g $NaHCO_3$ 40ml EtOAc + 20g Na_2SO_4 Heat to 35^{o}C, 5 min, with stirring	100	99	**99**

*** refers to sample/solvent ratio**

The problematic step with regard to recovery of the analytes was the extraction step, probably because of the dry nature of the matrix and its small particle size. The dispersive solid phase extraction clean-up step with primary secondary amine sorbent and anhydrous magnesium sulphate, as described in both of the above methods, can be employed without significant loss of analyte.

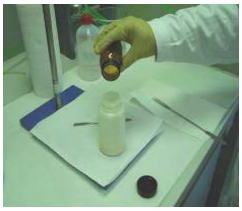
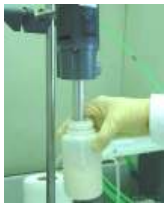
EXTRACTION






mix water and flour sample & add $NaHCO_3$


Add EtOAc and heat the mixture to 35°C, with stirring

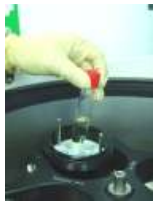




Add Na_2SO_4, homogenise with ultra turrax, centrifuge at 2500 rpm

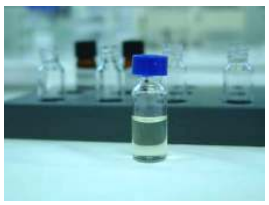
CLEAN-UP OF RAW ETHYL ACETATE EXTRACTS







Add EtOAc extracts to PSA/ $MgSO_4$ mixture, vortex and centrifuge at 1900 rpm




Transfer the final extracts to auto sampler vial & analyze by GC-ECD and NPD

Figure 1. The IAEA- Ethyl acetate method to determine pesticide residues in wheat flour

Increasing the solvent ratio in the extraction step did not improve the extraction efficiency. However, the results indicated that increasing the temperature and stirring the mixture did improve extraction efficiency. This is probably due to the expansion of the dough formed from the flour/water, allowing the extraction solvent to permeate the matrix and easily interact with the analytes.

Based on the recovery data shown in Table 1, procedure [5], using a flour/water to ethyl acetate ratio of 1:2 and with heating to 35°C, was selected as optimal and was validated for 24 representative analytes and for ^{14}C-chlorpyrifos in wheat flour at three different levels. Recovery data for each fortification level for both the extraction and clean-up steps are presented in Table 2. The average ^{14}C-chlorpyrifos recovery for wheat flour was 88 % with a relative standard deviation of 6 %.

Table 2. ^{14}C-Chlorpyrifos recoveries (Q) and repeatability of the recovery (as RSD) at different levels related to each step of the method

Fortification level (mg/kg)	Extraction		Clean-up		Overall	
	Q (%)	RSD (%)	Q (%)	RSD (%)	Q (%)	RSD (%)
0.03	89.4	6.14	94.0	1.53	**84.1**	**7.32**
0.3	94.5	2.95	95.1	0.59	**89.9**	**3.34**
3	94.8	2.05	96.5	1.41	**91.5**	**1.51**

Recovery results for each analyte at various fortification levels

GC-NPD and GC-ECD chromatograms of blank and fortified wheat flour extracts and matrix matched standards containing the 24 target analytes are shown in Figures 2 and 3. All analytes produced measures chromatographic peaks at all fortification levels.

Individual analyte recoveries of the replicates for different levels were calculated using weighted linear regression. The recovery data are summarized in Table 3. The typical recovery of the method, at all levels and for 23 of the analytes in wheat flour, was 94 % with a relative standard deviation of 9 %.

Table 3. Overview of some method validation characteristics

Fortification level (mg/kg)	Accuracy		Precision	
	Recovery (%)	Codex accept-able ranges	CV_A (%)	Codex accept-able ranges
0.03	95	70-120	10	20
0.3	94	70-110	9	15
3	94	70-110	8	10
Overall	**94**		**9**	

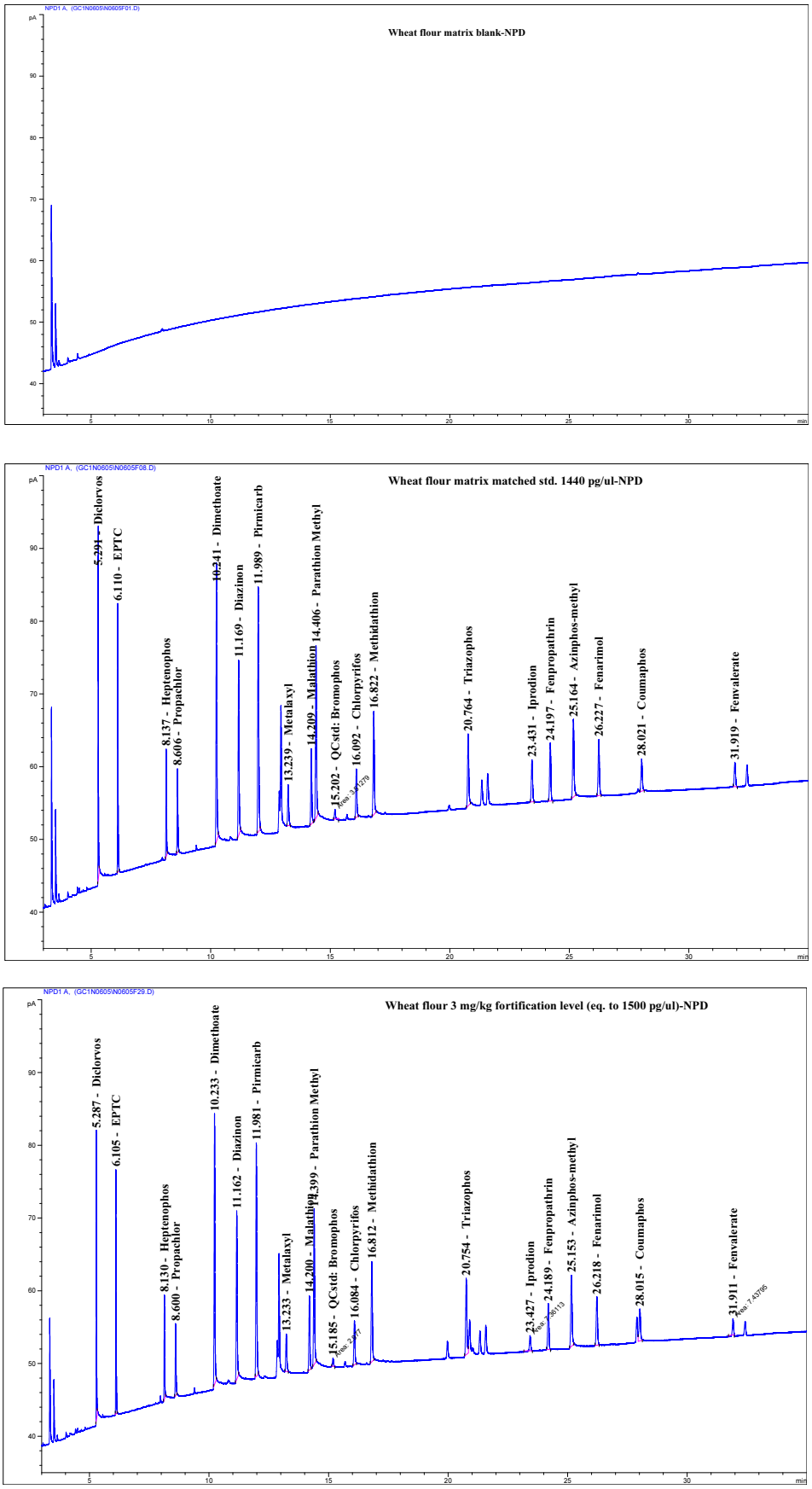


Figure 2. Representative GC-NPD Chromatograms of pesticides

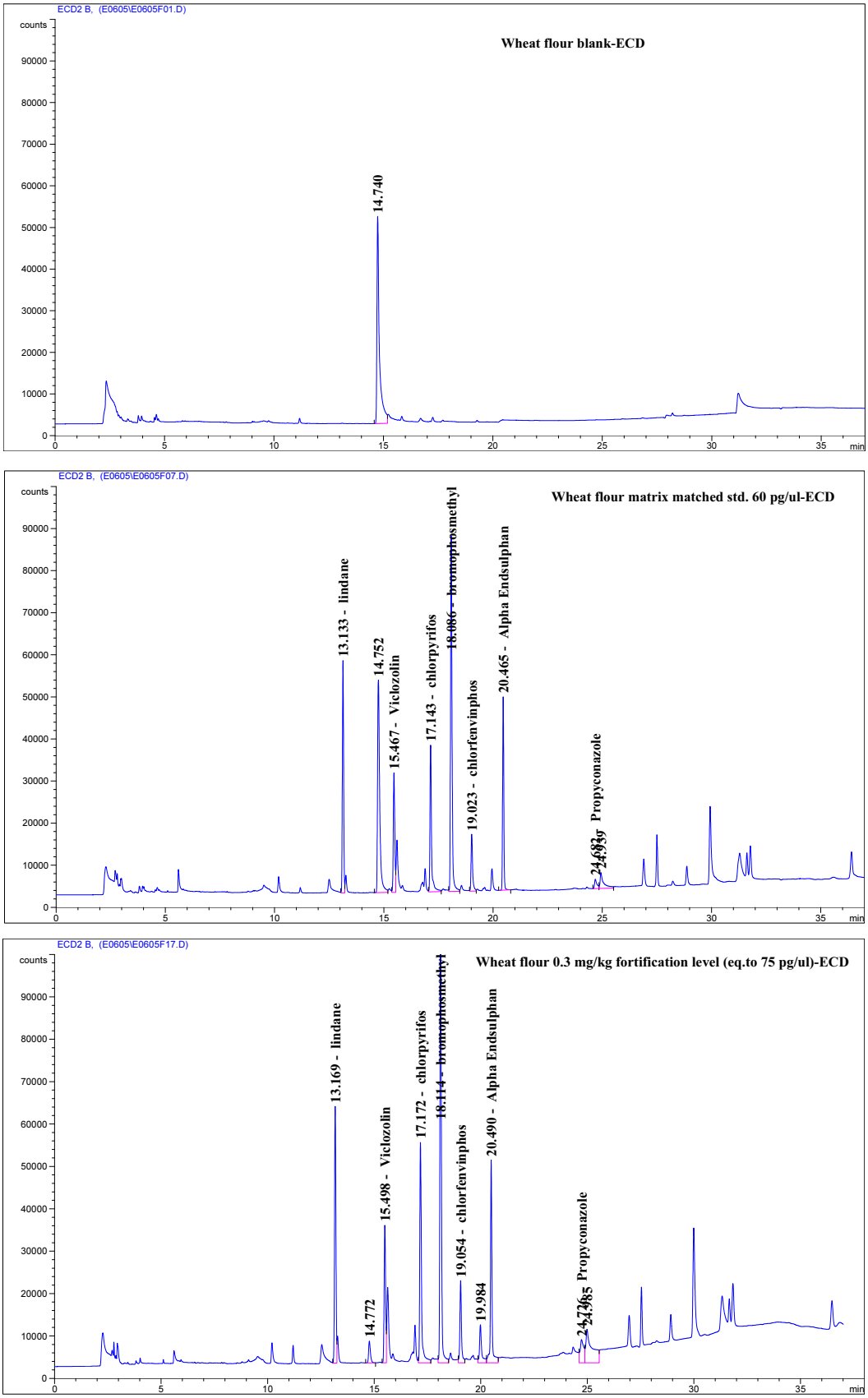


Figure 3. Representative GC-ECD Chromatograms of pesticides

Coumaphos determination at the level of 0.03 mg/kg was compromised by an interfering peak from the matrix, but the method performance for this compound at higher fortification levels was satisfactory (Figure 4).

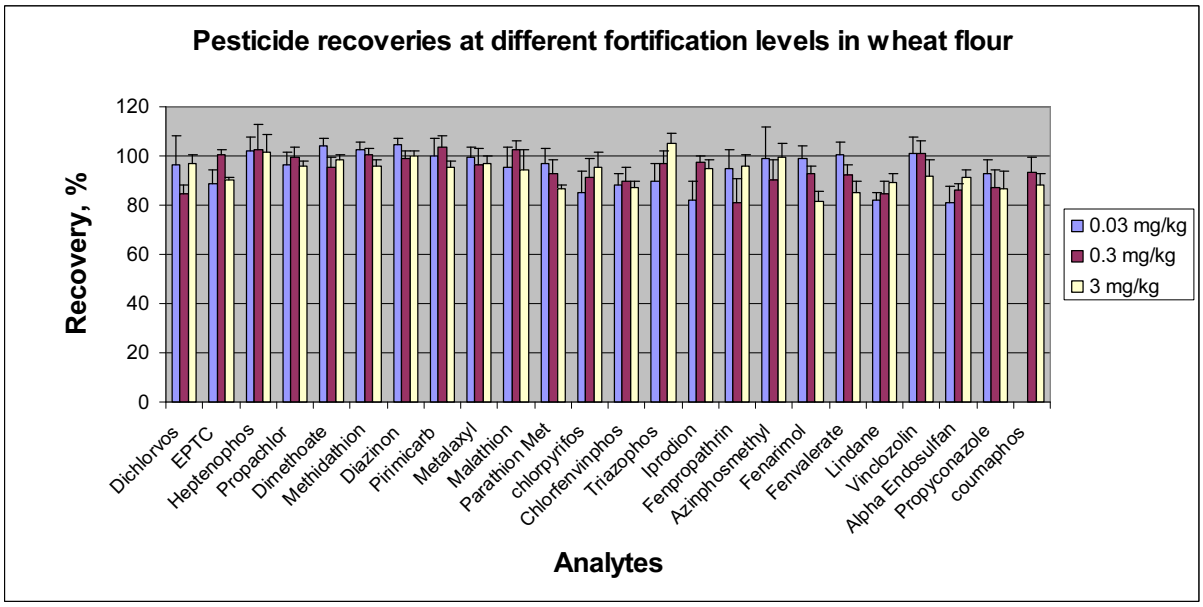


Figure 4. Individual pesticide recoveries at different fortification levels in wheat flour

Limit of Detection (LOD)

The LOD is another important parameter to be determined in method validation experiments. The LOD was estimated using calibration curves prepared in an extract of the wheat flour test matrix. The standard deviation of relative *y* residuals (Srr), which is a decisive parameter in internal quality control, should be less than 0.1 for the calibration curve. This was the case for all analytes in the study. Srr values and LOD values for each analyte are summarized in Table 4.

Table 4. Srr values of weighted regression calibrations and LOD values of the analytes

Analyte	Srr*	LOD (mg/kg)	Analytes	Srr*	LOD (mg/kg)
Dichlorvos	0.10	0.048	Triazophos	0.09	0.036
EPTC	0.06	0.024	Iprodion	0.04	0.022
Heptenophos	0.08	0.024	Fenpropathrin	0.08	0.038
Propachlor	0.06	0.028	Azinphos methyl	0.07	0.026
Dimethoate	0.08	0.028	Fenarimol	0.03	0.016
Diazinon	0.10	0.042	Coumaphos	0.05	0.042
Pirimicarb	0.09	0.038	Fenvalerate	0.05	0.034
Metalaxyl	0.04	0.014	Lindane	0.02	0.001
Malathion	0.09	0.030	Vinclozolin	0.07	0.003
Parathion methyl	0.08	0.024	Chlorfenvinphos	0.05	0.004
Chlorpyrifos	0.04	0.018	α-endosulfan	0.02	0.001
Methidation	0.08	0.028	Propiconazole	0.06	0.002

Conclusions

The method is considered fit for purpose since the mean recoveries and relative standard deviations at all fortification levels were within the specified acceptance criteria (70 %≤ Q ≤ 120 % and RSD ≤ 20 % for 0.03 mg/kg fortification level; 70 %≤ Q ≤ 110 % and RSD ≤ 10-15 % for 0.3 and 3 mg/kg fortification levels) for 23 compounds in wheat flour.

The advantages of the IAEA-ethyl acetate method in terms of quality of the results (accurate, repeatable and reproducible) and practical aspects (simplicity, low cost and waste, environmentally safe) make it suitable for application in food safety regulatory laboratories both in developed and developing countries.

A TC Fellow from Tanzania participated in the adaptation and validation of this method. A paper on the study was presented at a conference on "Pesticide Use in Developing Countries: Environmental Fate, Effects and Public Health Implications" organised by ANCAP (African Network for Chemical Analysis of Pesticides) and SETAC (The Society of Environmental Toxicology and Chemistry) Africa Branch, in Arusha, United Republic of Tanzania, 16-20 October 2006.

2.2. A multiresidue method for tetracyclines residues in pig liver

Tetracyclines are an important group of antibiotics used therapeutically in humans and animals and as prophylactics and growth promoters in livestock production. Oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC) and doxycycline (DC) are the most commonly used compounds.

Tetracyclines have a broad-spectrum activity against bacteria. The occurrence of residues of the compounds in human food, arising from their veterinary use, is a cause of concern to consumers worldwide, because of possible toxic or allergic reactions and the possibility that pathogenic organisms could become resistant to these drugs, thereby reducing their effectiveness when used in human medicine. It is necessary for analytical laboratories to have suitable methods in place to monitor the concentrations of these residues in edible tissues to ensure that good agricultural and production practices are followed, thus minimising the risk of the development of resistant organisms, safeguarding public health, and avoiding export-import disputes.

Chlortetracycline, OTC and TC have been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) and assigned Codex maximum residue limits (MRL) of 600 µg/kg in liver, 200 µg/kg in muscle and 1200 µg/kg in kidney of cattle, sheep, pig and poultry. In the European Union, the tetracyclines are included in Annex 1 of Council Regulation (EEC) 2377/90 of 26 June 1990, with MRLs of 100 µg/kg for muscle, 300 µg/kg for liver and 600 µg/kg for kidney for all food-producing animals. The EU has considered the fact that the tetracycline compounds can exist in animal tissues in a number of different isomeric forms, the main forms being the parent drug and its 4-epimer. The residue of interest in the EU legislation is the sum of each parent compound and its 4-epimer, except for DC where the MRL is set only for the parent drug.

