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Nuclear Techniques in Food and Agriculture

Agrochemicals Unit
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Agency's Laboratories, Seibersdorf

http://www.iaea.org/OurWork/ST/NA/NAAL/agri/agr/agriAGRmain.php

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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 pesticides and their residues, and mycotoxins. With the appointment of a new Unit Head in July 2004, the scope has been broadened to include residues of veterinary drugs. The main areas of activity in the pursuit of the objectives are; applied research and development, technology transfer, training, and support for the development of international guidelines. This report includes some activities from 2004, when the new Unit Head took up his position.

Several analytical methods were developed or adapted and validated for transfer to Member States and/or to provide data to support the development of guidelines. Radiolabelled compounds, when available, provided a comparative advantage as a quality control tool during method development.

Methods were developed and studies carried out for the pesticides indoxacarb and metalaxyl in kale and cypermethrin and chlorpyriphos in mango fruit as part of a coordinated project to evaluate the variability of pesticide residues in crop units. A multiresidue method for the determination of a range of pesticides using GC ECD/NPD was developed and validated in several matrices. The analytical protocol was tailored to permit its application as a regulatory or research method in a wide range of laboratories in developing countries. The method has been included in training courses.

Multiresidue HPLC methods were developed and validated for residues of a range of sulphonamide veterinary antibiotics and macrocyclic lactone anthelmintic drugs in animal tissues. Method uncertainty, as required for ISO17025 accreditation, and method performance characteristics as specified by the EU were estimated for each method. Both methods are suitable for application in regulatory laboratories in developing countries and two Fellows were trained in their application.

A method was adapted and validated for the quality control of the trypanocidal drug, isometamidium, as part of a FAO/IFAH project. The method will be transferred to regional reference laboratories in sub-Saharan Africa to be set up under the project, with the objective of controlling the use of counterfeit and poor quality trypanocides which have severe implications for animal health, the development of drug-resistant trypanosomes and food safety.

Studies were completed to estimate the uncertainty of reduced analytical portions for pesticides analysis in soil and fumonisin B1 in maize. Method performance parameters were calculated for small portion analysis, which can greatly reduce the cost and environmental impact of the analytical process.

A collaborative project with the Austrian Research Centre on the effect of climate change on pesticide behaviour was commenced and support was provided for a Coordinated Research Project.

Training activities at Seibersdorf included a 4-week inter-regional training workshop on QA/QC in pesticide residue analysis, which had 26 participants from 23 developing countries, the training of 3 Fellows and one Scientific Visitor 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. A regional workshop on "Food safety requirements for the international market: strategies for residues programmes" was organised and held in Chile.

Support for the development of international guidelines focused mainly on Codex Alimentarius. Guidelines on the use of mass spectrometry for the confirmation of residues, drafted by the Unit in 2004, went through the Codex procedure and were adopted by the Commission. Draft Guidelines on the estimation of uncertainty of results were advanced to step 6 of the Codex procedure. The Unit also became involved in the efforts of Codex to address the problem of trade barriers due to veterinary drugs without ADI/MRL. Studies on pesticide residues using methods developed in the Unit provided data for collation with data from field trials in 13 countries to produce estimates for sampling uncertainty and help develop sampling guidelines for pesticide residues. A technical report on this study was completed by a consultant. Work was completed on the analysis of 2000 maize samples for the mycotoxin, fumonisin B_1, for a Nigerian project. The data will be incorporated into a mycotoxin sampling manual to be published by the Agency in 2006.

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. Networking has also been successful, with technology transfer agreements between Brazil and South Africa and between Mongolia and Korea being examples of international cooperation fostered by the ACU activities.

1. INTRODUCTION

1.1. Sub-programme and Unit objectives

Ensuring the safety and quality of food supplies is an integral part of food security and consumer protection. It is also essential for countries wishing to pursue social and economic objectives through greater access to world markets in food and agricultural commodities. 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". There is an increasing demand from FAO and IAEA Member States for support and technical advice in implementing this concept. It is becoming increasingly important to harmonize national food safety and phytosanitary regulations around the standards and guidelines established by the FAO/WHO Codex Alimentarius Commission, since these are used as references by the WTO for trade related issues under the Sanitary and Phytosanitary (SPS) Agreement. There is also a need to implement related international codes of conduct to promote good agricultural practice (GAP) and good manufacturing practice (GMP) and thereby reduce the chances of unacceptable risks to health arising, for example, from inappropriate use of pesticides or veterinary drugs and the consequent presence of unacceptable residues in food and the environment. Sub-programme Project 2.E3.02 aims to improve the services provided by national food safety and pesticide regulatory institutions in support of consumer and environmental protection. The main objective is to assist Member States to comply with Codex Alimentarius food safety standards through improved analytical methods and capacities to assess and manage the risks associated with contaminants and residues.

Analytical laboratories are an integral component of food control systems, providing the fundamental support for risk assessment and risk management by providing policy makers at national, regional and international levels with the necessary data to support decision making. Within this framework, the objectives of the Agrochemicals Unit are to assist laboratories and regulatory authorities in developing countries to meet the required standards in terms of analytical methods, trained analysts and regulators, quality assurance systems and feedback systems to improve production practices. From the responses of approximately eighty pesticide residue laboratories to a questionnaire circulated by the Unit in 2005, greater than 50% of those who replied stated that their country's export of food and agricultural produce was hampered by non-conformity with WTO agreements and with importing countries maximum residue limits (MRLs), and almost 60% stated that trade was hampered by failure to comply with Codex MRLs.

More than 80% identified a lack of training opportunities for staff as a major constraint to residues testing. About 80% of the respondents identified the availability of validated methods as a major influence on the choice of methods used, second only to availability of instrumentation. To address these issues, the Agrochemicals Unit's activities focus on applied research, method development and validation, technology transfer, training and support for the development of international guidelines, mainly those promulgated by Codex.

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

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Consultants	**From**	**To**	
Alfredo Montes Niño	15 Jan 2005	15 July 2005	
Bruno Doko	15 Mar 2005	14 Apr 2005	
Gesa Schad	14 Nov 2005	21 Feb 2006	
Bruno Magalhaes Carniero	1 Dec 2005	28 Feb 2006	

In addition to regular staff, the Agrochemicals Unit had four temporary staff members at various periods during 2005. Dr. Alfredo Alfredo Montes Niño worked with the Sub-programme for 6 months, with his time divided between the Agrochemicals Unit and the Food and Environmental Protection Section. Dr. Montes' duties included support for technical cooperation projects in the field of veterinary drug residues and assistance with the revision of the quality system in the Agrochemicals Unit. Dr. Bruno Doko, a former member of the Unit, worked at Seibersdorf for 1 month to complete the analysis of maize samples from Nigeria for fumonisin B_1 (see section 4.5). Ms. Gesa Schad joined the Unit in November to work on the adaptation and

validation of a method for the quality control of trypanocidal drugs (see section 2.3). Mr. Bruno Carniero joined the Unit in December to provide expertise on methodologies for veterinary drug residue monitoring and complete robustness testing of the method for sulphonamide analysis validated in the Unit (see section 2.2.1).