Tetracyclines (TCs) can be determined in various biological matrices using high performance liquid chromatography (HPLC) in reverse-phase mode, with different detection techniques, such as ultra-violet (UV), fluorescence and mass spectrometry. Detection methods using UV generally have low sensitivity, while mass spectrometry still requires costly instruments. In general fluorescence detection is both sensitive and selective. The purpose of this work was to adapt a multi-residue method to determine tetracycline residues in pig liver, for transfer to Member State laboratories via training courses and fellowship training at Seibersdorf. Various methods from the scientific literature

were examined and a decision was taken to base the study on the method originally developed by Blanchflower et al[4].

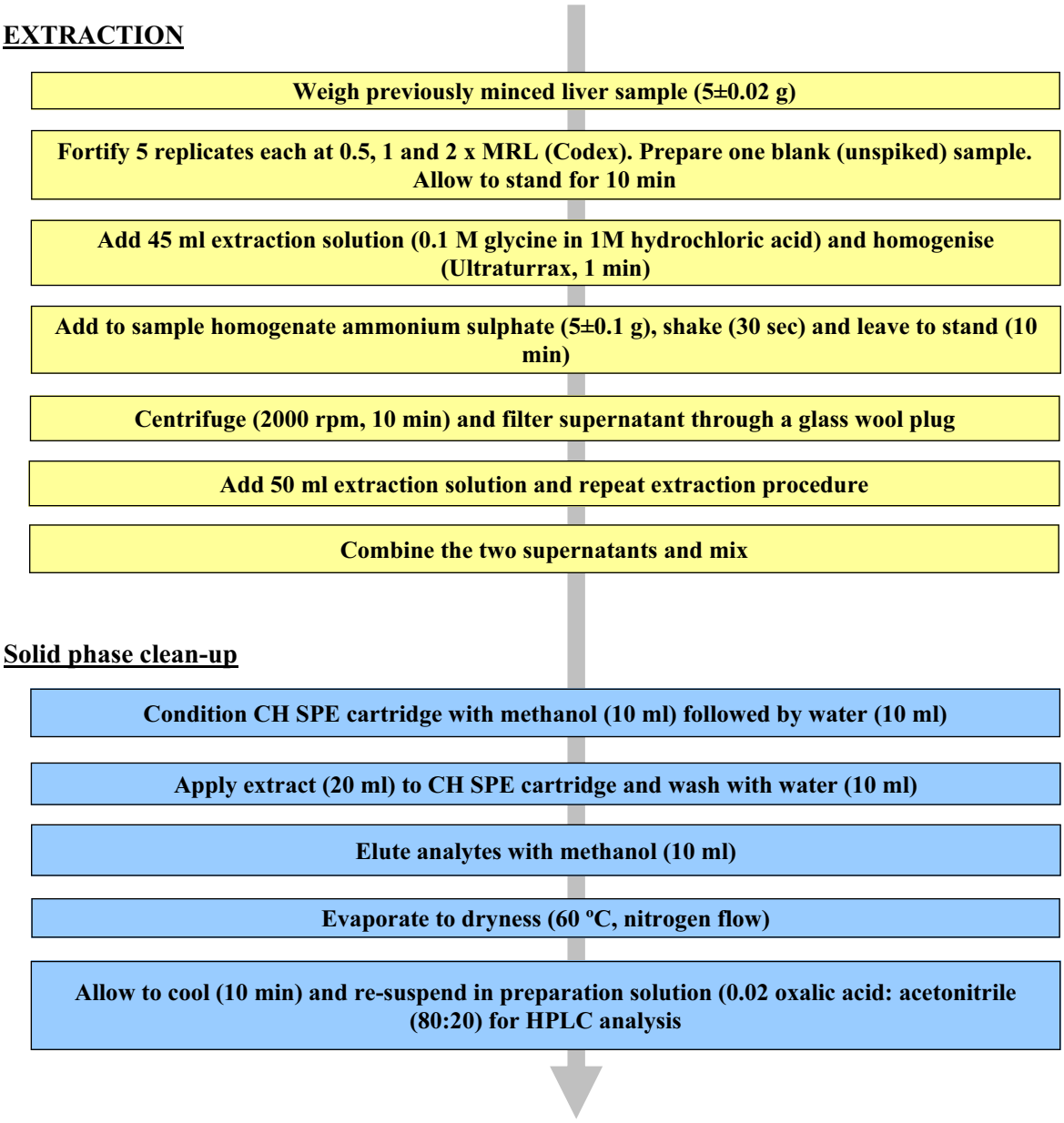


Figure 5. Sample preparation procedure for tetracyclines analysis

[4] Blanchflower, W.J., McCracken, R.J., Haggan, A.S., Kennedy, D.G. (1997). Confirmatory assay for the determination of tetracycline, oxytetracycline, chlortetracycline and its isomers in muscle and kidney using liquid chromatography-mass spectrometry. J. Chrom B, 692, 351-360

Results

Different clean up regimes using alumina, charcoal, cyclohexyl (CH), and a combination of the above, were investigated in an attempt to reduce the background signal and optimize the chromatography for the analysis of the target analytes. It was found that CH solid phase extraction (SPE) gave the best results, and additional clean-up steps did not improve the final chromatograms with respect to interferences. It was noted that there are differences in the batch to batch quality of the SPE cartridges.

A preliminary validation exercise was performed using replicate blank pig liver samples fortified with a mixture of OTC, TC, CTC, 4-epi-CTC and DC at three different levels; 300 µg/kg, 600 µg/kg and 1200 µg/kg. The results are shown in Table 5. Although the recoveries are relatively low, especially for OTC and TC, this is typical for these compounds, which are difficult to analyse in a multiresidue method because of their complex molecular structure, their existence in different isomeric forms (epimerization to the 4-epi form in aqueous solution between pH 2 and 6, tautomerisation between keto- and enol- isomers in aqueous solutions) and their avidity as chelating agents with metal ions.

Quantification was carried out using matrix matched calibrators prepared by spiking extracts of the same blank liver matrix, prepared using the same extraction and clean-up procedure, as the samples and included in the same batch. It was found that matrix effects were significant, especially for OTC, and therefore quantification using matrix matched calibrators was necessary to give realistic results.

Table 5: Repeatability of the method for pig liver

Spike (µg/kg)	n=5	OTC	TC	4 epi-CTC	CTC	DC
300	Rec (%)	49.9	41.3	82.3	67.0	54.1
	CV (%)	33	30	21	18	20
600	Rec (%)	49.4	42.3	77.3	71.9	61.0
	CV (%)	10	14	6	7	4
1200	Rec (%)	49.4	35.2	72.0	67.4	59.2
	CV (%)	5	9	4	3	2
overall	Rec (%)	**49.2**	**40**	**78.2**	**68.4**	**57.3**
	CV (%)	**22**	**23**	**16**	**12**	**14**

Representative chromatograms of an extract of a negative pig liver (A), a mixed tetracycline reference standard (B), and an extract of a negative pig liver fortified at 1200 µg/kg (C), are shown in Figure 6. The analytes are chromatographically resolved and free from significant background interferences. It is interesting to note that the liver used for this validation experiment was obtained from the market and contains a low concentration of OTC. This is not surprising, given the extremely widespread use of these drugs. The concentration found was well below the EU MRL for OTC in liver.

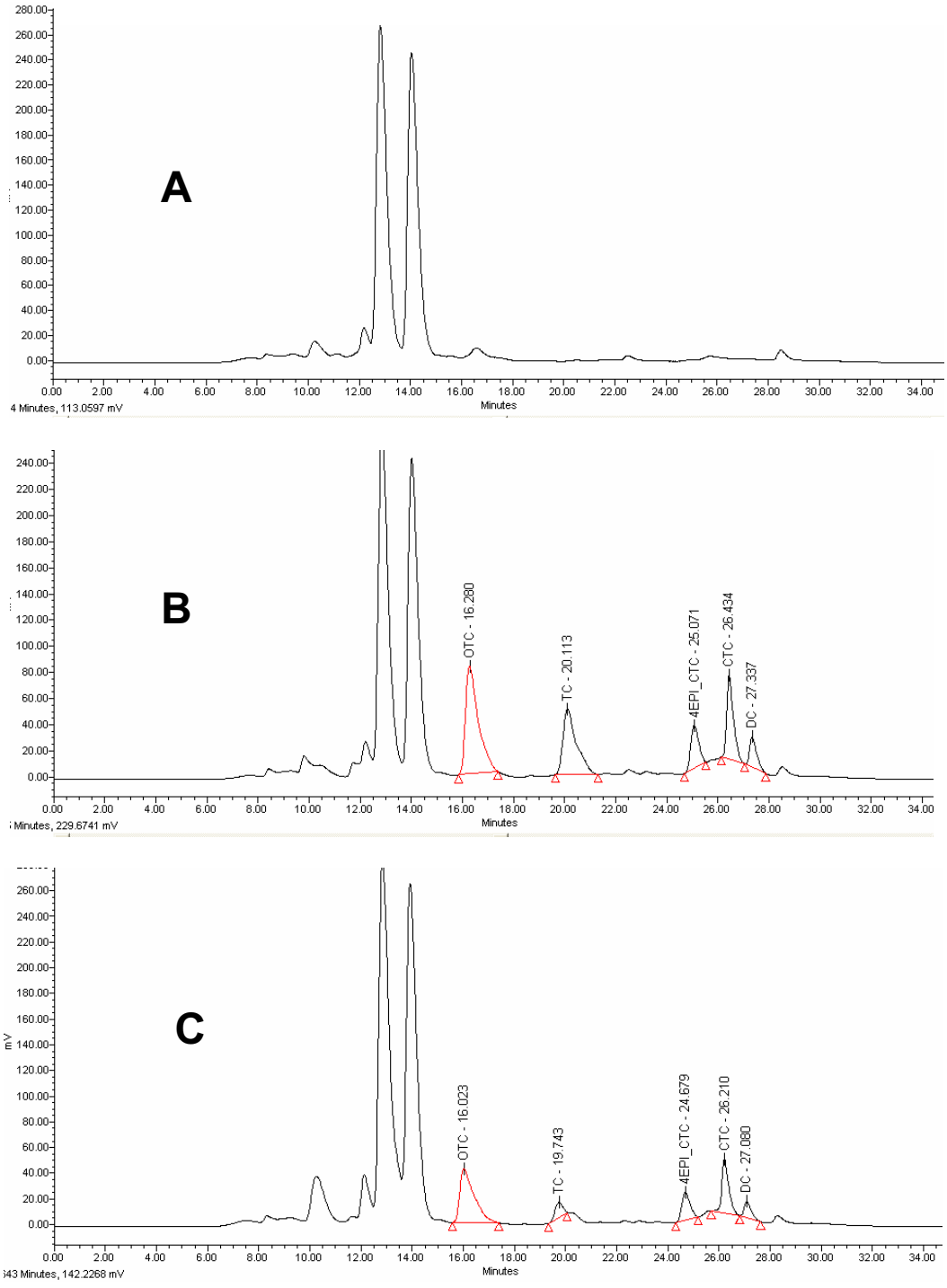


Figure 6. Representative chromatograms of (A), an extract of a blank pig liver (this is a market sample and there is contamination with OTC); (B), a mixed tetracycline matrix-matched reference standard; and (C), an extract of pig liver fortified at 1200 µg/kg.

Conclusions

The method was successfully adapted and has undergone a preliminary validation on a single day and with a single matrix. The results demonstrate good chromatographic resolution of the compounds. The recovery values for the method are relatively low, especially for OTC and TC, but are typical for analytical methods for these compounds and similar to values published in the scientific literature.

The method requires further investigation to improve the recovery values. The use of radiolabelled tetracyclines would be extremely helpful in this respect, but because of the complexity of these molecules, radiolabelled versions are extremely expensive to synthesize and are not currently available commercially.

Two trainees from an FAO project in Algeria were trained during the development and initial validation of this method.

2.3. Liquid chromatography-tandem mass spectrometry (LC-MSMS) confirmatory method for 13 sulphonamides

The sulphonamides are amongst the most widely used antimicrobial drugs in food-producing animals, both therapeutically and at sub-therapeutic levels as growth enhancers. Codex and other National and Regional bodies have set maximum residue levels for these substances in various animal tissues.

In 2005 a simple and inexpensive method was developed in the Agrochemicals Unit for the analysis of seven sulphonamide antibiotics in animal tissues and milk by HPLC with post-column derivatisation of the analytes and UV detection (reported in the Agrochemicals Unit Annual Report 2005). The method was extensively validated in-house, with the help of two TC Fellows from Montenegro and a cost-free consultant from Brazil, and was demonstrated to be suitable for screening and quantitation of sulphonamide residues in regulatory food safety laboratories. However, current regulations and guidelines require that, when samples are suspected, on the basis of a screening result, to contain concentrations of substances that are non-compliant with respect to international or national regulations, highly specific and selective methods must be available to confirm the presence of those substances. The technique of choice is mass spectrometry in combination with chromatography, since this provides the best combination currently available to the analyst of sensitivity, selectivity and specificity based on molecular information derived from the analyte. Considering the possible impact that non-compliant results may have on the farmer as well as on trade, product image and food safety perception by the customer, the necessity to use conclusive confirmatory techniques based on mass spectrometry is evident. To provide a method which meets these requirements for Member State laboratories, the HPLC method validated in 2005 has now been further developed as a confirmatory method using liquid chromatography-tandem mass spectrometry (LC-MSMS). The method meets the identification criteria specified by the European Union in Commission Decision 2002/657/EC, and included in the draft revised Codex guidelines[5], for confirmatory methods for compounds licensed for use in food-producing animals.

The sulphonamide drugs are analyzed using electrospray ionization (ESI), the most appropriate technique for polar, ionic, thermo-labile compounds, in positive ion mode using a triple-quadrupole mass analyzer. The triple-quadrupole analyzer consists of two quadrupoles separated by a collision cell. The first quadrupole can be set to select and transmit a specific ion, the 'parent' ion, characteristic of the analyte. This specific ion can be fragmented by collision with inert gas atoms in the collision cell, and the fragments, or 'daughter ions' are selectively transmitted by the second quadrupole to the detector. The detection of these highly specific fragments, which have arisen from a characteristic ion in the analyte, provides unequivocal identification of the substance. Using the instrument in this manner is known as multiple reaction monitoring (MRM).

[5] Proposed Draft Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the Use of Veterinary Drugs in Food Producing Animals, ALINORM 06/29/31 Appendix VII, http://www.codexalimentarius.net/web/index_en.jsp

One advantage of LC-MSMS, apart from the high sensitivity and specificity of the technique, is the possibility of significantly reducing analytical run times because individual analytes do not always need to be chromatographically resolved. To illustrate this, Figure 7 shows a chromatogram of seven sulphonamides at a concentration of 1.0 ng/µl, generated using the previously validated HPLC method with post-column derivatisation and UV detection. The peaks are well resolved in a run time of approximately 20 minutes, facilitating accurate peak area measurement for quantitation. Figure 8 shows MRM chromatograms for the same seven sulphonamides run by LC-MSMS. Because the mass spectrometer measures the characteristic ions of each analyte separately, there is no need for chromatographic baseline resolution, allowing a much shorter run time (about 7 minutes in this case), achieved using a shorter analytical column with a narrower bore and a reduced mobile-phase flow rate, thereby reducing both solvent/reagent usage and waste disposal costs. The system also allows the measurement of two characteristic daughter ions generated from the primary 'parent' ion for each compound, the ratios of which can be measured, thus fulfilling the above mentioned identification requirements for confirmatory methods.

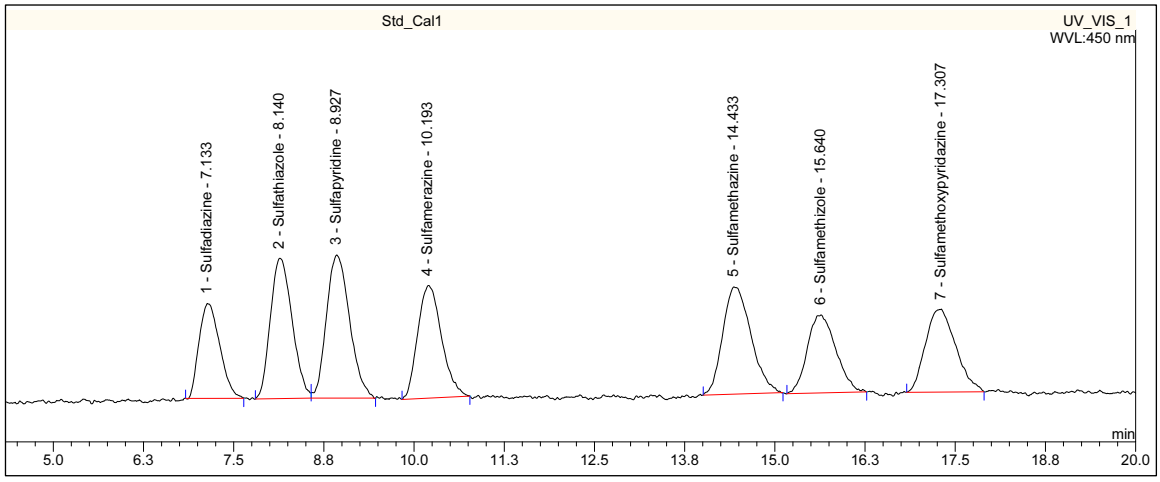


Figure 7. HPLC–UV chromatogram of seven sulphonamides at 0.1 ng/µl. The runtime is 20 minutes.

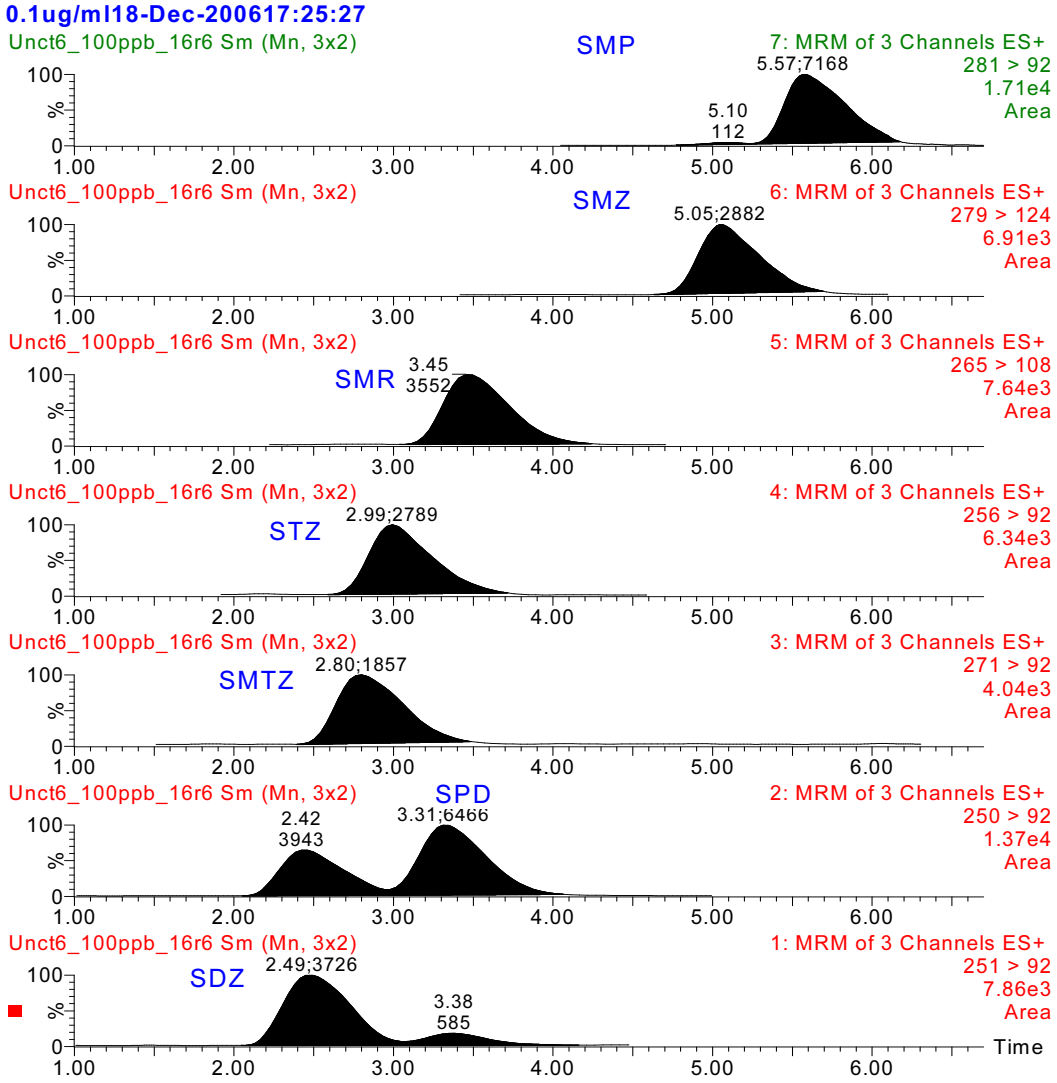


Figure 8. LC-MSMS MRM chromatograms of seven sulphonamides at 0.1 ng/µl. The run time is 7 minutes.

Work is currently under way to extend the scope of the confirmatory method to include six more members of the sulphonamide class of drugs. Various parameters must be optimized to meet the sensitivity and the reproducibility requirements for analytical results. One problem that must be resolved is the phenomenon of ion suppression, which is a competition effect exhibited when a sample contains a high concentration of salts or another co-eluting substance arising from the matrix being analysed that can ionize under given operating conditions, thus affecting the relative abundance of ions produced from the analyte under study. Such matrix effects can cause difficulties in quantitation of the analytes if calibrators prepared in pure solvent or mobile phase are used. Current results obtained using different mobile phases show that there is clear ion-suppression for most of the the sulphonamides when using the standard calibrators prepared in matrix extracts. Figure 9 shows that there is an obvious decrease in ionization response of some selected sulphonamides when the matrix is present. It is therefore necessary to measure the concentration of analytes found in the sample using matrix-matched standard calibration since an underestimation of the concentration would result if the instrument is calibrated using standards prepared in solvent. Another means of overcoming this problem in mass spectrometric techniques is by inclusion of one or more stable

isotope internal standards in an isotope dilution format. This option will be investigated for the sulphonamide method in future work.

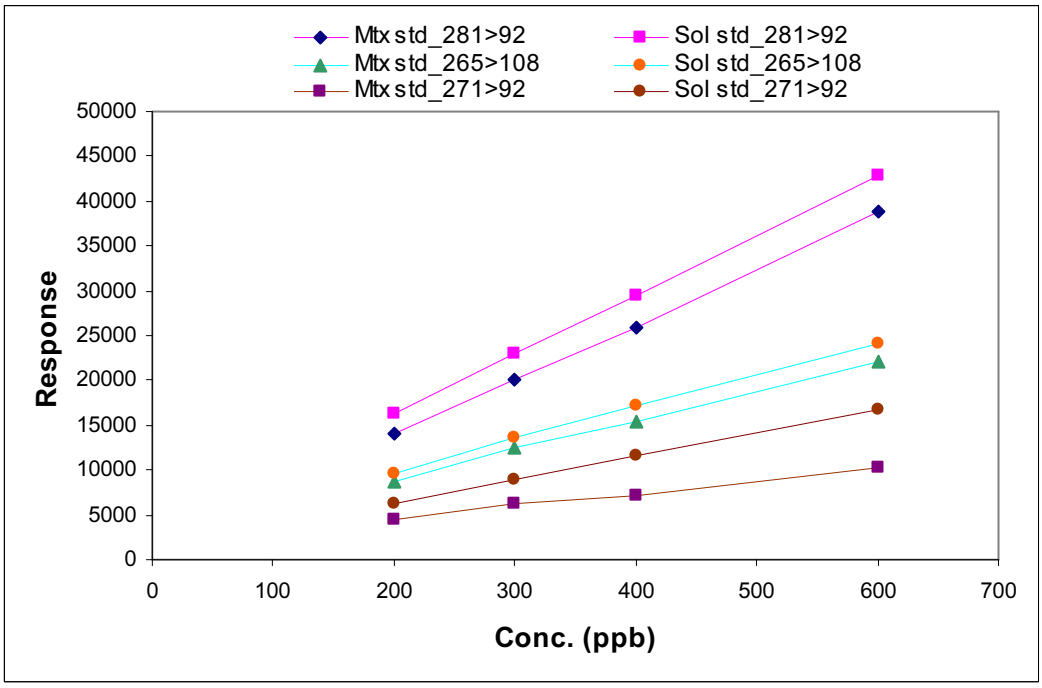


Figure 9. Matrix and solvent standard calibration points (4 levels) of 3 different daughter ions of selected sulfonamide analytes.

The method is being developed further and has currently been extended to include thirteen sulphonamides, all of which are licenced for use in animals in different regions of the world. The compounds covered are sulphadiazine, sulphathiazole, sulphapyridine, sulphamerazine, sulphamethazine, sulphamethizole, sulphamethoxy-pyridazine, sulphaguanidine, sulphanilamide, sulphachloropyridazine, sulphadoxine, sulphadimethoxine and sulphaquinoxaline. Typical MRM chromatograms for two daughter ions of each compound are shown in Figure 10. Though the compounds are not chromatographically separated, each ion gives reproducible results and all are eluted in less than 10 minutes under the isocratic conditions used here. The ratios of the abundances of the two daughter ions in each case were within the limits specified in the identification criteria for confirmatory methods by the EU and the revised Codex Guidelines.

This method will be finalized and validated in 2007. The confirmatory method employs the simple extraction protocol developed for the HPLC method, using an inexpensive extraction solvent (ethyl acetate) and with no solid-phase clean-up step, and will therefore provide a cheap and robust LC-MSMS confirmatory method for a range of sulphonamides.

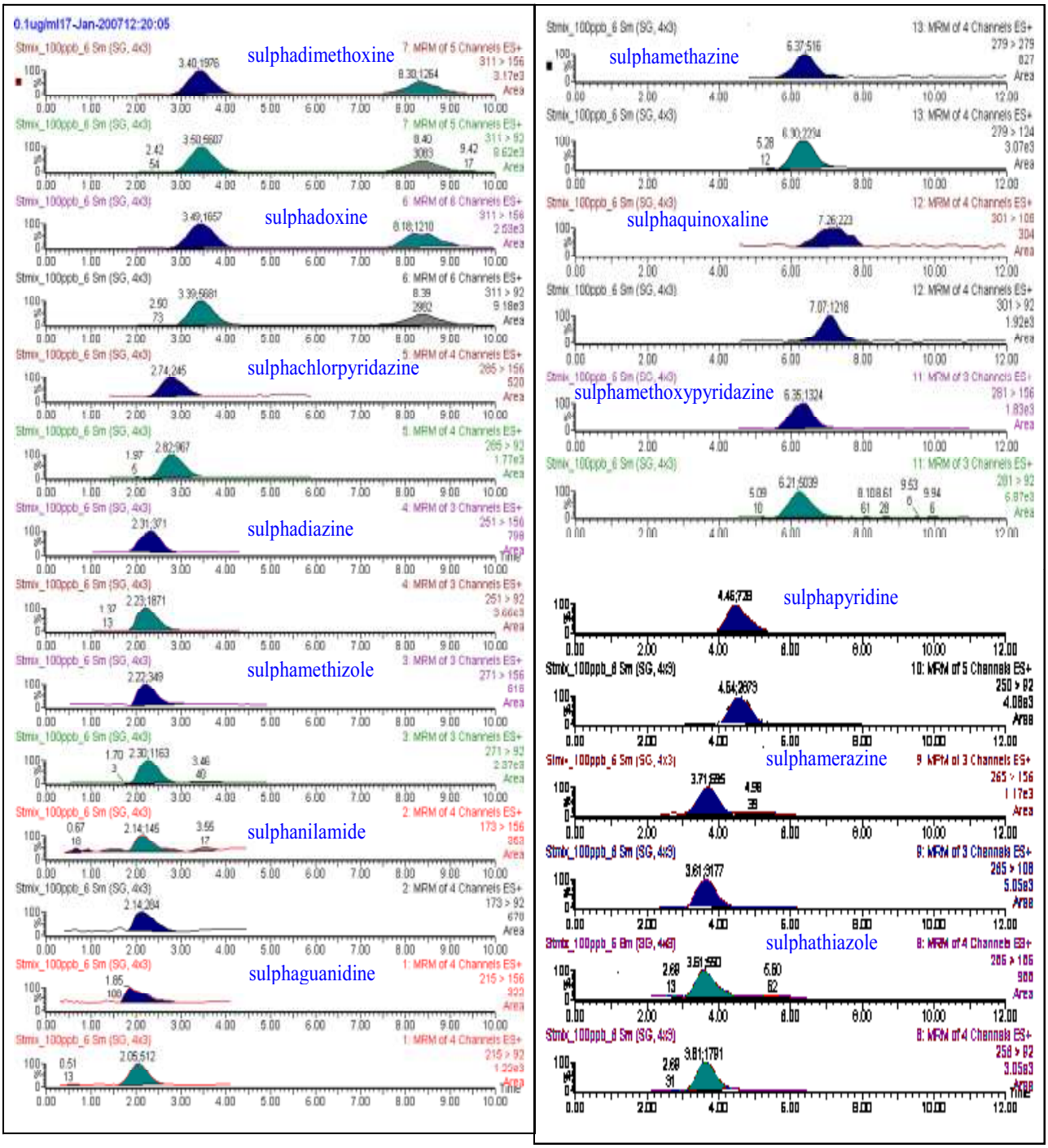


Figure 10. LC-MSMS multiple reaction monitoring chromatograms of two daughter ions for each of 13 sulphonamides at 0.1 ng/µl

2.4. Comparison of Methods for the Estimation of Measurement Uncertainty for an Analytical Method for Sulphonamides

To conform to Codex Standards and national and international regulations, analytical methods used to test compliance with regulatory limits for contaminants in food must be validated to demonstrate that they are 'fit for purpose'. The validation includes parameters such as accuracy, sensitivity, ruggedness and limit of detection, but it is now necessary also to estimate the measurement uncertainty associated with a method. Measurement uncertainty is defined[6] as 'a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand'. It is a requirement for laboratories working under the ISO 17025 quality system that the measurement uncertainty associated with a result should be available and reported if it is required by the client, is relevant to the validity of the test results, or if it mat affect compliance with a specification, for example compliance with a maximum residue limit for veterinary drugs or pesticides in food.

The traditional method for estimating measurement uncertainty is by a 'bottom-up' approach, as described by EURACHEM/CITAC[7]. This approach aims to estimate the individual contribution of every step and input to the analytical process to the overall uncertainty, and is time consuming and difficult to apply to analytical methods for food contaminant regulation. A more practical 'top-down' approach has more recently been recommended[8], which uses data obtained from interlaboratory studies, but this method is also not ideal for application in residues laboratories, because it assumes that a single standardized method is used in all laboratories, which is not true for residues analysis. One option is to apply a 'top-down' approach to the data generated by the in-house validation of the method. This approach is relatively simple and requires no extra practical work, using data which are already generated for the method validation. However, this method provides no information on the relative contribution of individual steps of the method to the overall uncertainty of the result, and is not yet accepted by most inspection/auditing bodies for accreditation to ISO 17025.

The purpose of this study was to estimate the measurement uncertainty associated with a simple liquid chromatographic method for the determination of seven sulphonamides in animal tissues (reported in the Agrochemicals Unit Annual Report 2005). Two methods were used for the estimation – a 'top-down' method, based on in-house validation data, and a hybrid 'bottom-up/top down' approach, based on the approach of Štěpán *et al*.[9], which aimed to simplify the traditional 'bottom-up' approach. The hybrid approach was also used to identify critical steps in the analytical procedure, which comprised an ethyl-acetate extraction in the presence of acid and sodium sulphate, evaporation/concentration, reconstitution in acidic methanol/water, hexane wash, and analysis by HPLC with post column derivatisation and UV detection. Six replicates of porcine kidney were fortified at 100 µg/kg at different stages of the analytical procedure: extraction, evaporation, and HPLC analysis. The uncertainties of the gravimetric and volumetric measurements, as well as the standard purity were estimated and integrated in the calculation of the total combined uncertainty.

[6] ISO, International Vocabulary of Basic and General Terms in Metrology, International Standards Organisation, Geneva, 1993.
[7] Quantifying Uncertainty in Analytical Measurement (Guide 4), Eurachem/Citac, 2000.
[8] ISO/TS 21748, Guidance for the Use of Repeatability, Reproducibility and Trueness Estimates in Measurement Uncertainty Estimation, 2004.
[9] Štěpán, R., Hajšlová, J., Kocourek, V. and Tachá, J. (2004). Uncertainties of gas chromatographic measurement of troublesome pesticide residues in apples employing conventional and mass spectrometric detectors, Analytica Chimica Acta, 520, 245-255.

In both approaches, it is considered that under real-life conditions, uncertainty of each analytical step consists of random and systematic error components, hence each of component was quantified and incorporated into the combined standard uncertainty.

Hybrid bottom-up/top-down approach

For the hybrid approach, the major steps for the analytical procedure were identified as: extraction (F1), evaporation (F2), and HPLC detection (F3). Eighteen replicates of porcine kidney were used in the experiment, six replicates were fortified at 100 µg/kg at each of the three different stages of the analytical procedure: extraction, evaporation, and HPLC analysis. The uncertainties of the gravimetric and volumetric measurements, as well as the standard purity were estimated and integrated in the calculation of the total combined uncertainty, calculated using equation 1.

$$u_c(\%) = \sqrt{u_{cEx}^2 + u_{cEv}^2 + u_{HPLC}^2 + u_{Bal}^2 + u_{Dil}^2 + u_{Std}^2} \qquad \text{Equation 1}$$

Using this method, the major contributor to the total combined uncertainty was identified as the extraction step, with values ranging between 3.8% - 8.4%. Figure 11 shows the individual contributions of each step in the total procedure for sulphonamides analysis. The uncertainty related to weighing, dilution and standard purity did not contribute significantly to the total uncertainty estimate. Two of the analytes, sulphathiazole and sulphamethizole, had a high variance during the

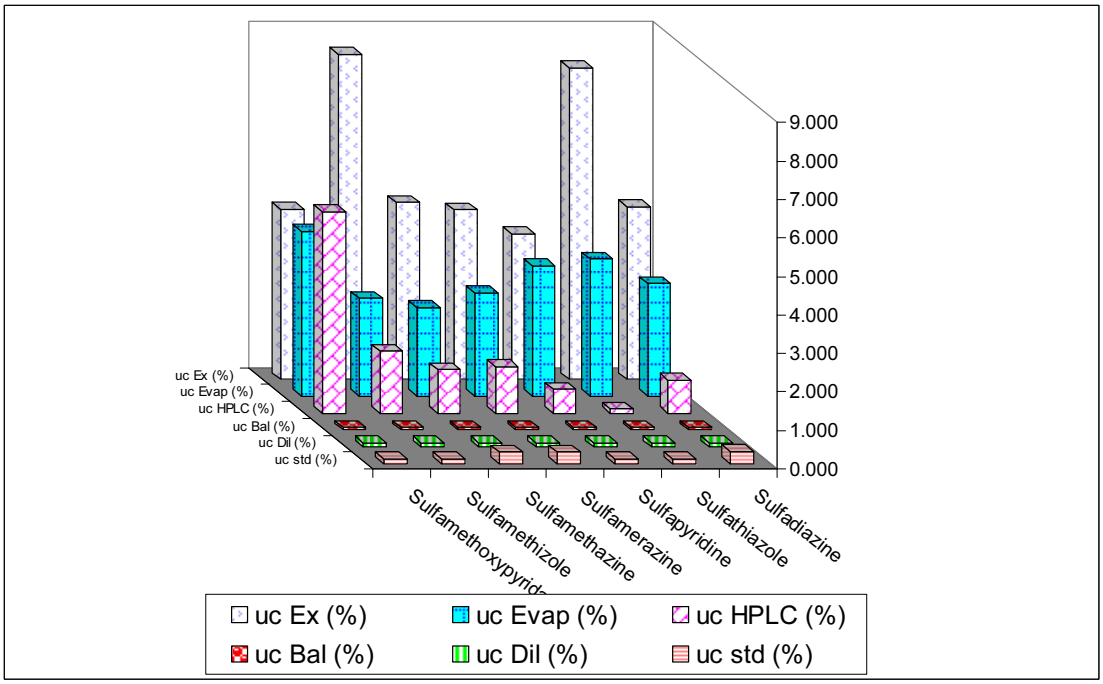


Figure 11. Contributions of individual uncertainty to the sulphonamides method

The uncertainty of the recovery for each individual stage of the method (extraction, evaporation and HPLC analysis) is calculated by first estimating the uncertainty associated with the recovery from each fortification step using equations 2, 3 and 4. The uncertainty due to HPLC analysis is equivalent, in this study, to the uncertainty of recovery from the final fortification step. The uncertainties are tabulated in Table 6.

$$u_{(R)1-3}(\%) = \frac{0.5\,x\,(100 - R_{F1-3})}{\sqrt{3}} \qquad \text{Equation 2}$$

(assuming a rectangular distribution)

Where: $\mathbf{R_{F1-3}}$ is the average recovery of the analyte from the first to the last stage of fortification (n=6).