2. APPLIED RESEARCH AND DEVELOPMENT

2.1. Pesticide residues

2.1.1. Multiresidue method for pesticide residues by GC-ECD/NPD

It is a regulatory requirement that analytical methods be available to determine pesticide residues in crops, feeds and food commodities and environmental samples. Methods may be adapted or modified to match the requirements and capabilities of the laboratory or the purpose for which they are being used. Following its development, a method must be validated to demonstrate that it is fit for the purpose for which it is intended.

The objective of this study was to develop and validate a rapid, simple and inexpensive method for the analysis of a range of pesticide residues in various plant matrices, suitable for use in developing country laboratories.

The protocol was based on the QuEChERS method (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe), which was introduced by M. Anastassiades *et al*.[1] in 2003. The QuEChERS method employs a simple extraction with acetonitrile, clean-up by dispersive solid phase extraction to remove interfering polar matrix components, such as organic acids, polar pigments and sugars, and analysis by gas chromatography-mass spectrometry (GC-MS). However, many laboratories in developing countries do not yet have access to mass spectrometry and are equipped with more conventional GC detectors such as the electron capture detector (ECD) and the nitrogen-phosphorous detector (NPD). The method was therefore modified to permit the use of GC with ECD/NPD by employing ethyl acetate for extraction. In validation experiments, tomato, apple and frozen green bean were each fortified with 25 selected pesticides. Six replicates of each matrix fortified at each of 3 levels from 0.05-5 mg/kg were analysed to provide validation data. To facilitate evaluation of the performance at each step in the method, ^{14}C-chlorpyrifos was applied in all matrices and at all fortification levels.

Composition of the pesticide mixture:

Dichlorvos, EPTC, Mevinphos, Heptenophos, Propachlor, Dimethoate, Diazinon, Pirimicarb, Vinclozolin, Fenitrothion, Chlorfenvinfos, Folpet, Methidation, Triazophos, Propyconazole, Fenpropathrin, Iprodion, Azinphos-methyl, Fenarimol, Coumaphos, Cyfluthrin, Fenvalerate, Lindane, α-endosulfane, ^{14}C-Chlorpyrifos.

Experimental

The method protocol is outlined in Figure 1. Briefly, a portion of comminuted sample was extracted with ethyl acetate, anhydrous sodium sulphate (Na_2SO_4) and sodium bicarbonate ($NaHCO_3$) using a probe blender. After centrifugation, clean-up and removal of residual water were performed simultaneously by dispersive solid-phase extraction, a rapid procedure in which primary-secondary amine (PSA) sorbent and anhydrous magnesium sulphate (Mg_2SO_4) were mixed with an aliquot (5 g sample equivalent) of the ethyl acetate extract. After clean-up, 1 ml of the supernatant extract was removed for analysis by GC-ECD/NPD (1 µl injection volume). The extracts for the samples fortified at the lowest level were concentrated (x 5) by evaporation for

[1] Anastassiades, M., Lehotay, S.J., Štajnbaher, D. and 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.

GC-NPD analysis. Matrix matched standards and weighted linear regression were used for quantification of the pesticide content.

For evaluation of individual sample preparation steps, portions were taken from extracts after the sample extraction and clean-up steps. Scintillation cocktail was added and the activity measured on a liquid scintillation counter. Activities were compared with the expected activities from the added ^{14}C-chlorpyrifos to determine the efficiency and repeatability of the method.

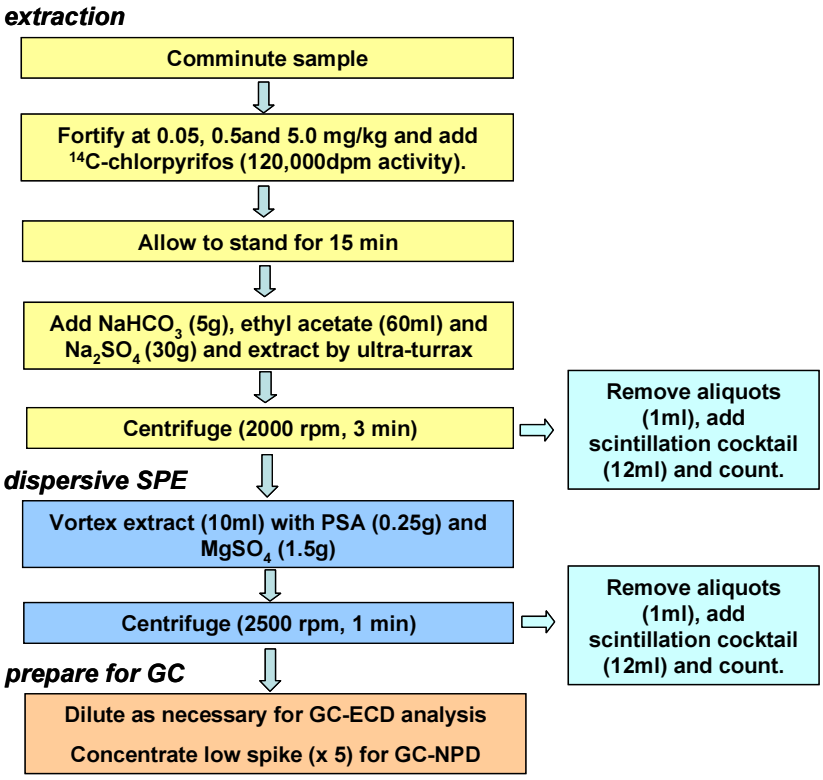

FIGURE 1. Sample preparation flow diagram for the method validation

Results

The method was successfully validated for 22 of the 25 compounds. Dichlorvos recoveries were significantly lower than the recoveries of the other analytes especially in tomatoes and green bean; iprodion determination in green bean was obstructed due to matrix interference and phorate determination was compromised due to degradation of the compound.

Mean recoveries and relative standard deviations (RSDs) for each matrix are presented in Table 1 and individual recoveries for each compound are presented in Figure 2. The overall average recovery of the method was 93% with a RSD of 10% (n=1182), for 22 analytes at all 3 fortification levels in all 3 commodities. The mean recoveries and RSDs for the 22 compounds at all fortification levels and in all matrices were within the acceptance criteria specified by CODEX (mean recovery 70-120% and RSD ≤ 20 % for 0.05 mg/kg fortification level; mean recovery 70-110 % and RSD ≤ 15 % for 0.5 and 5 mg/kg fortification levels).

Table 1. Analytical recoveries (22 analytes)

Matrix	Recovery (%)	RSD (%)
Tomato	94	9
Apple	96	8
Green bean	88	11

Conclusions

The method was successfully validated by a single laboratory using GC/ECD and GC/NPD for analysis. This method is well suited to the analysis of pesticide residues in non-fatty foods, especially in developing country laboratories which are not equipped with expensive mass spectrometry instrumentation. The method was also applied to further studies in the Unit (for example, see section 2.1.2), was used for practical exercises in the training workshop "Introduction to QA/QC measures in pesticide residue analytical laboratories" (section 3.1 of this report), and training in its application will be given by Ms. Aysal at a TC training course in Costa Rica in 2006. The method protocol and validation data are available on the Sub-programme web page.