$\mathbf{u_{(R)1-3}}$ is the uncertainty of recovery from the first to the last stage of fortification

$$u_{(R)Ex}(\%) = \sqrt{u_{(R)1}^2 - u_{(R)2}^2 - u_{(R)3}^2} \qquad \text{Equation 3}$$

$$u_{(R)Evap}(\%) = \sqrt{u_{(R)2}^2 - u_{(R)3}^2} \qquad \text{Equation 4}$$

Where: $\mathbf{u_{(R)Ex}}$ is the uncertainty of the recovery of associated with extraction

$\mathbf{u_{(R)Evap}}$ is the uncertainty of the recovery of associated with evaporation

Table 6. Uncertainty of recoveries for the individual steps of the procedure

Spiked:	Before extraction	Before evap	Before reconst			$u_{(R)HPLC}$		
For recoveries	R_{F1} (%)	R_{F2} (%)	R_{F3} (%)	$u_{(R)1}$ (%)	$u_{(R)2}$ (%)	$u_{(R)3}$ (%)	$u_{(R)Ex}$ (%)	$u_{(R)Evap}$ (%)
Sulphadiazine	89.0	97.5	101.5	3.2	0.7	0.4	3.1	0.6
Sulphathiazole	74.0	93.0	99.8	7.5	2.0	0.1	7.2	2.0
Sulphapyridine	88.3	97.8	101.2	3.4	0.64	0.35	3.3	0.5
Sulphamerazine	89.2	97.8	102.2	3.1	0.6	0.6	3.0	0.0
Sulphamethazine	89.3	97.2	101.7	3.1	0.8	0.5	2.9	0.6
Sulphamethizole	75.8	93.2	102.8	7.0	2.0	0.8	6.7	1.8
S'methoxypyrid	80.3	88.2	94.5	5.7	3.4	1.6	4.3	3.0

When blank extracts were fortified directly after extraction, the recoveries of sulphathiazole and sulphamethizole were 93.0% and 93.2%, respectively, whereas when the replicate portions were spiked before the extraction, their recoveries were only 74.0% and 75.8%.This, in conjunction with the high uncertainty values for these two analytes (Figure 11), would indicate that the extraction step is the probable cause of the poor recovery of those analytes during method validation. This illustrates how the bottom-up approach is useful in helping the analyst to evaluate the possible sources of analytical problems and in improving the method during development.

Top-down approach

In the 'top-down' approach, the uncertainty is calculated using the data from the method validation study. In order to compare the results of both approaches, the same spiking level was chosen. The repeatability of the method was calculated as relative standard deviation and the uncertainty of

recovery was also determined in order to obtain the combined standard uncertainty. The average of the six replicates for each analyte was used in the calculations. The total combined uncertainty was calculated as the quadratic mean of combined standard uncertainties derived from the validation data from three different occasions.

Table 7 shows the uncertainty values of the seven sulphonamides analytes for each individual step of the procedure and the average values obtained for the hybrid and the 'top-down' method. There was no statistical difference between the uncertainty values obtained by either approach, so for this analytical method the analyst would be justified in applying the 'top-down' estimation using method validation data, rather than performing additional experiments to obtain uncertainty data.

Conclusions

The hybrid approach simplifies the traditional 'bottom-up' approach to some extent whilst retaining the advantage that critical steps can be identified, allowing further improvement of the method. After the method development process has been completed, it is much simpler and more cost-effective to apply the 'top-down' approach to uncertainty estimation. This would be especially relevant in situations where a laboratory adapts a Codex endorsed method, or a method from the literature, which requires validation but no method development. The results from this study have shown that the estimates of combined uncertainty using both methods give comparable results.

Table 7. Comparison of uncertainty values between hybrid approach and the "top-down" method

Combined uncertainties obtained using the hybrid approach								**Top down**
Sulphonamide	$u_{c(ex)}$ (%)	$u_{c(evap)}$ (%)	$u_{c(HPLC)}$ (%)	$u_{c(bal)}$ (%)	$u_{c(dil)}$ (%)	$u_{c(std)}$ (%)	**u_c** (%)	**u_c** (%)
Sulphadiazine	4.491	2.930	0.866	0.069	0.109	0.318	**5.4**	**6.2**
Sulphathiazole	8.096	3.567	0.110	0.067	0.109	0.131	**8.8**	**9.3**
Sulphapyridine	3.790	3.389	0.624	0.066	0.109	0.131	**5.1**	**5.9**
Sulphamerazine	4.406	2.698	1.207	0.066	0.109	0.316	**5.3**	**5.9**
Sulphamethazine	4.605	2.316	1.129	0.065	0.109	0.315	**5.3**	**5.4**
Sulphamethizole	8.453	2.548	1.617	0.065	0.109	0.130	**9.0**	**9.5**
S'methoxypyrid	4.423	4.294	5.239	0.064	0.109	0.130	**8.1**	**7.5**

2.5. Influence of climate change on the environmental behaviour of s-metolachlor in a soil-plant-water system

A poster entitled "Influence of climate change on the environmental behaviour of s-metolachlor in a soil-plant-water system" (authors B. Wimmer, E. Kaltenbrunner, M. Schweikert Turcu, F. Strebl) was presented at the workshop "Lysimeters for Global Change Research: Biological Processes and the Environmental Fate of Pollutants", 4th - 6th October 2006 at the campus of the GSF- National Research Center for Environment and Health in Neuherberg, Germany. The poster reflected the results of a collaborative project between Agrochemicals Unit and the Department of Agricultural Research of the Seibersdorf Austrian Research Center (ARC). The analytical methodology for the measurement of s-metalochlor was adapted in the Agrochemicals Unit and sample analysis was then carried out in the Unit by a student of the Fachhochschule Wels (University for Biotechnology and Environmental Engineering), who was assigned to the project through the ARC.

The experimental design, using monolith lysimeters, facilitated an investigation into the effect of climate warming changes anticipated by many scientists on the behaviour of pesticides in the environment. Simulated increased temperature (+3˚C) and higher precipitation intensities in fewer irrigation events positively influenced soy plant germination and growth, but no significant effect was observed on pesticide leaching and degradation rates in soil.

2.6. Internal quality control procedures for the analysis of fumonisin B1 in primary samples of corn from Nigeria

The fumonisins are a group of structurally related compounds mainly produced by *Fusarium verticillioides* and other *Fusarium* fungal species, which are field pathogens of maize (Zea mays) and other cereals. More than ten types of fumonisins have been isolated and characterized. Of the six structurally related metabolites (Fumonisin B1, B2, B3, B4, A1 and A2) isolated from cultures of *F. moniliforme*, fumonisin B1 (FB1), fumonisin B2 and fumonisin B3 are the major compounds usually present both in maize fungal cultures and in naturally contaminated maize. The most prevalent of these mycotoxins in contaminated maize is fumonisin B1, which is believed to be the most toxic (EFSA[10], 2005).

The most significant crop in which fumonisins occur is maize, particularly when grown in warmer regions. Maize has become Africa's most important staple food crop, with production and consumption of maize exceeding that of other cereals such as wheat or sorghum. Maize has been in the diet of Nigerians for centuries. Furthermore, as a primary staple human food in West Africa, maize may be consumed up to three times a day and is used as a weaning food for babies. Therefore, it is extremely important to be able to detect and control mycotoxins residues in food to ensure public health.

The objective of this work was to set up internal quality control procedures (IQC) for the analysis of fumonisin B1 in maize samples in order to enable the analyst to decide whether the results were acceptable.

Sampling Locations

Maize kernel samples were purchased in Nigeria from markets, retail outlets, and cereal stores in Lagos, Ibadan, Maiduguri, Kadana, and Enugu. These five locations are dispersed through out Nigeria and are identified on the map in Figure 12. The study design specified that 20-shelled maize lots be identified at each of the five locations (100 lots) and a two kg bulk sample would be taken from each maize lot. The 100 x two kg bulk samples were identified by location and lot number and sent to the Agrochemicals Unit for analysis. Each 2 kg bulk sample was divided into twenty 100 g primary samples.

[10] EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the Commission related to fumonisins as undesirable substances in animal feed. http://www.efsa.eu.int. The EFSA Journal (2005) 235, 1 – 32

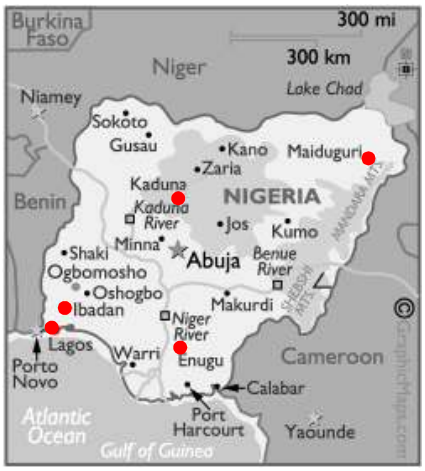


Figure 12. Map of Nigeria with sampling locations indicated in red

Sample preparation and extraction

Each 100 g primary sample of maize kernels was finely ground using a RAS II Romer Mill and thoroughly mixed. A 25 g analytical portion was taken from each comminuted primary sample. Fumonisin was extracted from the 25 g analytical portion with 50 ml-methanol-water (3+1) into a 500 ml Duran screw-cap glass container, using a Certomat SII rotary shaker (B. Braun Biotech International), 1hr. at 170 rpm, and then filtered throughout Whatman number 4 filter paper. The samples were cleaned up by solid phase extraction on Bond-Elut strong anion-exchange (SAX) cartridges (Varian, Harbor City, CA). The cartridges were fitted to a 12-port Supelco solid-phase extraction (SPE) manifold (Supelco, Bellefonte, PA) and conditioned, prior to application of the sample extracts, by the successive passage of methanol (5 ml) and methanol: water (3:1, 5 ml). The cartridge was not allowed to dry. Ten ml of the filtered extract was applied to SAX cartridges while maintaining the flow rate below 2 ml/min. The SAX cartridges were washed with methanol:water (3:1, 8 ml) followed by methanol (3 ml). Fumonisin B1 was eluted with 14 ml 0.5% methanolic acetic acid, at flow rate £ 1 ml/min into conical glass screw-cap test tubes. The eluates in conical glass test tubes were evaporated to dryness at ca 60°C, under a moderate stream of nitrogen, using a Turbovap LV Evaporator (Zymark). The dry samples, once cooled to room temperature, were tightly closed and stored dry at 4°C pending HPLC analysis.

Fumonisin B1 analysis

The residue was redissolved in acetonitrile:water (1+1). An aliquot (100 µl) of this solution was derivatized with 400 µl o-phthaldialdehyde (OPA) solution, obtained by adding 5 ml 0.1 M sodium tetraborate and 50 µl of 2-mercaptoethanol to 1 ml methanol containing 40 mg OPA. The solution was vortex-mixed and the fumonisin-OPA derivatives (40 µl) injected within one minute onto a reversed-phase HPLC/ fluorescence detection system. The HPLC system consisted of a Waters 717-plus auto sampler (Waters Corp., Milford, MA 01757), Waters 515 HPLC pump (flow rate set at 1 ml/min) connected to Waters 474 Scanning fluorescence detector with Millennium analytical data processing system. Chromatographic separations were performed on a stainless steel LC Novapak C18 reverse-phase column (150 x 3.9 mm id., 5µm) in line with a C18 column guard. Methanol-0.1 M sodium dihydrogen phosphate (75:25; pH 3.35) solution was used as mobile phase, at a flow rate of 1.0 ml/min. Fluorescence of the FB1-OPA derivatives was recorded at excitation and emission wavelengths of 335 nm and 440 nm, respectively. Fumonisin B1 quantitation was performed by area measurements, against a reference standard solution. Fumonisin B1 was reported as µg/g (parts per million). The FB1 concentration in each lot was estimated by averaging all individual individual

sample FB1 results from the same lot. The limit of detection of the analytical method was 0.01 µg/g.

Results

The fumonisin B1 concentration among all 87 lots marketed in Nigeria averaged 0.91 µg/g and ranged from 0.01 to 2.98 µg/g .Summary results are presented in table 8.

Table 8. FB1 distribution in maize lots (µg/g)

Location	Average	Median	Max	Min
Ibadan	0.67	0.62	1.16	0.45
Enugu	0.70	0.39	2.39	0.01
Maiduguri	0.97	0.90	2.36	0.13
Kaduna	1.06	0.99	2.98	0.11
Lagos	1.15	0.73	2.71	0.41

IQC procedures

A number of quality control checks were included in our work. Amongst others, they included analysis of spiked samples (recovery samples), analysis of replicate analytical portions (replicate samples), and checking the goodness of calibration .

Recovery samples

To detect possible procedural errors and show up any bias in the method performance, one recovery sample (a blank sample spiked with a known amount of FB1) was included in each batch analysed. The recovery results were plotted on a control chart, constructed using the standard deviation and the typical recovery values established during method validation. An example is shown in Figure 13.

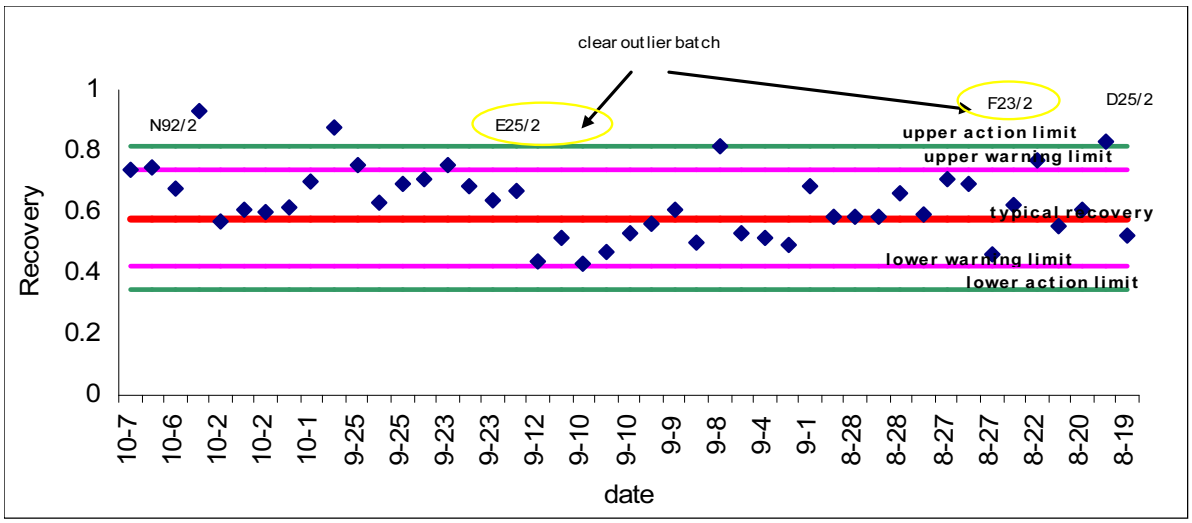


Figure 13. Control chart of recovery samples

Goodness of calibration curve

Quantitation was performed by calibration using weighted linear regression. Weighted regression was applied because the errors in the measurements rise as the concentration increases. This means that the calibration points do not all have equal weight or importance. It is more important for the regression line to pass close to the points with the smallest *y*-errors than it is for it to pass close to the points where the *y*-errors are largest. The straight line determined is referred to as a weighted calibration curve. The goodness of fit of the calibration curve was established by checking that the standard deviation of the relative residuals (Srr) from the weighted regression calculation was less than 0.1 and that the R^2 value was greater than 0.999 (Table 9).

Table 9. Weighted linear regression parameters. The goodness of fit of thecalibration curve is given by the values of Srr and R^2

Weighted linear regression			
a	-16806.232	**r**	0.99702
b	230916.219	**R^2**	0.999744
Srr	0.07199018	**R'^{2}**	0.999693
LOD	0.011		

Repeat samples

In each analytical batch, a positive sample from a previous batch was re-extracted and analysed, so that routinely two analytical portions from the same positive primary sample were analysed on two different days. This provided an additional tool to verify method performance, with more stringent acceptability criteria than through recovery data alone.

To use this approach, it was necessary to first verify that the sample was homogeneous and that the uncertainty of sample processing was minimal.

To judge the acceptability of the results of measurements of the repeated samples, the extreme range or critical difference was calculated as:

$$CD = f*SL = f*CV_L*R_{ave}$$

taking the initial CV_L from the results of recoveries (R_{ave}) from method validation, and a value of f = 2.8. The use of f=2.8 was acceptable as the degrees of freedom of the calculated standard deviation was based on n=62.