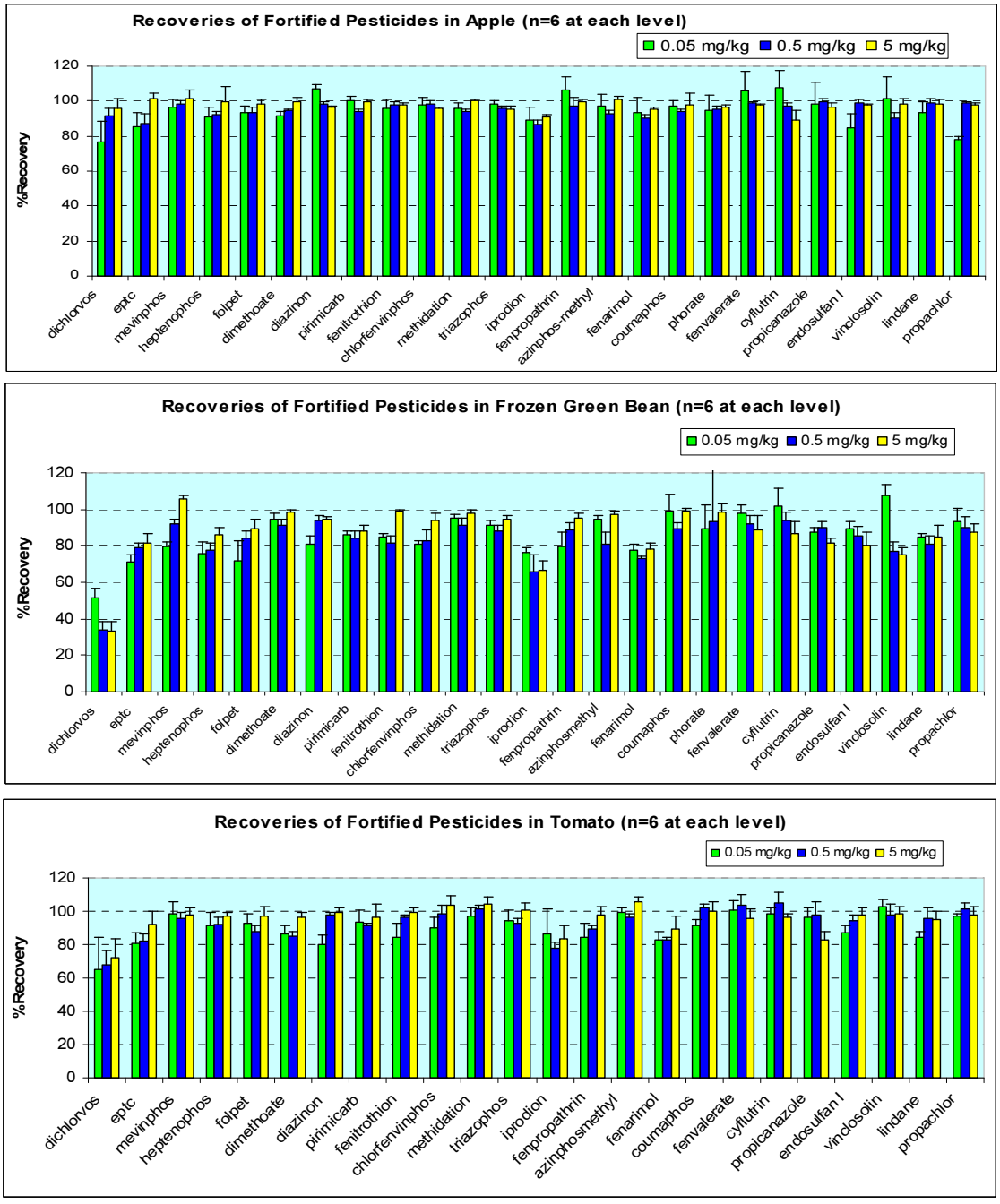

FIGURE 2. Recovery of pesticides from fortified sample matrices.

2.1.2. Determination of chlorpyriphos and cypermethrin in mango by GC-ECD

As part of a project on "sampling guidelines for fruits and vegetables" (see section 4.4), a study on pesticide residue analysis in mango was performed. Mango, a popular fruit produced mostly in tropical countries, is a major contributor to the export economy of these countries. However, trade barriers may arise if the residual content of pesticides in the mango exceeds the maximum residue limits (MRL) set by the main importing markets like the U.S.A, Japan and Europe. A major contributory factor to the variation in the measured residual content of any batch of produce is the variation in sampling. Sampling strategies may exist in these developing countries, but may need to be improved and properly implemented in the field in order to comply with the required standards.

A variety of matrix/pesticide combinations was chosen for the project on sampling guidelines. For this study, two pesticides were selected; chlorpyriphos, a non-systemic organophosphorous insecticide active against fruit pests, and cypermethrin, a synthetic pyrethroid insecticide widely used in fruit, cereals and vegetables and also used for food storage and animal husbandry applications. Cypermethrin is an unresolved mixture of its alpha, beta, theta and zeta isomers.

Experimental

Method validation

An analytical procedure was adapted and validated before the analysis of the field samples. A validation procedure based on 5 fortification levels was performed for both active ingredients. The validation levels were selected to cover the range of expected concentrations in the field samples.

The analytical procedure comprised sample processing, extraction, clean-up and analysis. After removal of the stones and weighing, the mango samples were initially processed by chopping in a Stephan blender for about 30 seconds, then homogenized using a P-chopper, with the addition of distilled water if necessary to obtain a homogeneous pulp. Aliquots (25 g) of sample were fortified for validation, 25g of sodium sulphate added and the mixture was extracted with 25 ml ethyl actetate using a probe blender. The extracts were centrifuged. Portions of the supernatants (5 ml, representing about 5 g mango sample) were loaded onto ENVI-Carb SPE cartridges, preconditioned with toluene (5 ml), acetonitrile (5 ml), acetone (5 ml), and 15% acetone in hexane (5 ml). The analytes were eluted with 20 ml acetone:hexane (15+85) at a flow rate of approximately 1ml/minute. The extracts were evaporated almost to dryness at 40^{o}C and the residues dissolved in acetone:iso-octane (15+85) to give a final volume of 5 ml (final concentration 1 g/ml extract). The extract was analysed by gas chromatography with electron capture detector for the identification and quantitation

Processing of mango samples

of analytes present. The instrument was programmed with a temperature profile to permit the resolution and quantitation of chlorpyriphos and the four isomers of cypermethrin in approximately 32 minutes. A five-point calibration was used to quantify the analytes. Cypermethrin results were calculated as the sum of the four isomers present.

Field samples

Mango samples treated with chlorpyrifos and cypermethrin in the plantation according to regular agricultural practice were received from Malaysia. Samples were prepared and analysed in batches of 20 using the procedure described for the fortified samples. Internal quality control was achieved by analysing, typically, 2 samples spiked at different levels, 2 previously analysed samples and one blank sample with every batch.