Table 10. Calculation of the critical range (CD)

Date of extraction	Repeated sample	Residue in AP/2	Residue in AP/1	average	D	SD	Acceptable range	
12 Nov	P73/2	0.437	0.576	0.507	0.139	0.093	0.261	Pass
12 Nov	P74/2	0.050	0.043	0.047	0.007	0.009	0.024	Pass
7 Oct	Q88/2	0.299	0.235	0.267	0.064	0.049	0.138	Pass
7 Oct	Q70/2	0.291	0.311	0.301	0.020	0.055	0.155	Pass
6 Oct	C93/2	0.508	0.500	0.504	0.008	0.093	0.260	Pass
2 Oct	L86/2	0.389	0.361	0.375	0.028	0.069	0.193	Pass
2 Oct	L73/2	0.290	0.233	0.261	0.058	0.048	0.135	Pass
1 Oct	H96/2	1.625	1.892	1.758	0.267	0.323	0.906	Pass
1 Oct	H85/2	2.167	2.231	2.199	0.065	0.405	1.133	Pass
30 Sept	F/32/2	0.592	1.164	0.878	0.572	0.162	0.452	Fail

As shown in table 10, one repeated sample analysed within a batch of samples on 30 September was outside the critical range, with the consequence that the entire batch had to be re-analysed.

Conclusions

Applying the IQC measures and acceptability criteria described above increased confidence in the analytical results. Similar procedures can be easily applied in all regulatory/testing laboratories.
All samples tested were found to contain FB1, indicating that contamination of maize with FB1 is widespread in Nigeria. In some cases the FB1 was found at concentrations greater than the EU MRL of 2 µg/g for unprocessed maize.

Fumonisin B1 and mycotoxins in general represent a severe health risk to consumers and a potential barrier to trade in food products. Therefore, it is extremely important to have analytical procedures and internal quality control procedures in place, along with preventative strategies such as the disposal of visibly damaged kernels, cleaning procedures, and wet milling processing, to prevent consumers from exposure to harmful levels of the toxins in foods.

This work was presented as a poster at the AOAC conference 'Foods to dye for – contaminants – sampling, analysis, legal limits', Limassol, Cyprus, 6-7 November 2006.

2.7. Coordinated Research Projects

2.7.1. The development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries (D3.20.22)

Technical Officer: Andrew Cannavan

The Agrochemicals Unit Head was the Scientific Secretary for the 4th and final Research Coordination Meeting (RCM) of the FAO/IAEA Coordinated Research Project (CRP) "Development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries" (D3.20.22), which was held in Freising, Germany, 27 November – 1 December 2006.

The RCM was hosted by a research agreement holder, Prof. Dr. Heinrich Meyer, Professor of Physiology in the Centre for Life Sciences, Munich Technical University, Weihenstephan, Freising, and was officially opened by Prof. Dr. Anna Maria Reichlmayr-Lais, representing the President of Munich Technical University. Eleven of the twelve research contract holders, two research agreement holders, two technical contract holders and the Scientific Secretary attended the RCM and presented the results of their research. In addition, Dr. Iris Lange, a former technical contract holder and Head of the Veterinary Drug Residue Laboratory of the Bavarian State Institute for Food and Hygiene (LGL) participated in the meeting as an observer and conducted a tour of the LGL laboratories. Dr. John McEvoy, of the European Commission (EC) DG Sanco Food and Veterinary Office (FVO) also participated in the meeting for one day (funded by the EC) and Dr. Andreas Daxenberger, Head of the Certification Unit of the Food and Feed Department of TÜV SÜV Management Service GmbH, Munich, gave a guest presentation.

Summary of CRP results

Development, characterization and comparison of immunoassay screening methods

Good quality polyclonal antibodies against chloramphenicol were produced by the research group in Kenya in several species, most significantly in camels, potentially providing a plentiful supply of antisera for all members of the CRP and for the African region. Protocols were elaborated for enzyme-linked immunosorbent assays (ELISA) using these antisera for chloramphenicol residues in sheep tissues and serum. The ELISA methods were applied to elaborate pharmacokinetic parameters of chloramphenicol in sheep and have been used to monitor tissues of sheep at slaughter for chloramphenicol residues. Polyclonal antibodies were also produced in Indonesia and were successfully lyophilized for long-term storage. The antisera were tested in an ELISA format which enables screening for chlorasmphenicol residues in approximately two hours. This method will be developed by the research group into kit format for transfer to regional laboratories in Indonesia. In Malta, commercial ELISA test kits and extraction procedures for chloramphenicol were evaluated and compared to identify and address problems with poor kit performance (low optical density readings, high false positive rate, poor precision). With the assistance of one of the commercial manufacturers, the main cause of the problems was identified as the handling and storage of kits during delivery to the laboratory. Whilst this could be overcome to some degree in Malta, it was concluded that in many countries these problems would render the kits ineffective. Other causes of problems with kit performance included operator errors in pipetting, cross contamination of wells, timing of operations and carry over of solvents from the extraction phase. Appropriate training in the use of the kits could minimize these factors. The commercial kit was validated in-house for the analysis of chloramphenicol in milk. Commercial kits were also compared and validated by the research group in Sri Lanka and Cyprus The research group in Cyprus, in addition to optimizing extraction/clean-up and reagent stabilization procedures, produced swine tissues incurred with chloramphenicol at various levels for use by the CRP partners in method development and validation and for possible future use in inter-laboratory comparison studies.

The research group in Brazil successfully expanded the scope of a commercially available radioimmunoassay (RIA) kit for the analysis of animal tissues for the β-agonist, clenbuterol, to include a range of β-agonists by utilizing the cross-reactivity of an available antiserum with the structurally related β-agonists. Tritiated clenbuterol was used as the label in the competitive assay, and extraction and clean-up techniques were developed to facilitate the analysis of both phenolic- and aniline-type β-agonists. The method was exhaustively validated for 7 analytes by applying the validation protocol developed in the CRP, and can now be routinely applied for regulatory screening purposes in Brazil. This research group has also undertaken the elaboration of a novel ^{125}I-RIA for chloramphenicol which was developed by a technical contract holder in the first phase of the project. A member of the Brazilian group has now been trained in the protocol at the laboratory of the technical contract holder in Munich Technical University. This work has been transferred from the original research group in Turkey, who withdrew from the CRP after that group failed to perform satisfactorily due to various factors including lack of support from their top management. The development has not, therefore, been completed, but work is ongoing even after completion of the CRP and the results will be published in due course.

Confirmatory methods

Confirmatory methods for chloramphenicol residues in animal tissues and honey using liquid chromatography–tandem mass spectrometry (LC-MSMS) were developed and validated in Thailand, Argentina and Korea. Methods for the major metabolites of the four main nitrofuran drugs were also developed and validated in Thailand and Argentina. These laboratories are now capable of providing confirmatory analyses for residues in food of the compounds that have been the major causes of trade disputes over the past few years, in the regions worst affected by those disputes.

A number of investigations into the possible natural occurrence of chloramphenicol in poultry litter were carried out by the researchers in Thailand, using the sensitive LC-MSMS method developed. The objective was to test the hypothesis that poultry found to contain residues of chloramphenicol were contaminated by the antibiotic produced naturally by Streptomyces venezuelae rather than through illegal use of the drug. After exhaustive experimentation, no evidence was found that chloramphenicol was naturally produced in chicken litter under normal production conditions. This is an important result which has significant implications with regard to the illegal use of the drug in food-producing animals in various countries. Chromatographic screening/quantitative methods.

Extraction and clean-up techniques for the analysis of nitrofuran metabolites by high performance liquid chromatography (HPLC) were developed in South Africa and Namibia. The application of flurometric detection was investigated in South Africa, but was demonstrated to have no advantage over previously published HPLC methods using ultra-violet (UV) detection. Nitrofuran metabolite derivatives for use in the HPLC-UV method were prepared in Namibia and shown to be of similar quality to commercially available, but expensive, products. After completion of the CRP, the two groups will continue to work together to develop the method using the South African clean-up procedure and the Namibian analytical procedure, which promises to provide increased sensitivity over the previously published HPLC methods, thereby meeting the requirements for screening for these banned compounds. An HPLC method was also developed and validated for chloramphenicol in Indonesia. The method was applied to samples from aquaculture production and some positives were detected in both shrimps and shrimp feed.

Quality control and sampling

The method validation protocol developed in the CRP is now being applied by all partners both for commercially available kits and for methods developed in-house. The institute of the research group in Korea has implemented a laboratory quality system and has attained accreditation to the ISO 17025 standard, which is an important factor in maintaining international trade capabilities for animal-derived food commodities. The same institute has initiated a collaboration, facilitated through participation in the CRP, with the Residues Section of the State Central Veterinary Laboratory (SCVL) in Ulaan Baatar, Mongolia (currently a TCP counterpart laboratory) resulting in the training of two Mongolian Scientists in Korea and provision of chromatography equipment to the SCVL with funding from the Korean International Cooperation Agency. Other important outcomes of the CRP include the instigation of an agreement between the institutes of the partners in Brazil and South Africa, resulting in exchange visits of personnel and transfer/sharing of methods and quality assurance protocols to the benefit of both institutes, training of personnel from South Africa and Brazil in Munich Technical University, and the establishment of a collaborative network comprising the CRP research groups, technical contract and agreement holders and other partners who have become involved throughout the duration of the project.

The Scientific Secretary wishes to express his gratitude to Prof. Heinrich Meyer, Dr. Iris Lange, Ms. Martina Reiter, Ms. Renata Schöpf and the local TUM organizing team for their assistance in holding the meeting and to acknowledge the support of Bayern-Leverkeusen GmbH for sponsorship of coffee breaks/lunches and the RCM dinner.

A TECDOC reporting the main results of the CRP will be published in 2007.

Conclusions

The overall objective of the CRP was to assist member states to meet international trade requirements for veterinary drug residues in livestock and livestock products. Specific objectives included the identification and comparison of suitable screening methods; development, and validation of screening and confirmatory assays, and; development of appropriate laboratory quality assurance procedures and sampling protocols for residues monitoring. These objectives have largely been met.

The CRP has resulted in the development and validation of screening and/or confirmatory methods for some important compounds and in-house production of good quality immunoassay reagents and protocols which should ensure sustainability of the methods and overcome the problems, also elucidated within the CRP, encountered with commercial screening test kits in many countries. A protocol for the validation of immunoassay methods has been produced and successfully applied within the CRP. Various quality control/quality assurance documents have been made available to the participants and one laboratory has achieved accreditation to ISO 17025 during the course of the project. Swine tissues incurred with chloramphenicol have been produced and made available for method development and interlaboratory comparison purposes. The Codex Alimentarius sampling guidelines published in CAC/GL/16/93 were accepted by all participants and the on-line material developed by the EC, including spreadsheets for sample numbers/matrices/compounds to be tested for, were demonstrated.

Work on the development of the biotin-streptavidin ^{125}I-radioimmunoassay for chloramphenicol has now been transferred to the research group in Brazil. Although the results of this work will be outside the time-frame of the CRP, the method is expected to offer some advantages over current immunoassays, including flexibility of format (RIA with ^{125}I or ^{3}H labels, ELISA), wide applicability due to the use of labeled biotin rather than labeled analyte, and robustness. Further development and application of this technique would make a good basis for a future CRP.

The consensus of the meeting was that this project was extremely useful not only in terms of the methods and protocols and research results produced, but also in providing the background, means and contacts to perform research and development in the field of veterinary drug residues in order to assist in capacity building to meet modern requirements for participation in international trade and protect public health. An important outcome of the CRP is the networking between the participants, ensuring that further research and capacity building in this field need not be carried out by any one partner in isolation.

The outputs and outcomes of the CRP were recognized as significant by the European Commission in the context of their “better training for safer food’ initiative as applied to third countries, especially developing countries. This was reflected in the participation in the final RCM of a representative of the EC DG Sanco Food and Veterinary Office.

Six research papers have been identified for preparation and submission for publication in the peer-reviewed scientific press. One paper has already been published and posters have been presented at three international conferences.

The meeting recommended that a new CRP should be initiated in the field of residues/contaminants in food. Suggestions included emphasis on antibiotics and anthelmintics, and also mycotoxins, given the high impact of, for example, aflatoxin residues both as an acute and often fatal health hazard, and in terms of the effect of contamination on trade in food commodities.

2.7.2. Coordinated Research Project on "Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale", CRP D5.20.35

Technical Officer: Britt Maestroni

One of the key recommendations from the Food and Environmental Protection Sub-programme's 2005 consultants' meeting on "The Role of Analytical Laboratories in the Application of good agricultural practice (GAP) in the Production of Fresh Fruits and Vegetables and Animals and Animal Products" was to strengthen the capabilities of laboratories and laboratory networks in assessing the implementation of GAP for internal and external markets. As a consequence the Subprogramme elaborated a proposal for a CRP on "Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale".

Introduction

Agriculture is a dominant component of the global economy, and the pressure to produce enough food for the world's ever growing population has had a worldwide impact on agricultural practices. The challenge of securing a sufficient food supply was highlighted in Agenda 21 of the United Nations Conference on Environment and Development in 1992. As a result, the use of fertilizers and pesticides has steadily increased over the years to ensure and sustain high crop yields.

Pesticides can have adverse non-target effects especially in the hydrologic system. Water is the primary pathway by which pesticides are transported from their application areas into the environment. Once pesticides are displaced, they can be widely dispersed into streams, rivers, lakes, reservoirs, and oceans.

Agriculture is both a cause and a victim of water pollution. It is a cause through the discharge of pollutants (pesticides, fertilizers, etc.) to surface and/or groundwater. It is a victim through contaminated water being used for irrigation, for example. The economical, social, environmental and public health implications due to decreasing water quality are a common worldwide threat. Surface waters not only supply a large amount of drinking water to populations, they are also vital aquatic ecosystems that provide important environmental and economic benefits. Fresh water is predicted to become the principal limitation for sustainable development within this century[11].

Preventing and controlling pollution of water resources, both surface and underground, is a government function that has lead to the adoption of a variety of legislative approaches[12]. Legislation has mainly dealt with the control of "point source" pollution, i.e., pollution that can be tracked to a specific entry point with sufficient accuracy, such as industrial discharges, domestic sewage or municipal wastewater effluents or treatment plants. Reduction of "non-point source" pollution, on the other hand, can be achieved through the application of precautionary measures, including GAP and

and through adherence to national requirements on the use and applications of pesticides in the field.

In order to be able to support the control of water pollution, quality data are needed: one can only base management decisions on reliable and scientifically sound measurements. Effective monitoring schemes are necessary to identify specific pollutants, their sources and occurrences, to develop preventive measures, and to assess the efficacy of corrective actions. Developing countries face many problems in establishing appropriate monitoring schemes to evaluate surface water pollution by pesticides, and in producing valid analytical results.

With respect to contaminants in water, the U.S. Geological Survey (USGS)[13] stated that "there is the need for long-term monitoring studies which include a larger number of pesticides and their transformation products". The major difficulty, as pointed out by Ongley (1994)[14] is that "a common observation amongst water quality professionals is that many water quality programmes, especially in developing countries, collect the wrong parameters, from the wrong places, using the wrong substrates and at inappropriate sampling frequencies, and produce data that are often quite unreliable".

Difficulties in developing monitoring schemes arise because pesticide concentrations in surface waters follow strong seasonal patterns that result from the timing of pesticide applications and runoff conditions (rainfall, soil permeability, soil infiltration rate, interflow, etc.). In water, pesticides may be transported as dissolved material or by adhering to suspended matter, such as particulates and sediments. Therefore not only water, but also particulates and sediments, should be considered as part of the research project.

Studies on "non-point source" pollution showed that the primary transfer mechanism from land to water of nutrients, sediments and pesticides is runoff (FAO, 1996)[15]; however air drift of pesticides, applied in the fields, can also contaminate streams and waterways.

Rainfall, following a pesticide application in the field, may result in runoff and a pulse of high pesticide concentration continuing downstream until it eventually reaches a lake or a containment area. In lakes or basins, the concentration of stable pesticides are likely to remain elevated much longer than in streams, because the pesticides will not be flushed from the system as quickly, and may be observed for a long time after nearby agricultural applications have been suspended.

The Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia, have developed a "Pesticide Impact Ranking Index" (PIRI) software package to rank pesticides in terms of their relative pollution potential to soils, ground and surface water, and to compare different land uses in catchment areas in terms of their relative impact on water quality. The PIRI software will be used to process data on the application of pesticides in the field to assess the pollution risks to surface and groundwater. Data on concentrations of pesticides gathered through water monitoring will be used to validate the PIRI risk assessments and expand the scope of the software to a variety of agro-ecological zones.

[11] FAO Irrigation and Drainage Paper Nr 55, D.Ongley, 1996.
[12] FAO Legislative Study paper Nr 80, S.Burchi and A. D'Andrea, 2003
[13] USGC web page: http://ca.water.usgs.gov/pnsp/rep/fs97039/index.html
[14] E.D. Ongley, Global water pollution: challenges and opportunities, Pub. 3, Stockholm Water symposium, 10-14 Aug. 1993, Stockholm, Sweden, pp.23-30, 1994.
[15] FAO Irrigation and Drainage Paper Nr 55, D.Ongley, 1996.

This CRP proposal addresses the use of agrochemicals to provide an adequate and safe food supply whilst ensuring environmental sustainability under the agricultural production system applied. One tool that can be developed through this CRP is to indirectly assess the effects of Good Agricultural Practices used in the field by monitoring pesticide contamination of surface waters.

Overall objectives

This CRP will contribute to the Joint FAO/IAEA project on "Technologies and Capacity Building to Identify Good Agricultural Practices for the Management of Food and Environmental Hazards" (E3.02) which focuses on the development of principles, indicators and guidelines for agricultural practices that promote food safety and quality and environmental sustainability.

Close linkages will be fostered with activities of the Soil and Water Management and Crop Nutrition Section which is working on tools and approaches to address runoff and erosion at the catchment scale, and is also developing a new program (E.1.08) on "Technologies and practices for efficient agricultural water use and conservation".

The overall objective of this CRP is to help member states develop means of sustainable agricultural development, through the assessment of the effectiveness of GAPs.