Results

The repeatability and reproducibility of the method were determined using five different fortification levels, in five replicates and on different days. The results are summarized in table 2. The performance of the method was well within the acceptable criteria according to Codex Alimentarius Guidelines. The overall mean recovery for all fortification levels was 113.9% (RSD = 9.3%, n = 49) for chlorpyriphos and 115.0% (RSD = 12.2%, n = 41) for cypermethrin. The limits of quantitation were 0.001mg/kg and 0.005mg/kg for chlorpyrifos and cypermethrin, respectively.

Table 2. Method repeatability and reproducibility for chlorpyrifos and cypermethrin

Chlorpyriphos

Fortification level (mg/kg)	**0.001**	**0.05**	**0.5**	**1.0**	**3.0**
Reproducibility (RSD, %)	7.0	9.6	5.2	7.1	5.0
CODEX Criteria	≤53.0	≤32.0	≤23.0	≤23.0	≤16.0
Repeatability (RSD, %)	7.3	7.6	5.2	3.4	2.8
CODEX Criteria	≤35.0	≤20.0	≤15.0	≤15.0	≤15.0

Cypermethrin

Fortification level (mg/kg)	**0.05**	**0.5**	**1.0**	**3.0**	**5.0**
Reproducibility (RSD, %)	17.7	9.1	6.1	8.9	6.9
CODEX Criteria	≤32.0	≤23.0	≤23.0	≤23.0	≤23.0
Repeatability (RSD, %)	11.7	5.2	6.9	3.1	6.9
CODEX Criteria	≤20.0	≤15.0	≤15.0	≤15.0	≤10.0

Conclusions

The method developed was validated satisfactorily and proved to be suitable for the analysis of residues of the two target compounds. The method was applied to the analysis of 136 field-treated samples and the results produced were evaluated and collated with the results from other collaborating countries conducting similar field trials to produce an estimation of sampling uncertainty (see 4.4).

The method protocol has been circulated to TCP counterparts and made available on the Sub-programme web page.

2.1.3. Determination of metalaxyl and indoxacarb in kale by GC-MS

The aim of this study was to determine the distribution of metalaxyl and indoxacarb residues in kale which were harvested two days after last treatment, according to an experimental protocol designed to represent normal agricultural practices. The study was part of a project designed to evaluate the variability of pesticide residues in crop units in order to formulate sampling guidelines (see section 4.4.).

Kale is a leafy green vegetable that belongs to the Brassica family, a group of vegetables including cabbage, collards and brussels sprouts that have gained recent widespread attention due to their health promoting, sulphur-containing phyto-chemicals. It is easy to grow and can grow in colder temperatures where a light frost will produce especially sweet kale leaves. The leaves of the kale plant provide an earthy flavor and more nutritional value for fewer calories than almost any other food. Although it can be found in markets throughout the year, it is in season from the middle of winter through the beginning of spring when it has a sweeter taste and is more widely available.

Kale, a high water, high chlorophyll content leafy vegetable

For the project on sampling guidelines, a range of matrix/pesticide combinations was chosen. For this study, an insecticide and a fungicide were selected. Indoxacarb is a non-systemic, synthetic oxadiazine insecticide, used as an organophosphate replacement to control sucking insects. It is used on a range of crops, including fruits, vegetables, soyabeans, alfalfa and peanuts. Metalaxyl is a systemic acylamino acid or anilide fungicide used in mixtures as a foliar spray, as a soil treatment for control of soil-borne pathogens and as a seed treatment.

Experimental

Method validation

Fortification experiments were carried out with 5 replicates at three levels (0.5, 2.0 and 5 mg/kg) to assess mean recovery and precision of the method. The extraction and clean-up efficiency of the method for kale was assessed by using ^{14}C-chlorpyrifos at 0.05 mg/kg (n=5).

Method

A portion (30 g) of comminuted sample was extracted with 30 ml ethyl acetate (indoxacarb) or 60 ml acetonitrile (metalaxyl) using a probe blender, dried with 30 g anhydrous sodium sulphate (Na_2SO_4) and neutralised with 5 g sodium bicarbonate ($NaHCO_3$). The extracts were centrifuged and cleaned-up by dispersive solid-phase extraction, as described in section 2.1.1. After clean-up, extracts were analysed using gas chromatography – mass spectrometry (GC-MS). To be able to determine 0.05 mg/kg residue levels in the field kale samples, a further evaporation/concentration step was applied before GC-MS analysis. Two different GC-MS systems were used. For metalaxyl analysis, GC with a mass selective detector (GC-MSD) was employed, operated in selected ion monitoring mode for the protonated molecular ion at m/z 280 and fragment ions at m/z 206, 160 and 132. For indoxacarb, GC with ion-trap mass spectrometric detector (GC-ITMS) was used in selected ion storage mode for the fragment ions at m/z 499, 417, 264, 218 and 203. Mass calibration of the instruments

was performed by introducing perfluorotributylamine (PFTBA); air and water leaks and total ion count were checked before the analysis.

Matrix matched standards were used for quantitation. Individual recoveries, limits of determination for both analytes and standard deviations of relative residuals were calculated using weighted linear regression templates.

Field samples

Field trials for this study were conducted in Sri Lallang, Malaysia. A mixture of metalaxyl and indoxacarb was applied four times over two weeks, starting from 36 days after seeding. Control samples were collected before the last spray and treated plants were sampled 2 days later. Samples were transferred to Agency's Laboratories, and stored at - 80^{o}C pending sample processing. Samples were comminuted and 30g portions extracted and analysed as described above.

Results

The efficiency of the extraction and clean-up steps of the method for kale were assessed individually by using ^{14}C-chlorpyrifos at 0.05 mg/kg (n=5). The extraction recovery was found to be 70.5%, clean-up recovery 100.7 % and total recovery of the method 71.0 %.

The mean recoveries and RSDs for the three fortification levels are presented in Table 3. All parameters complied with the Codex acceptance criteria. Since the differences in recovery between fortification levels and between analytes were found to be statistically not significant, overall recovery was calculated as the average of 34 recovery data. The overall recovery for the method was 87.3% (RSD = 14.1%). The LOQs, defined here as the lowest calibration level for each analyte, were 0.12 mg/kg for indoxacarb and 0.08 mg/kg for metalaxyl.

Table 3. Performance characteristics of the analytical method used for determining pesticide residues in kale

	Metalaxyl			Indoxacarb		
Fortification level (mg/kg)	**0.5**	**2**	**5.0**	**0.5**	**2**	**5.0**
Average recovery (%)	97.1	73.0	94.0	94.5	88.4	76.8
RSD (%)	5.3	13.1	4.3	11.6	6.4	9.5
n	5	6	6	5	6	6

During the analysis of samples from the field study, quality control of the method performance was achieved by analysing fortified blank samples with each analytical batch. Mean recoveries (n=12) from fortified portions were 88.5% (RSD = 13.4%) and 92.8 (RSD = 13.8%) for metalaxyl and indoxacarb, respectively. These results were within the Codex acceptable range (recovery 70-110%, RSD $\leq$ 15%).