Specific objectives

- Establish laboratory capacity and indicators to assess the effectiveness of good agricultural practices at catchment scale

Expected outcomes of the project

- To establish and operate cost-effective and sustainable quality monitoring schemes for surface water, including particulate matter.
- To establish a mechanism to "feed back" the results of laboratory analysis to the primary producers community/extension services.
- To exchange information and to pursue horizontal cooperation among countries in terms of harmonized analytical methods and water monitoring schemes.

Outputs of the project

- Harmonized protocols for sampling of surface water.
- Laboratory protocols for the analysis of water and particulate matter using nuclear and related analytical technologies.
- Baseline and trend data on the type and amount of pesticide contamination in surface water and particulate matter in defined geographical areas.
- Validated PIRI outputs verified by analytical investigations.
- Geo-referenced databases of monitored pesticide residue data.
- Guidelines for establishment of monitoring schemes for pesticide contamination of surface water.
- Guidelines for evaluation of monitoring data.
- Distance learning material on analytical methods, instrumentation and their use to generate data that reflects the effectiveness of GAP.
- Establishment of regional centres for "hands-on" training of laboratory personnel to monitor objective indicators for the adoption of GAP.

Summary

This CRP brings together laboratories which have the required analytical capabilities and are working as members of wider groups (primary producers and other stakeholders) that intend to apply good agricultural practices (GAP), and that have joined the project to evaluate and optimize the effect of GAP on environmental sustainability as measured by the presence of selected high impact-ranking pesticides in surface water and sediments at a catchment scale. Immediate benefits to individual groups include assistance from IAEA/FAO to improve laboratory competence for the specific requirements of the project and the opportunity to interact with groups working on comparable problems in different environments. Further benefits include the opportunity to establish quality-assured competence to evaluate indicators[16] of GAP performance by environmental monitoring at catchment scales and strengthening of multi- disciplinary/stakeholder groups.

The CRP aims to integrate risk assessment tools and targeted analytical monitoring as a cost-effective option for developing countries to identify specific water pollutants, their sources and occurrences and to use this information to critically evaluate and, where necessary, improve production practices. Nuclear and related techniques will assist in generating outputs such as harmonized protocols for sampling and analysis of surface water. Georeferenced data, guidelines, and access to eLearning courses will accelerate capacity building and lead to three major outcomes: (1) cost-effective, sustainable and catchment-targeted monitoring schemes for surface water; (2) mechanisms to "feed back" the results of laboratory analysis to the primary producers community/extension services; and (3) information exchange on harmonized analytical methods and water monitoring schemes to improve pesticide management practices and the production of safe food.

Activities

A web site for exchange of information within the CRP was set up at the following address:

http://elearning.iaea.org/ATutor/bounce.php?course=82

The site is currently protected with access only for contract and agreement holders

A consultants' meeting, having the objectives of elaborating the protocols and activities for the CRP, took place in Vienna from 6-9 June 2006. Details can be found at the following link:

http://www-naweb.iaea.org/nafa/fep/meetings/2006-ConsultantMeetingCRP.pdf

A call for proposals for the abovementioned CRP on "Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale" was issued in July 2006. The response was good and 24 research contract proposals and 5 research agreement proposals reached the IAEA Research Contracts Administration Section by October 2006. The quality of the proposals was in general very good, and the selection criteria were quite strict, based on demonstrated experience in pesticide residue analysis availability of instruments at the analytical laboratory, i.e. GC-ECD/NPD(FPD) and preferably HPLC DAD/FLUO, GC/MS, a quality system in place (preferably according to ISO/IEC 17025), experience in residue analysis, linkages with GAP and watershed activities, capability to conduct field work, an ongoing water quality programme, adequate funds for monitoring activities, and internet access and capability/willingness to conduct training and undertake risk communication.

Ten research contract holders and 5 agreement holders have been positively evaluated and recommended for contract awards to the IAEA Research Contracts Administration Section. All recommended contracts were awarded to participating laboratories on 14 December 2006. The first research coordination meeting is planned for 9-13 July 2007 in San Jose, Costa Rica.

[16] OECD (1999) Environmental indicators for agriculture Volume 1 Concepts and Framework (http://www1.oecd.org/agr/biodiversity/volume1.pdf).

3. TRAINING

One of the major activities of the Food and Environmental Protection Sub-programme is training. The Agrochemicals Unit at Seibersdorf is the central laboratory of the FAO/IAEA Training and Reference Centre for Food and Pesticide Control (TRC), which was established in 1998. An additional training laboratory facility, funded by FAO and through donations from Austria and Sweden, was opened in 1999.

The TRC was established to strengthen the analytical capabilities of developing country Member States and to assist in the control of food quality and safety, especially with respect to meeting international requirements for safe, quality assured products and in order to participate in international trade. It also helps introduce and implement quality assurance and quality control systems in testing laboratories in Member States.

The Agrochemicals Unit contributes to the activities of the TRC through laboratory-based training in subjects such as laboratory quality assurance and quality control (based on the principles of ISO/IEC 17025 and OECD Good Laboratory Practice), pesticide residue analysis and veterinary drug residues analysis. Workshops and training courses are designed for national officials involved in planning, decision making and supervision, as well as analysts working at the bench. Participants in the training programmes gain experience which they can use to improve the capabilities of their own institutes, and are encouraged to further spread the training by organising workshops in their own countries. They may also become potential lecturers in regional IAEA training courses or workshops.

Training is also provided to Fellows and Scientific Visitors funded through the Department of Technical Cooperation. Fellows will spend a period of time, from 1–12 months training "on-the-job" in subjects such as residue analysis or instrumental methods of analysis. Scientific visitors gain experience in specific aspects of residues monitoring programmes or other managerial aspects of the regulatory process through short visits to the Unit, typically of 1 week.

3.1. Introduction to Quality Assurance/Quality Control Measures in Pesticide Residue Analytical Laboratories, 11 Sept – 6 Oct 2006

The training workshop "Introduction to Quality Assurance/Quality Control Measures in Pesticide Residue Analytical Laboratories" was held from 11 September to 6 October 2006 at the Seibersdorf Laboratories. The workshop was announced in February 2006 and more than 80 applications were received by the end of April.

The goal of the workshop was to provide a basic understanding of the principles of laboratory quality management systems and the quality control procedures necessary to apply such systems. The programme comprised lectures, discussion and feedback sessions, and practical exercises in the laboratory. The lectures covered topics such as basic statistics, quality principles and systems, accreditation, documentation of laboratory work, method validation, measurement uncertainty, reporting of results, sample extraction and clean-up, and new developments in pesticide residue analysis. The practical sessions included demonstrations of sample preparation, extraction and clean-up techniques and group sessions on TLC, HPLC, GC, GC-MS and LC-MSMS methods. The emphasis was on identifying, discussing and demonstrating quality control issues such as system suitability checks and the use of recovery samples and control charts. Lectures were presented by staff of the Agrochemicals Unit and the Food and Environmental Protection Section and other IAEA staff, and by invited lecturers from Germany, Hungary, the Czech Republic and Thailand. It is noteworthy that the lecturer from Thailand had attended the previous workshop in 2005 as trainee.

Participants and staff of the training workshop 2006

The workshop also included presentations and demonstrations on HPLC, GC troubleshooting and GC-MSMS, provided by personnel from Agilent and Waters Corporation, and a visit to the Austrian Health & Food Safety Agency (AGES) laboratories in Vienna, where workshop participants viewed the procedures in place for sample reception, processing and analysis in an accredited national laboratory.

Agrochemicals Unit staff were involved in preparation for the workshop from early 2006, with intensive activities from June 2006, including preparation of lecture material, design and testing of laboratory practical sessions, identification of and correspondence with invited lecturers, evaluation of nominations and selection of workshop participants, interaction with TC with regard to fellowship awards for several nominees, organization of the external visit to AGES, negotiations with analytical instrument manufacturers to provide instruments for demonstration during the workshop, and general administrative activities.

Nineteen candidates from developing countries were selected for the training workshop. Qualified candidates not selected are retained in the data base of the Agrochemical Unit, so that they may be informed of other training events. The selection was based on objective criteria such as age, gender, qualifications, years of experience in pesticide residue analysis, type of work, experience in quality systems, and English language capability. The spread of selected candidates by region and pesticide residues analysis experience is illustrated in Figure 14.

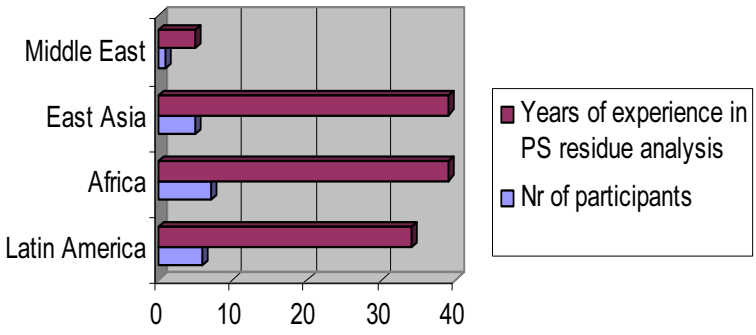


Figure 14. Selected workshop participants by regions and experience

The workshop opened on 11 September 2006 at Vienna International Centre with introductory key lectures on Codex Alimentarius and Food Safety, the latter given by a guest speaker from AGES, the Austrian Health and Food Safety Agency. The participants, although varying to some degree in experience and background, proved to be well informed, enthusiastic, and interactive during the whole duration of the course. The working style of the participants benefited from a team-building

exercise on the first day of the workshop, which set a good working climate. Team building associated with soft skills such as presentation skills and report writing proved to be very successful and resulted in good interaction and information exchange between the participants from the start of the workshop.

In the first week of the course participants gave individual presentations on their pesticide residue laboratories and identified analytical and quality control aspects they wanted to target during the workshop.

Sample preparation exercise

Break-out sessions were organized during the final week of the workshop, in which the participants, in four working groups, performed a critical review of the SANCO document 10232/2006 relating to "quality control procedures for pesticide residue analysis". Each group presented their analysis of the document and its applicability in developing country laboratories, and common problems and possible solutions were discussed amongst the participants.

The final morning of the workshop was taken up by a presentation and round-table discussion session, which included representatives of FAO and AgroVet. To introduce the round-table session, each of the four working groups of participants gave a presentation on the role of the quality assured analytical laboratory in the implementation of good agricultural practices in relation to pesticide management, food safety and trade.

The session closed with the presentation of certificates, all participants having fulfilled the criteria for successful completion of the workshop.

Participants in the programme gained experience which should enhance their professional capabilities. They should be able to use the experience and knowledge gained to improve conditions in their home laboratories through better implementation of quality assurance and quality control measures, thereby assuring the quality of pesticide residue data produced. It is also envisaged that many of the participants will spread the information further by organizing training workshops and seminars in their own institutes and involving their colleagues. The workshop organizing team hopes to receive feedback in the near future from work shop participants regarding the status of implementation of quality systems in their laboratories and, where appropriate, the achievement of accreditation.

Overall, the workshop was considered very successful, due in no small part to the enthusiasm and interaction of the participants. Initial feedback from participants and lecturers alike has been very good and the organising team are using the lessons learned and incorporating good suggestions into the programme for the next workshop, which is scheduled for September 2007. Some results from a final questionnaire completed by all participants are presented in the Figures 15-17.

Dr. Anastassiades leading a lab exercise

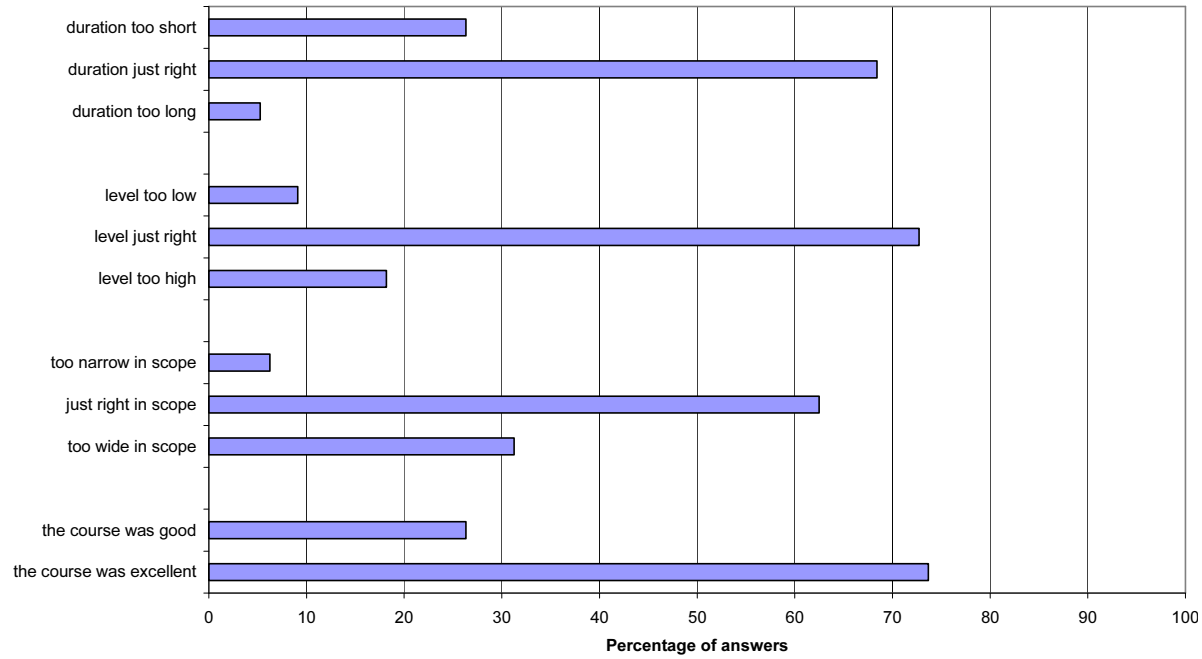


Figure 15. Opinion of trainees on the training workshop 2006: overall feedback

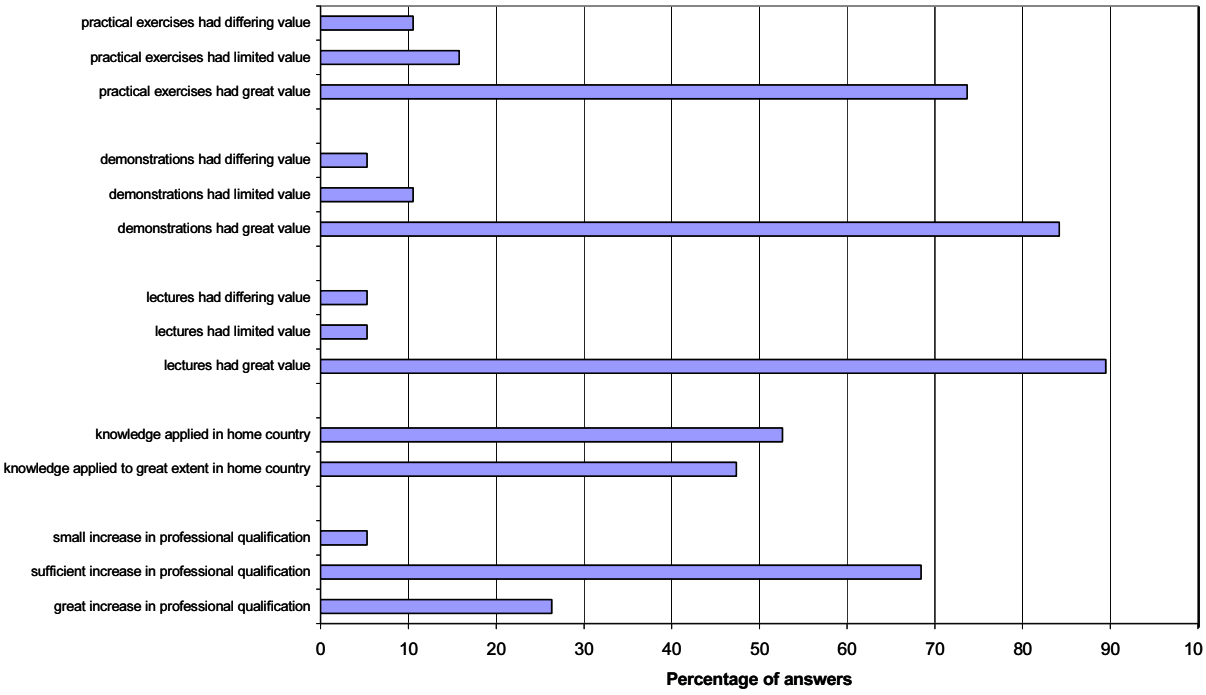


Figure 16. Opinion of trainees on the value of the practical training and the usefulness of the work shop in their professional career

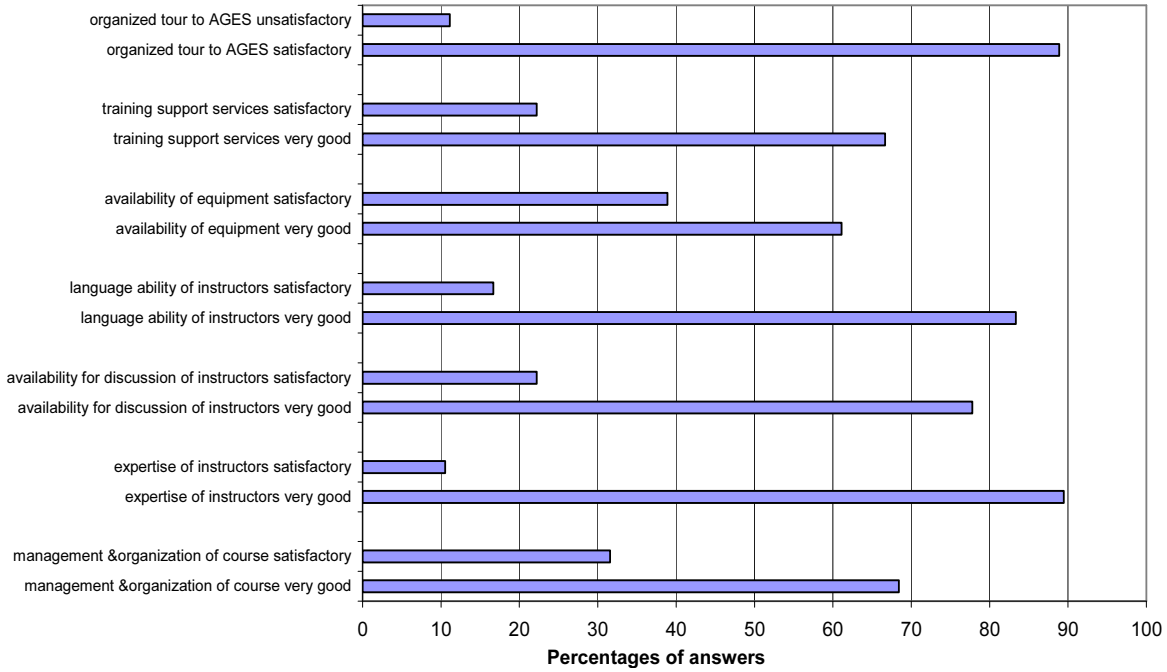


Figure 17. Opinion of the trainees on the organisation and the management of the training workshop

3.2. Asia/Pacific Food Safety Summit, Singapore, 15-17 October, and Food Safety Seminar, Bangkok, Thailand, 18-19 October 2006

These events were co-sponsored by Waters Corporation and the Joint FAO/IAEA Programme, represented by the Agrochemicals Unit Head. The food safety meetings provided platforms for discussion on experiences, problems encountered, trends and methodologies related to the regulatory control of residues and contaminants in food. The programme at each event comprised a series of lectures on regulatory and analytical issues, open discussion and panel discussion sessions.