Conclusions

The method developed was validated satisfactorily and proved to be suitable for the analysis of residues of the two target compounds. The results produced for the 130 field-treated samples were evaluated and collated with the results from other collaborating countries conducting similar field trials to produce an estimation of sampling uncertainty (see 4.4). The method protocol has been circulated to TCP counterparts and made available on the Sub-programme web page.

2.1.4. Estimation of sample processing uncertainty for small portion soil analysis

The aim of this study was to verify the applicability and estimate the uncertainty of sample processing and analysis for reduced analytical portions - up to 10 times smaller than the standard procedure. Reduction of the analytical portion size and down-sizing of the analytical procedure allows a significant reduction of cost through reduced solvent consumption and waste disposal. It is also in line with the concept of environmental protection.

A multiresidue method for the analysis of pesticides in soil was previously developed in the Agrochemicals Unit. The method has certain advantages: a) it can be widely applied in any analytical laboratory equipped with a gas chromatograph, and b) it avoids the use of the banned reagent dichloromethane, which was the most commonly used reagent for soil analysis in the past. In this method, the samples are extracted by shaking with acetone and ethyl acetate rather than dichloromethane.

In the present study, a radio-labelled tracer, ^{14}C-chlorpyrifos, was used to determine the typical recovery, precision and robustness of the analytical method. The method was also characterised for a mixture of 10 pesticides by GC-NPD.

Composition of the pesticide mixture:

propazine, promethrin, terbuthrin, terbutilazine, dimethenamid, pendimethalin, oxyfluorfen, chlorfenvinphos, chlorpyriphos ethyl and azinphos ethyl.

Experimental

Method validation.

The method was validated based on 3 levels of fortification (with the lowest level at the LOQ) on 4 types of soil, with 7 replicate analytical portions per sample. Each trial was performed on 3 or 4 occasions.

Procedure

Briefly, 300 g sieved soil was fortified with ^{14}C-chlorpyrifos and allowed to stand for 30 min to allow matrix-analyte interaction. The sample was homogenized with water in a blender and analytical portions were taken equivalent to 2 g and 20 g of dry soil. Ammonium chloride solution (0.2 M) was added – 0.3 ml to the 2 g sample equivalent analytical portion and 2.8 ml to the 20 g portion - and the samples mixed. The analytical portions were extracted on a mechanical shaker for 30 min with 2 ml or 20 ml acetone, then 2 ml or 20 ml ethyl acetate added and the tubes shaken for a further 30 min. The tubes were centrifuged, the supernatants decanted, and portions were taken and added to scintillation cocktail. The ^{14}C activity was measured on a beta-counter.

Samples spiked with a mixture of pesticides were extracted in the same way and analysed by GC-NPD.

Results

Data obtained from the ^{14}C measurements showed low variation between replicates for all analytical portions considered, with RSDs between 0.2 and 2.9 %. The recovery for ^{14}C-chlorpyrifos was between 78.4 and 94.3%, depending on soil characteristics.

The chromatographic determination of pesticides provided a greater variation in recovery, depending on the strength of the interaction between the pesticide and the

soil and/or on the compound chemistry. The overall average recovery established during the validation ranged from 57 to 120%. The variations between replicates (maximum 20%) indicate that homogeneity was achieved in the sample processing. A typical chromatogram of a soil extract is shown in Figure 3. The 10 pesticides extracted were chromatographically resolved and free from interfering peaks.

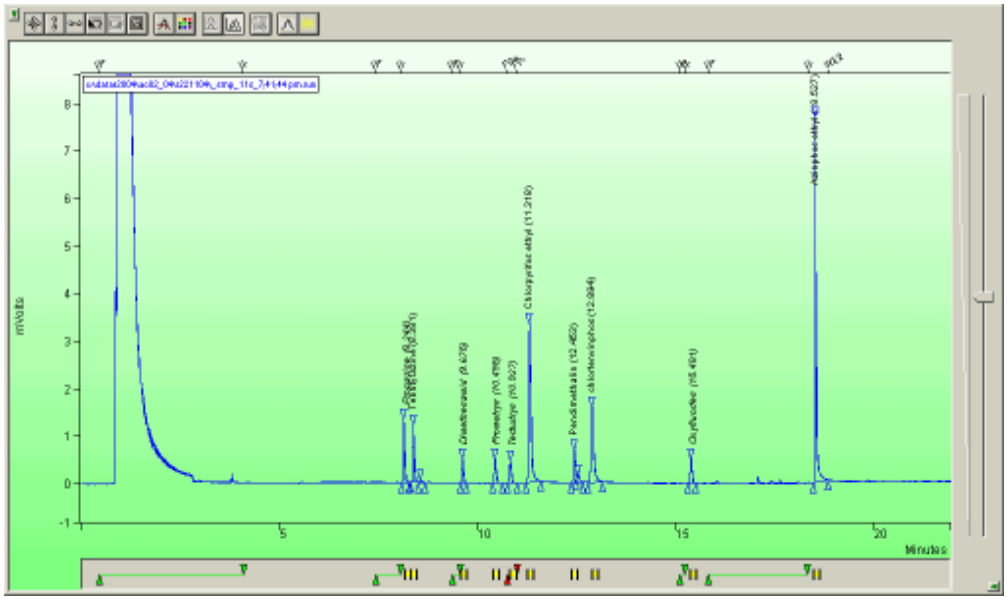

FIGURE 3. Typical chromatogram for 10 pesticides extracted from soil.

Compounds such as chlorpyrifos, propazine, promethrin, terbuthrin and terbutilazine gave higher and reproducible values for all applied doses. The recovery values for compounds such as dimethenamid, pendimethalin or oxyfluorfen were lower (with individual recoveries down to 40 % in some cases), probably due to partial degradation during the analysis.

For the calculation of residues an in-house developed excel template was used. This facilitates the calculation of the standard deviation of the concentrations of up to 150 analytes in 20 samples.

Conclusions

This study has demonstrated that for a properly processed/homogenised sample the analysis of reduced analytical portions of 2-5 g provides reliable results within the parameters established during the method validation phase. As stated above, reducing the analytical portion reduces the cost of the assay, is more environmentally responsible, and the reduced scale can also simplify the sample handling during the laboratory procedure.

The method protocol has been circulated to TCP counterparts and made available on the Sub-programme web page.

2.2. Veterinary Drug Residues

2.2.1. A multiresidue method for sulphonamide residues in animal tissues by high performance liquid chromatography (HPLC)

The sulphonamides are an important class of antibacterial drugs that are widely used worldwide in animal production as therapeutic agents and as growth promoters. The most commonly used sulphonamide drug in veterinary medicine is sulphamethazine (sulphadimidine). Sulphamethazine has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and assigned a Codex maximum residue limit (MRL) of 100 μg/kg in cattle muscle, liver, kidney and fat. In the European Union, the sulphonamides are included in Annex 1 of Council Regulation (EEC) 2377/90, with a MRL of 100 μg/kg for the combined total residues of all substances within the group in a range of matrices. A withdrawal period must be observed after treatment of animals with the drug to ensure that the MRL is not exceeded. Violative residues may be found when the withdrawal period has been insufficient. It has also been shown that violations can occur following the administration of sulphonamide contaminated feedstuffs prior to slaughter or as a result of exposure of unmedicated animals to a sulphonamide contaminated environment. Because of their widespread use, sulphonamide MRL violations resulting in export-import detentions and/or trade disputes are relatively frequent. It is necessary for countries wishing to export animal-derived food products to have in place a reliable method for the determination of sulphonamides in animal tissues. A high performance liquid chromatography (HPLC) method was developed and validated for the analysis of tissue samples for seven of the sulphonamide drugs that are licensed for use in food producing animals. The compounds covered are sulphadiazine (SDZ), sulphathiazole (STZ), sulphapyridine (SPY), sulphamerazine (SMR), sulphamethazine (SMT), sulphamethizole (SMZ) and sulphamethoxy-pyridazine (SMP). In addition to the classical validation data such as specificity, recovery, repeatability, reproducibility and robustness, the validation protocol permitted the estimation of the decision limit (CCα) and the detection capability (CCβ), method performance characteristics defined by the European Commission in Decision 2002/657/EC. CCα represents the value at or above which a sample would be regarded as non-compliant.

Experimental

Tissue samples were minced in a blender and 3 g aliquots taken for analysis. The tissue was extracted with 9 ml ethyl acetate in the presence of anhydrous sodium sulphate and hydrochloric acid. After centrifugation, a 6 ml portion of the extract was evaporated to dryness under a stream of nitrogen and the residue dissolved in methanol/acetic acid/water. The solution was washed with hexane to remove fats, centrifuged and the aqueous/polar portion used for analysis. The extract was analysed, without further clean-up, by isocratic reversed-phase HPLC. The sulphonamides were subjected to post-column derivatisation by reaction with p-dimethyl-aminobenzaldehyde with ultra-violet detection at 450 nm. The post-column reaction was carried out in a simple reaction coil made from a 5 metre length of 0.5 mm ID PEEK HPLC tubing, with the derivatisation reagent delivered by a second isocratic HPLC pump and mixing with the column effluent achieved via a low dead-volume T-piece.

Results

Representative chromatograms of a mixed sulphonamide reference standard, an extract of negative chicken muscle fortified at 100 µg/kg with the seven sulphonamides, and an extract of negative chicken muscle are shown in Figure 4. The analytes are resolved and free from interfering peaks.

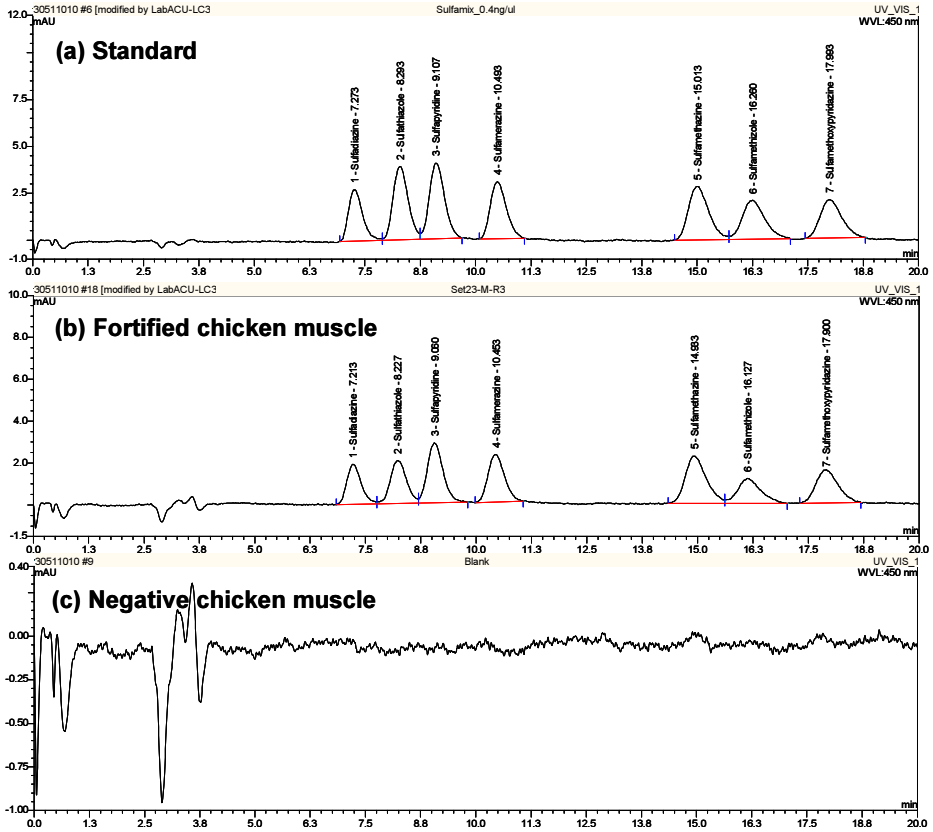

FIGURE 4. Representative chromatograms of (a), a mixed sulphonamide standard; (b), an extract of chicken muscle fortified at 100 µg/kg; and (c), a negative chicken muscle extract.

The method was validated by extracting and analyzing aliquots of negative tissue fortified at 3 levels (50 µg/kg, 100 µg/kg and 150 µg/kg, corresponding to 0.5 x MRL, 1 x MRL and 1.5 x MRL), with 6 replicates at each level. To provide reproducibility data, the validation experiments were repeated on two further days. The recoveries of each compound at each fortification level are illustrated in Figure 5. The reproducibility for each compound, expressed as the RSD of 18 analyses (6 replicates on each of 3 days), is represented by the error bars.

The robustness of the method was checked by having a different analyst perform a single-day validation for each matrix using a different HPLC system. There were no significant differences between analysts or instrument used.