Opening of the Food Safety Summit

The meeting in Singapore was attended by approximately 80 participants, with delegates from China, Indonesia, Japan, Korea, Malaysia, Philippines, Thailand, Taiwan, Singapore, the United Kingdom and the United States of America. Four keynote speakers introduced sessions on global food safety issues, legislative and regulatory requirements, analytical methodology and meeting challenges in food safety testing laboratories. Other participants gave presentations on various analytical methods, technologies, data handling and advances in screening techniques by mass spectrometry, all of which addressed issues of food safety, mainly focusing on residues of veterinary drugs and pesticides and other contaminants such as mycotoxins and dioxins in foods.

The seminar in Bangkok had approximately 120 participants, mainly from regulatory authorities and laboratories in Thailand. It was noteworthy that several of the participants were former trainees on courses run by the Agrochemicals Unit at Seibersdorf, and some participants were also collaborators with the Agency through Technical Cooperation Projects or as CRP contract holders. At this meeting, two keynote speakers gave presentations on global food safety issues and analytical methodology, followed by presentations on the same themes as those given in Singapore.

At each meeting the Unit Head represented the Joint FAO/IAEA Programme as co-sponsor of the event and presented the first keynote address on global food safety issues and trends. The Unit Head also participated in both group and individual discussions on analytical methodology, regulatory guidelines and legislation and the research, capacity building and training activities of the Joint Programme. Information folders on the Joint Programme (prepared in advance of the IAEA General Conference) were distributed to the meeting participants. The training courses and workshops held through the FAO/IAEA Training and Reference Centre for Food and Pesticide Control at Seibersdorf have established a good reputation and it became clear that places on the courses are much sought after. Many of the delegates requested information on future courses and workshops.

Following the seminar in Bangkok, the Unit head visited the Laboratory Center for Food and Agricultural Products (LCFA). This is a modern, advanced analytical laboratory with a wide range of analytical expertise and equipment, including gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. The laboratory operates a quality system and has been accredited by the Bureau of Laboratory Quality Standards, Ministry of Public Health, to the ISO 17025 standard. The LCFA management was positive and enthusiastic regarding the possibility of using their laboratory for any future regional training courses that may arise. Dr. Sasitorn Kanarat, Director of the Veterinary Public Health Laboratory, which is similarly well equipped and accredited, also offered to host training courses in the future.

The Food Safety meetings provided an excellent opportunity for the dissemination of information on regulatory and technical aspects of food safety in Asia to targeted individuals with influence in this field. Co-sponsorship of the events with Waters Corporation facilitated contact with a wider audience than would be normally be achievable with internal funding. It is recommended that this collaboration should be maintained and similar collaborations with other enterprises should be sought in the future.

Good contacts were made and a basis for future collaborations in the region, both in research and in capacity building, was founded. Given the great need and demand for training in analytical techniques for food safety, the LCFA or VPHL laboratories in Bangkok would provide excellent venues for future regional training courses.

3.3. Training in Costa Rica on pesticide analysis using radiotracer technique and the IAEA multi-residue method

A training mission, funded under TCP COS5026, was undertaken by Ms. Perihan Aysal to the Centro de Investigación Ambiental (CICA), San Jose, Costa Rica, 3-15 July 2006. The objectives were to transfer the IAEA-ethyl acetate multi-residue method for the determination of pesticide residues in fruits and vegetables; to provide training in the use of radiotracer technique for quick review of the method or method adaptation/validation; to provide guidance on laboratory sampling and sample processing of medium size fruits and vegetables; and to provide advice on laboratory sub-bench ventilation system and other laboratory features.

Analysts being trained in the CICA laboratory

Practical demonstrations of the method were given and discussion sessions in the above mentioned and other related subjects were held with Dr. Elizabeth Carazo, Director of CICA, and 8 scientists of CICA. Laboratory experiments were performed for the demonstration of the method using two pesticide mixtures provided by CICA, containing a total of 27 compounds and ^{14}C-chlorpyrifos in pineapple.

The crude ethyl acetate extracts of blank and fortified samples were analysed by Gas Chromatography (GC) with electron capture detector (ECD), nitrogen-phosphorus detector (NPD) and mass spectrometry (MS). Advice was given on the calculation and interpretation of results and an excel spreadsheet template was adapted for calculation of results generated by the IAEA method.

The use of ^{14}C-labeled pesticides in pesticide residue analysis was elaborated, and information was provided on pesticide degradation in soil; on use of bio flasks and the biological oxidizer; on the isotope dilution method in the radiotracer technique and on solid phase extraction (SPE) as an alternative clean-up method to two scientists who are responsible for soil analysis.

Discussion sessions were held on the use of system suitability test (SST) parameters, control charts and troubleshooting GC with ECD, NPD and MS detection systems, and on the concepts for the optimization of any chromatographic system in pesticide residue laboratories. The confirmatory criteria for pesticide residues using GC-MS were explained.

Ms. Aysal also gave a seminar on sample processing and sampling for the preparation of laboratory samples and analytical portions.

The IAEA-ethyl acetate multiresidue method is rapid and simple in comparison to the method being used routinely by CICA. Full validation and adoption of the IAEA method will improve the analytical capabilities and efficiency of the laboratory. The method performed very well for most of the compounds examined, although the clean-up step could not be performed because not all of the necessary chemicals and equipment were available.

The CICA director and scientists, who are responsible for the pesticide residue analysis on behalf of Ministry of Agriculture and the University of Costa Rica, agreed to perform further validation of the IAEA-ethyl acetate method to permit its routine application in place of their current method.

The mission was a very worthwhile event since CICA has been playing a very active and important role in Costa Rica and also in the region in terms of research, training, and routine analysis of pesticide residues in food commodities, and also providing services to farmers to help in implementing good agricultural practices, thus facilitating international trade and competitiveness of farmers. The FAO/IAEA eLearning system, which is established in the University of Costa Rica, is a good source which is being used to accelerate capacity building in addition to other training activities such as fellowship programs.

3.4. Regional workshop on improving the assessment of good agricultural practices at a catchment scale using laboratory analytical support

Ms. Maestroni participated as technical resource person in a workshop on the formulation of a regional TC Project on “Improving the assessment of good agricultural practices at a catchment scale using laboratory analytical support” that was held in Mendoza, Argentina, February 27 - March 3, 2006. Activities included preparation of background materials to aid preparation for and follow-up from the workshop[17]; provision of technical backstopping for the good agricultural practice (GAP) project concept team; provision of *ad hoc* training on PIRI[18] and the QuEChERS[19] method for analysing pesticide residues; assistance to national experts in formulating a draft regional log framework .

[17] http://elearning.iaea.org/ATutor/bounce.php?course=69
[18] Pesticide Impact Rating Index program http://www.cmis.csiro.au/envir/Research/PesticideRisk/)
[19] Quick, Easy, Cheap, Effective, Rugged and Safe

Technical discussions examined the reason(s) for the increase pesticide sales in Latin America (up 30% since 2003) and 937 pesticide import detentions by the USFDA between November 2004 to October 2005. It was concluded that:

- pesticides are an advanced technology that is being used in Latin America often without the "safety net" that exists in developed countries;
- an integrated approach to the management of land, water and external inputs, when fully developed, has the potential to value add to commodity trade and foster the adoption of GAP and agribusiness from farm-to-fork;
- implementation requires education, accelerated capacity building and multidisciplinary teams to address barriers;
- success depends on knowing where and when to intervene and on learning lessons identified from good and bad case studies.

Fresh fruits and vegetables were targeted, since these represent the main export item for many developing countries and a major source of foreign exchange earnings. The participants agreed that the goal must be to control hazards along the whole food chain with emphasis on prevention at the source rather than total reliance on end product testing. New or more stringent food and environmental standards were viewed as a catalyst for change. The challenge expressed by the participants was that most of their laboratories lacked modern instrumentation such as gas chromatography-mass spectrometry (GC-MS) and cost-effective methods. However, they viewed the laboratory as playing a key role in the implementation of GAP because analytical results can assure third parties (donors, distributors, consumers) of the reliability and safety of products produced using improved practices. This was underscored by the unanimous view that food safety, environmental safety, social welfare and human health were non-negotiable. The question of how to accomplish these goals given the limited resources available was the main topic of discussion.

Laboratory services were discussed. The main activities were screening, import/export control programmes and enforcement/compliance monitoring. Other services could include field residue trials to establish/monitor GAP and setting of maximum residue limits (MRL) at Codex meetings. Confirmation of analytical results was a key issue which was viewed as a challenge for laboratories, especially those without a well established quality management system. Ms. Maestroni presented and discussed the laboratory requirements for unequivocal confirmation of positive/non-compliant results.

Participants agreed that adding value to fresh produce and communicating analytical results to producers and consumers is essential. This can ensure the sustainability of laboratory operations and monitoring and enforcement activities. It was agreed that the first phase of the project should focus on harmonizing reporting and meeting stakeholder expectations—"end product testing", market demands, consumers and retailers food quality expectations. Latter phases of the project would emphasis the need for changing practices and monitoring relevant environmental indicators.

[20] Personal communication (Hance 2005)
[21] http://kc.iaea.org/livelink/livelink.exe/paper_05%2D10%2D032.doc?func=doc.Fetch&nodeId=6081451&docTitle=paper_05%2D10%2D032%2Edoc&viewType=1

3.5. OIRSA Workshop on Veterinary Drug Registration and Residue Control, El Salvador

A workshop was held in San Salvador to provide assistance with regard to building awareness in the Central American region on the issue of the control of veterinary drug residues in foods, as planned under the Programme of work for 2006 (activity E3.02.5). Three regional experts in the field of veterinary drug residues, from the European Commission, the United States and Latin America, were sponsored by the Joint FAO/IAEA Programme to convene a workshop from 31 August – 1 September on veterinary drug registration and residue control during the IX Reunion de Jefes de Registro de Productos Veterinarios, Biologicos y Alimentos de Uso Animal in San Salvador. The workshop was organized by the International Regional Organization for Plant and Animal Health (OIRSA) and was attended by twelve representatives from Belize, Costa Rica, El Salvador, Honduras, Mexico, Nicaragua and Panama. The participants were professionals with responsibility for veterinary drug registration, who had come together to promote harmonization of regulations in the region.

The programme structure, as drafted by Dr. Gudrun Gallhof of the European Commission in consultation with the Agrochemicals Unit Head, was adopted and implemented. The lecturers gave presentations on the registration and appropriate control of veterinary drugs, and strategies to control their residues in food in Latin America, Europe and the U.S.A., and demonstrated various information sources, such as the web sites of Codex, the European Union and the U.S. Food and Drug Administration, with information on regulations for veterinary drugs and the control of veterinary drug residues.

Following the presentations of the regional experts, two working groups were formed to formulate and approve conclusions for the meeting. The participants tabled a proposal to their countries and to OIRSA to promote harmonisation activities with regard to registration of veterinary pharmaceuticals and measures to control their residues in food.

The conclusions of the workshop were included in the conclusions and recommendations of the OIRSA meeting.

Lecturers sponsored by the IAEA:
Dr. Gudrun Gallhoff (European Commission), Dr. Richard Ellis (U.S.A.), Dr. Alfredo Montes Niño (Argentina)

3.6. Fellows and Scientific Visitors

During 2006, two TC Fellows were trained on instrumental techniques and pesticide residue analysis. As a result of this training, one of the Fellows, Mr. Gaspar Mushi from Tanzania, co-authored a paper which was presented at the conference on “Pesticide Use in Developing Countries: Environmental Fate, Effects and Public Health Implications” organised by ANCAP (African Network for Chemical Analysis of Pesticides) and SETAC (The Society of Environmental Toxicology and Chemistry) Africa Branch, in Arusha, United Republic of Tanzania, 16-20 October 2006 (see section 2.1.)

Six TC Fellows participated in a training workshop on QA/QC in pesticide residue analytical laboratories and two FAO TC trainees (see section 3.7.) were trained on pesticide and veterinary drug residue analytical techniques. Direct Fellowship training activities in the Unit amounted to a total of 12 months & 12 days.

FAO funded trainees

Two scientists, Ms. Hafidha Idir and Mr. Abdel-Nacer Zahi, from the Algerian National Institute for Veterinary Medicine, Algiers, were trained in the Agrochemicals Unit from 12 June to 7 July, with funding under an FAO Technical Cooperation Project.

In the first part of the training (week 1 & 2), the trainees worked on a study to elaborate the performance characteristics of the IAEA-ethyl acetate multiresidue pesticide method in melon. Comminuted melon samples were fortified at 0.03, 0.3 and 3 mg/kg fortification levels with the following 24 representative pesticides:

dichlorvos, EPTC, heptenophos, propachlor, dimethoate, diazinon, pirimicarb, vinclozolin, chlorpyrifos-ethyl, parathion-methyl, chlorfenvinphos, methidation, triazophos, propyconazole, fenpropathrin, iprodion, azinphos-methyl, fenarimol, coumaphos, fenvalerate, lindane, alpha-endosulfane, metalaxyl, malathion.

For a quick review of the method performance and to evaluate the individual analysis steps during method adaptation, ^{14}C-chlorpyrifos was also applied at all fortification levels (Table 11).

Table 11. ^{14}C-Chlorpyrifos recoveries (Q) and repeatabilities (as RSD) at different levels related to each step of the method

FORTIFICATION LEVEL, mg/kg	EXTRACTION		CLEAN-UP		TOTAL	
	Q (%)	RSD (%)	Q (%)	RSD (%)	**Q (%)**	**RSD (%)**
0.03	94	2.0	93	2.6	**87**	**2.0**
0.3	92	1.5	95	1.8	**87**	**2.7**
3	89	1.0	94	5.9	**83**	**5.9**

The trainees were able to use this study to gain experience in basic maintenance of GC instruments and evaluation of GC chromatograms, as well as the application of gas chromatography-mass spectrometry (GC-MS) as a confirmatory method and quantification in GC-MS systems by using ion spectra of some compounds.

In the second part of the training period (week 3 & 4), the trainees gained experience in HPLC, including column installation, mobile phase preparation, sample analysis, system suitability tests, related calculations and introduction to QA/QC measures. An HPLC method for tetracyclines in animal products was used to demonstrate various aspects of HPLC analyses, the preparation of standard solutions, and the use of calibration templates.

3.7. eLearning

Agrochemicals Unit staff continued to work in collaboration with the Food and Environmental Section to support the Sub-programme's database and eLearning initiatives. In 2005, work began on updating the TRC training material CD, which contains material developed and collected since 1997. The content and presentation needed to be updated and, in some cases, peer-review was necessary to ensure the quality/relevance of the materials. The transfer of this material from the CD to the eLearning system (http://elearning.iaea.org), from which it can be more readily updated and/or extracted at any time for offline usage, continued in 2006, including the upload of the presentations and training material prepared for the 2006 TRC training workshop "Introduction to QA/QC measures in pesticide residue analytical laboratories". Some of the major results expected from this work are:

- shortening of training courses, with substantial cost reductions;
- establishing a reusable knowledge base for FAO/IAEA, affiliated universities and other stakeholders;
- helping publicise the FAO/IAEA activities and address core mandates related to the provision of authoritative technical information.

One significant addition to the electronic resources of the Food and Environmental Protection Sub-programme was the "Revision of the list of methods for pesticide residue analysis available to the Codex Committee for Pesticide Residue (CCPR)". Several countries supplied updated methods through the FEP Section, NAFA, and these were formatted for online presentation and uploaded by Mr. Philipp Klaus. Materials where supplied by Canada (10 analytical methods), Germany (four German-EN methods) and the USA.

Agrochemicals Unit staff also provided major input to the development of new modules on Documentation of Laboratory Work, Ecological Risk Assessment of Pesticides, Handling Pipettes and Syringes, and Introduction to Conformity Assessment in Analytical Laboratories which became available in 2006 on the eLearning system.

Forty five students passed a total of 7 eLearning courses related to pesticide residues in 2006.