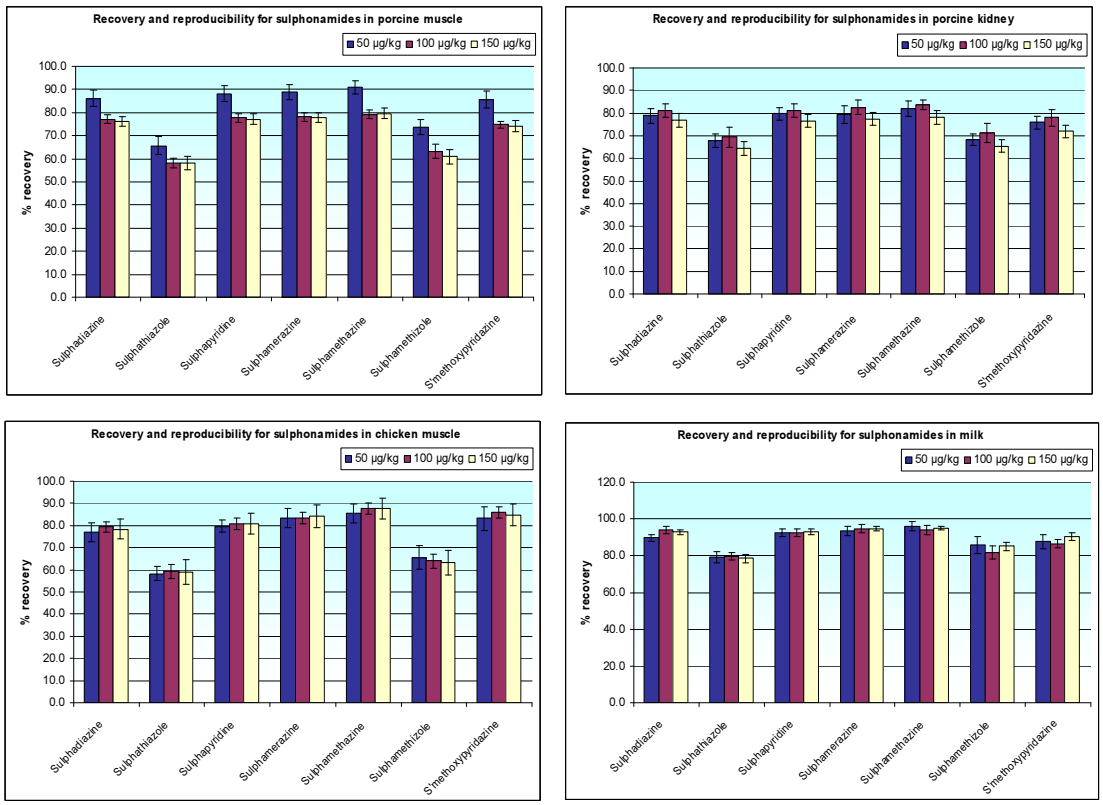

FIGURE 5. Recovery and reproducibility of the method.

CCα and CCβ were calculated by the calibration curve procedure according to ISO 11843, as outlined in European Commission Decision 2002/657/EC. Typical CCα and CCβ values for each matrix are shown in Table 4.

Table 4. Typical decision limit (CCα) and detection capability (CCβ) values for the method, expressed in µg/kg.

Matrix		SDZ	STZ	SPY	SMR	SMT	SMZ	SMP
Chicken muscle	CCα	108.3	110.2	109.2	113.7	111.2	112.9	109.5
	CCβ	118.1	121.3	120.9	128.6	123.3	129.3	119.0
Porcine muscle	CCα	105.4	109.0	105.5	104.1	103.6	110.3	103.7
	CCβ	110.3	117.5	110.4	108.7	108.4	120.2	108.7
Porcine kidney	CCα	109.0	108.5	107.1	109.2	107.5	107.4	108.9
	CCβ	120.2	118.9	116.5	120.9	117.7	117.5	118.8
Milk	CCα	103.1	105.0	104.6	104.2	103.7	107.8	106.4
	CCβ	108.0	110.4	108.9	109.2	108.2	115.0	113.3

Conclusions

A multiresidue method for the determination of residues of seven sulphonamide drugs in animal tissues was developed. The performance of the method was verified in a single laboratory. The method uses a single-phase extraction and avoids the use of solid-phase clean-up which is common to many published methods, thus minimizing both the cost of analysis and the complexity of the procedure. Post-column

derivatisation with p-dimethylaminobenzaldehyde for UV detection confers good selectivity upon the method. The post column derivatisation is performed in a simple apparatus constructed in the laboratory and does not depend upon expensive proprietary post-column reaction products. The method is, therefore, rapid and inexpensive and suitable for application in veterinary drug monitoring laboratories in developing countries.

The validation protocol used satisfies both the classical approach recommended by Codex and other international bodies such as IUPAC, and the approach adopted in the European Union in Decision 2002/657/EC. Many developing country veterinary drug residue monitoring laboratories prefer to adhere to the recommendations of the EU document since it is relatively specific in comparison with other international guidelines. This study shows that a carefully designed validation protocol can provide the data and performance characteristic parameters to comply with both approaches.

The method protocol has been circulated to TCP counterparts and CRP contract holders working on veterinary drug residue monitoring and has been made available on the Sub-programme web page. Two TC Fellows hosted in the Agrochemicals Unit were trained in this method in 2005.

2.2.2. A Multiresidue method for macrocyclic lactone anthelmintic drug residues in animal tissues

The avermectins and milbemycins are closely related 16-membered macrocyclic lactones derived from actinomycetes of the genus *Streptomyces*. They are extremely potent against nematode and arthropod parasites and are widely used throughout the world, including in developing countries, in the treatment of endoparasitic infections and ectoparasitic infestations in cattle, sheep, pigs and horses. The main structural difference between the groups is that the avermectins have a disaccharide moiety attached to the 13-position of the macrocyclic ring, whereas the milbemycins do not.

Each of the drugs consists of a major ($\geq$ 80%) and a minor ($\leq$ 20%) homologue. The major homologues are used as markers for residues of the drugs in edible tissues. Because of their widespread use, there is a potential for residues of the macrocyclic lactones to occur in edible animal tissues. JECFA has evaluated and recommend MRLs for five compounds of this group; eprinomectin (EPR), doramectin (DOR), abamectin (ABA), ivermectin (IVR) and moxidectin (MOX). The MRLs vary widely depending on the compound/species/matrix, from 5 µg/kg for doramectin in porcine muscle to 2000 µg/kg for eprinomectin in bovine liver. It is necessary for analytical laboratories to have suitable methods in place to monitor the concentrations of residues of these compounds in edible tissues to ensure that good agricultural practices in their use are being followed, thus minimizing the development of parasites resistant to the compounds, safeguarding public health, and avoiding export-import disputes.

Several chromatographic methods have been published for the analysis of one or more of this group of compounds. The published HPLC methods generally employ multi-step clean-up procedures, making sample analysis costly, and/or suffer from poor chromatographic selectivity for the more polar compounds which elute relatively quickly from reversed-phase columns. The objective of this study was to develop and validate a multiresidue method with relatively simple clean-up and good

chromatographic selectivity, permitting accurate quantification of all compounds. The method developed was based on the methods of Kennedy *et al*.[2] and Danaher *et al*.[3]

Experimental

A preliminary validation excercise was performed using bovine liver fortified with the 5 compounds. The tissues were extracted and analysed as described below.

The avermectins/milbemycins were extracted from the minced tissue samples with acetonitrile. The extracts were cleaned up by solid phase extraction on C18 SPE cartridges. The analytes were then dehydrated by reaction with trifluoroacetic anhydride with 1-methylimidazole as a nucleophilic catalyst, forming fluorescent derivatives. The derivatives were chromatographed on a reversed-phase column and detected by fluorimetry. The procedure is outlined in Figure 6.