3.8. Agrochemicals Unit staff training

Agrochemicals Unit staff participated in a number of training events, including:

- Basic Training on Waters Quattro Micro GC-MSMS by Mr. Martin Duff (Waters Corporation), ACU, 24 September 2006 - (M. Schweikert, B. Maestroni, N. Rathor)
- Training on LC-MSMS applied to pesticide analysis by consultant, Prof. N. Leepipatiboon, 25-28 September 2006 (M. Dabalus, N. Rathor, B. Maestroni, M. Schweikert)
- Agilent Vienna Analytical Forum 2006: New developments in pharmaceutical and biotechnological analysis techniques, Vienna, 8 February 2006 (N. Rathor)
- Agilent Vienna seminar on LC & LC-MS(MS), Vienna, 27 March 2006 (N. Rathor)
- 21 .11.06 Waters Vienna Sample preparation Workshop, Vienna, 21 November 2006 (N. Rathor)

- Agilent seminar on Applications of Chromatographic techniques, BOKU, Vienna, 7 February 2006 (M. Schweikert Turcu)
- GC-MS training by Restek, Vienna, 4 October 2006 (B. Maestroni)
- Agrochemicals Unit staff also participated in a number of internal IAEA training courses.

3.9. Agrochemicals Unit Quality System

In 2001, a Quality Assurance System was initiated in the Agrochemicals Unit. The system combined elements of OECD GLP and ISO 17025 to meet the requirements of the activities then being carried out in the Unit. During 2002, the system was continuously revised and improved to reflect experience gained since its implementation and to adopt to changes in the Unit's work. However, the system was very complex and did not comply completely with the either the GLP or ISO requirements. Since the Unit provides training in the implementation of laboratory quality assurance and quality control, and there is a constant demand for advice and the provision of quality system documentation from training workshop participants, TC Fellows and counterparts in Member States, it was decided in 2005 to completely revise the system.

The appropriate standard upon which the quality system should be based is the ISO 17025 standard, since the training provided by the Unit is largely targeted at regulatory laboratories carrying out testing of commodities to comply with national or international limits. Implementation of the ISO 17025 system would provide the necessary credibility for the Unit's training activities and underpin the quality of the analytical results produced for individual projects, research, and in support of the development of international standards. The ultimate objective is to attain accreditation of the quality system, as recommended by the FAO Autoevaluation of Programme Entity 215P1. One major problem is that, although it is accepted that it is imperative to implement a suitable quality system and highly desirable to achieve accreditation, no resources have been made available to do this.

The revision of the quality system began in 2006, in collaboration with Mr. Fajgelj, the NAAL Quality Manager. The revision also provided an opportunity for harmonisation of the Unit's system with those being implemented by other NAAL Units.

During 2006, the working practices of the Unit and the individual responsibilities of the Unit members were examined to provide a basis for the revised system. A revision of all standard operating procedures was initiated, involving the Unit Quality Officer and all staff members. More than 40 documents were revised, with shortened, more simplified text that better reflects the actual workflow. The quality system documents will be further revised in 2007 with a tentative target of the end of that year for completion of the revised system.

4. GUIDELINES AND STANDARDS

4.1. Draft Guidelines on the estimation of the uncertainty of results

At the 38th Session of the Codex Committee on Pesticide Residues (CCPR), the proposed guidelines, originally drafted at a consultants' meeting organized by the Agrochemicals Unit, were discussed in great detail within the Working Group on Analytical Methods. The Guidelines were revised by the Working Group based on comments from several countries and forwarded for adoption by the Joint FAO/WHO Codex Alimentarius Commission.

4.2. Committee on Residues of Veterinary Drugs in Foods

At the 16th meeting of the CCRVDF (May 2006), the Agrochemicals Unit Head, representing IAEA, presented a summary (Conference Room Document 5) of the activities of the Joint FAO/IAEA Programme related to residues of veterinary drugs in food. The Committee was informed of a FAO/ IFAH project with inputs from the FAO/IAEA Joint Programme to build capacity in sub-Saharan Africa for the quality control of trypanocidal drugs and that, in the future, the scope of the project would be expanded to include the development and transfer of methods for quality control to a range of other veterinary drugs and methods for their residues in foods.

The Committee noted that, in response to a recommendation of the Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL, the FAO/IAEA Joint Programme was planning to hold an inter-regional training course in 2007 for developing countries on methods for veterinary drugs residues.

The Committee welcomed the offer to include on the Joint Division's website, Codex analytical methods in order to enhance the capabilities of developing countries to identify and implement suitable methods in support of residue monitoring plans. In response to this, Canada offered the method protocols in their method compendium, and UK has tentatively offered access to selected methods.

The Unit Head volunteered to become involved in *ad hoc* working groups on Risk Management (electronic working group to identify work topics and options), Residues of Veterinary Drugs without ADI/MRL and Methods of Analysis and Sampling.

The full report of the 16th CCRVDF is available at:
http://www.codexalimentarius.net/download/report/659/al29_31e.pdf.

4.3. Sampling manual for mycotoxins

The final draft of a sampling manual for mycotoxins was produced by two consultants in 2006, using the results of a survey carried out by the Agrochemicals Unit of maize samples from different regions of Nigeria for fumonisin B1 as a case study. The manual was delivered in late 2006 and is being edited by Agrochemicals Unit staff before publication in 2007. It is planned to introduce the manual at a round-table held by FAO to release a training video on sampling at the IUPAC Symposium on Mycotoxins in Istanbul, Turkey, May 2007. The FAO video and the sampling manual produced by the Joint FAO/IAEA Programme should be complementary and are expected to be of significant assistance to Member States in planning sampling schemes for the control of mycotoxins.

5. SELECTED COUNTRY ACHIEVEMENTS

The activities of the Agrochemicals Unit, as an integral part of the Food and Environmental Protection Sub-programme, have produced significant outputs leading to important outcomes in many countries. The "train the trainers" approach taken for the workshops and training courses organised by the Unit, along with other outreach activities such as joint sponsorship of regional food safety summits, has also had considerable impact on awareness-building and expansion of the knowledge-base in Member States. Some specific examples are summarised below:

Bangladesh – Dr. Md. Mazibur Rahman, a participant in the 2005 training workshop 'Introduction to QA/QC measures in pesticide residue analytical laboratories', presented the information from the workshop at a high-level National Seminar on 'Pesticide residue research and monitoring in the environment', 26 February 2006, Atomic Energy Centre, Dhaka. The seminar had approximately 100 participants including representatives of the Department of Agriculture, the Ministry of Science Information and Communication Technology, and the Bangladesh Atomic Energy Commission. The meeting recommended, *inter alia*, that pesticide residue monitoring must be continued because of its extreme importance in maintaining food and environmental safety and promoting trade in agricultural products; that training of personnel in pesticide residue analysis is essential; and that efforts must be made to create a National Centre which should become an accredited laboratory. A well equipped modern laboratory facility has been established at the Institute of Food and Radiation Biology in the Bangladesh Atomic Energy Commission, which is able to perform pesticide residue research and monitoring.

Dr. Rahman also organized a National Seminar entitled 'Introduction to QA/QC measures in pesticide residue analytical laboratories' at the Institute of Food and Radiation Biology in July 2006, where material from the ACU workshop was presented, including the role of the laboratory in providing feedback to farmers and producers. It was asserted that 'the lack of operational QA/QC systems in Bangladesh has circumscribed the entry of Bangladeshi agricultural and fishery products into International market'.

Thailand – In 2006, The Food and Veterinary Office (FVO) of the European Commission carried out an inspection of facilities in Thailand with respect to residues of veterinary drugs in food for export to the EU. The laboratory capabilities, including quality assurance aspects, put in place with assistance from ACU in training, methodology transfer and the participation of the Veterinary Public Health Laboratory in a CRP, satisfied the inspectors and trade in aquaculture and poultry products, a major source of income for Thailand (export of poultry and shrimps to the EU was worth more than €700 million in 2005), continues.

One of our training workshop participants, Ms. Leepipatboon, having planned and implemented training courses on method validation and instrumental analysis in Thailand in 2006, also presented lectures and practical sessions at the 2006 ACU Training Workshop in Seibersdorf.

Brazil – The EU carried out a follow-up visit to Brazil after the previous FVO inspection in 2005 found that the necessary assurances were not in place to guarantee that exports of food commodities to the EU were safe in terms of veterinary drug residues. Analytical methods and protocols provided by ACU and developed under the CRP 'Development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries' helped address the shortfall and maintain trade (beef and poultry exports to EU were worth €1.5 billion in 2005).

The impact of the Agency's training and capacity building activities in Brazil and other countries was acknowledged by an FVO inspector in a recent meeting with the ACU Unit Head.

Nigeria - Following training in a workshop on mycotoxins analysis in 2003, the mycotoxins laboratory in Lagos has now implemented testing for a range of mycotoxins in almost all agricultural commodities for export, for example aflatoxin M1 in milk, total aflatoxins in cereal and cereal products (oats, wheat, corn, groundnut, peanut, vegetable oil, etc.) and ochratoxin A in coffee, tea and rice. The laboratory is participating successfully in international proficiency tests (FAPAS). A very successful National Workshop on mycotoxins was held in 2005.

General - Based on data extracted from the responses to questionnaires sent to TC Counterparts, CRP participants and recipients of fellowship training or scientific visits, as part of the FAO Autoevaluation of the FEP Sub-programme, covering the years 2002-2005, 73% of TCP respondants, 63% of CRP respondants and 76% of fellows/scientific visitors said that their laboratory has the capacity to better comply with standards as a result of participation in the project/programme. Only 3% of CRP participants and 6% of fellows/scientific visitors responded in the negative, the remainder replied ‘not applicable’.

6. APPENDICES

6.1. Publications

Fodey, T., Murilla, G., **Cannavan, A**. and Elliott, C. (2007). Characterisation of antibodies to chloramphenicol produced in different species by ELISA and biosensor technologies. Analytica Chimica Acta, 592, 51-57.

Aysal, P., Ambrus, Á., Lehotay, S.J. and **Cannavan, A**. (2007). Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. Journal of Environmental Science and Health B, 42, 481-490.

Wesongah, J., Murilla, G., Guantai, A., Elliott, C., Fodey, T. and **Cannavan, A.** (2007). A competitive enzyme-linked immunosorbent assay for determination of chloramphenicol. Journal of Veterinary Pharmacology and Therapeutics, 30, 68-73.

Aysal, P., Mushi, G. and **Cannavan, A**. (2006). Adaptation of the IAEA-ethyl acetate multiresidue method to determine pesticide residues in wheat flour. Book of abstracts of the International Conference on Pesticide Use in Developing Countries: Environmental Fate, Effects and Public Health Implications, 16-20 October 2006, Arusha, Tanzania, 51.

Brodesser, J., Byron, D.H., **Cannavan, A**., Ferris, I.G., Gross-Helmert, K., Hendrichs, J., **Maestroni, B.M**., Unsworth, J., Vaagt, G., and Zapata, F. (2006). Pesticides in developing countries and the International Code of Conduct on the Distribution and the Use of Pesticides. Austrian Agency for Health and Food Safety (AGES) Meeting on Risks and Benefits of Pesticides, Vienna, Austria, 30 March 2006.

Maestroni, B.M., Ferris, I.G., Brodesser, J., **Cannavan, A**., Byron, D.H., Gross-Helmert, K. and **Rathor, N.** (2006). Integrated approaches to assess indicators of the effectiveness of pesticide management practices: Challenges and opportunities for developing countries. Austrian Agency for Health and Food Safety (AGES) Meeting on Risks and Benefits of Pesticides, Vienna, Austria, 30 March 2006.

Unsworth, J.B., Ferris, I.G., Gross-Helmert, K., **Klaus, P.M., Maestroni, B.M**. and Marsella, M. (2006). INFOCRIS and the IUPAC compendium of agrochemical information. Book of abstracts of the 11th IUPAC International Congress of Pesticide Chemistry, Kobe, Japan, 6-11 August 2006.

Posters

Maestroni, B. M., Rathor, N., Islam, M.D., Doko, M.B., Ogunbanwo, B.F. and **Cannavan, A.** (2006). Internal quality control procedures for the analysis of fumonisin B1 in primary samples of corn from Nigeria, AOAC conference 'Foods to dye for – contaminants – sampling, analysis, legal limits', Limassol, Cyprus, 6-7 November 2006.

Wimmer, B., Kaltenbrunner, E., **Schweikert Turcu, M**. and Strebl, F. (2006). Influence of climate change on the environmental behaviour of s-metolachlor in a soil-plant-water system, Workshop on "Lysimeters for Global Change Research: Biological Processes and the Environmental Fate of Pollutants", 4- 6 October 2006, GSF- National Research Center for Environment and Health, Neuherberg, Germany.

Suszter, G., Ambrus, A., **Schweikert Turcu, M. and Klaus, P.M.** (2006). Comparison of sample processing methods for analysing pesticide residues in soil, IUPAC conference,Kobe, Japan, 3-6 June 2006.

6.2. Travel

A. Cannavan

Middle East Food Safety Summit (organised by Waters Corporation), Manchester, UK, 4-6 May 2006. Keynote lecture, 'International guidelines and regulations for the control of veterinary drug residues in food'.

Sixteenth Session of the Codex Committee on Residues of Veterinary Drugs in Food, Cancun, Mexico, 8-12 May 2006. To represent the Agency and present a conference room document, participate in *ad hoc* working groups.

University of Peradeniya, Sri Lanka, 12-16 June 2006. To review progress and update the work plan for TCP SRL/5/039, provide advice on the application of HPLC to veterinary drug residue analysis and inspect the new laboratory facilities and recently installed equipment.

Seminar presented on 'Research and capacity building in animal science and food safety in developing countries', Munich Technical University, Freising, Germany, 26 July 2006.

Asia-Pacific Food safety Summit (co-sponsored by the FAO/IAEA Joint Programme and Waters Corporation), Singapore, 14-17 October 2006. Keynote address 'Global food safety – issues and trends'.

Food Safety Seminar, Bangkok, Thailand, 18-19 October 2006. (co-sponsored by the FAO/IAEA Joint Programme and Waters Corporation). Keynote address 'Global food safety – issues and trends'.

Food Safety Colloquium, Institute of Chemical Technology, Prague, Czech Republic, 22-24 November 2006. Keynote lecture 'Control of Veterinary Drug residues in Food: EU perspectives and global trade'.

Fourth RCM of the CRP "The Development of Effective Strategies for Monitoring Veterinary Drug Residues in Animals and Animal Products in Developing Countries", Freising, Germany, 27 November – 1 December 2006. Scientific Secretary.

B. M. Maestroni

AOAC conference 'Foods to dye for – contaminants – sampling, analysis, legal limits', Limassol, Cyprus, 6-7 November 2006. To present a poster on 'Internal quality control procedures for the analysis of fumonisin B1 in primary samples of corn from Nigeria'.

Mendoza, Argentina 27 February–3 March 2006. Regional Workshop 'Improving the assessment of good agricultural practices at catchment scale using laboratory analytical support' for the formulation of a regional TCP, RLA0021. Workshop attended by National Experts from Argentina, Bolivia, Chile, Costa Rica, Cuba, Ecuador and Uruguay.

La Paz, Bolivia, 6-8 March 2006. To support TCP BOL/5/015, 'Developing pesticide residue monitoring capabilities in support of cash crops'.

P. Aysal

San Jose, Costa Rica, 3-15 July 2006. To provide training on the IAEA multi-residue pesticide method and radioisotope techniques in pesticide residue analysis for staff in the Centro de Investigación Ambiental under TCP COS/5/026.

Arusha, United Republic of Tanzania, 16-20 October 2006. To give an oral presentation; 'Adaptation of the IAEA-ethyl acetate multiresidue method to determine pesticide residues in wheat flour', at the ANCAP/SETAC International Conference on "Pesticide Use in Developing Countries: Environmental Fate, Effects and Public Health Implications".

6.3. Fellows/Scientific Visitors

Fellows	**TC code**	**Dates**	Training
MUSHI, Mr. G.	URT/06016	01/03/06-12/06/06	Pesticide residue analysis
MATATA, Mr. Z.	URT/06015	01/11/06 – 30/11/06	Instrument troubleshooting and maintenance
MARCHESE, Ms. L.	BRA/06046	11/09/06-06/10/06	Training workshop on QA/QC in residue labs
PEREZ ROJAS, Mr. G.M.	COS/06008	11/09/06-06/10/06	Training workshop on QA/QC in residue labs
VIDELLA CAMPILLAY, Ms. X.M.	CHI/06013	11/09/06-06/10/06	Training workshop on QA/QC in residue labs
BOU KHOZAM, Ms. R.	LEB/06005	11/09/06-06/10/06	Training workshop on QA/QC in residue labs
CACERES CHOQUE, Mr. L.F.	BOL/06011	11/09/06-06/10/06	Training workshop on QA/QC in residue labs
HERNANDEZ, Mr. G.	PAN/06020	11/09/06-06/10/06	Training workshop on QA/QC in residue labs
FAO TC trainees			
IDIR, Ms. H	ALG	12/06/06 – 07/07/06	Pesticide and veterinary drug residue analysis
ZAHI, Mr. A	ALG	12/06/06 – 07/07/06	Pesticide and veterinary drug residue analysis

Other visitors to the Unit

Prof. Peter Sundin, Director, International Science Programme (ISP). Uppsala, Sweden.

Dr. Michelangelo Anastassiades, EU Community Reference Laboratory for Pesticide Residues (CVUA), Germany.

6.4. Technical Cooperation Projects

TC code	Title	Technical Officer
MON/5/012	Monitoring of residues in livestock products and surveillance of animal diseases	**A. Cannavan**
YEM/5/005	Monitoring of veterinary drug residues	**A. Cannavan**
SRL/5/039	Monitoring of chemical residues and food-borne pathogens	**A. Cannavan**
CHI/5/046	Certification of animal products using nuclear and other analytical techniques	**A. Cannavan** D.H. Byron
ANG/5/003	Veterinary drug residue monitoring programme	**A. Cannavan** D.H. Byron
BEN/5/003	Veterinary drug residue monitoring programme	**A. Cannavan** D.H. Byron
NIC/5/007	Determining drug residues in bovine meat exports	**A. Cannavan** D.H. Byron
PAN/5/015	Quality assurance in pesticide residue analysis for agricultural production	K. Gross **B.M. Maestroni**
MAK/5/005	Upgrading of food safety system	P.J. Brodesser **B.M. Maestroni**
BKF/5/005	Regulatory Control and Monitoring of Contaminants and Residues	P.J. Brodesser **B.M. Maestroni**

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