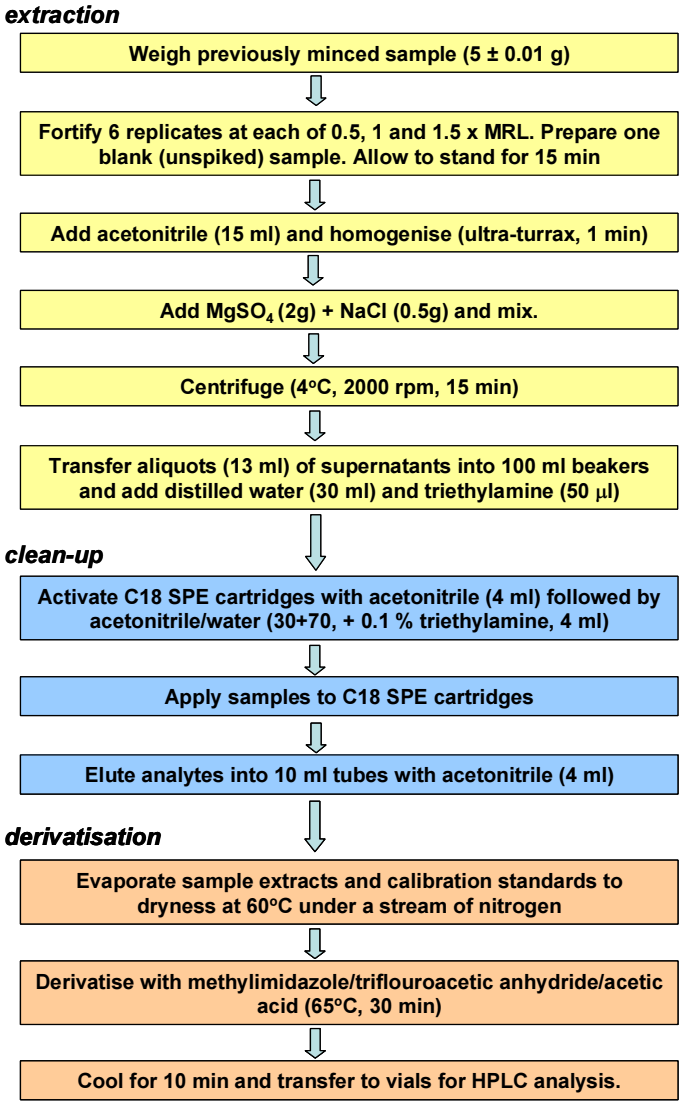

FIGURE 6. Sample preparation procedure for avermectins/milbemycins.

[2] Kennedy, D.G., Cannavan, A., Hewitt, S.A., Rice, D.A. and Blanchflower, W.J. (2001). Determination of ivermectin residues in the tissues of Atlantic salmon (*Salmo salar*) using HPLC with fluorescence detection..Food Additives and Contaminants, **10**, 579-584.

[3] Danaher, M., O'Keefe, M., Glennon, J.D. and Howells L. (2001). Development and optimization of an improved derivatisation procedure for the determination of avermectins and milbemycins in bovine liver. Analyst, **126**, 576-580.

Results

Chromatograms of an extract of blank liver extract, a mixed standard solution and an extract of a blank liver fortified at 100 µg/kg are shown in Figure 7. The analytes were well chromatographically resolved and there were no interfering peaks at the retention times of the target analytes.

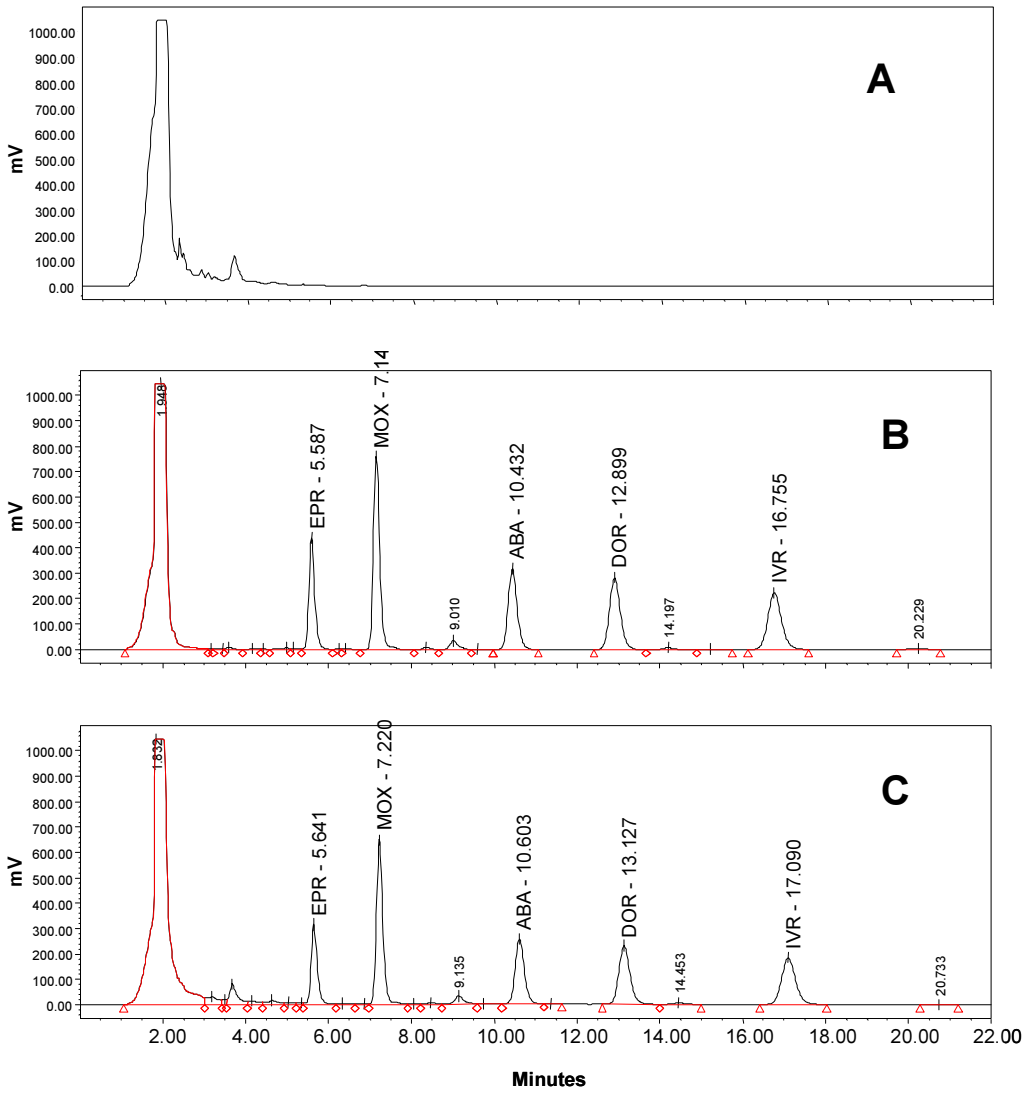

FIGURE 7. Typical chromatograms for (A), an extract of a negative liver sample; (B), a mixed standard solution; and (C), an extract of a negative liver sample fortified before extraction at 100 µg/kg.

For the preliminary validation of the method, six replicates of blank liver fortified at each of three levels, 50, 100 and 150 µg/kg were extracted and analysed. The results are shown in Table 5.

It was found that liver samples that had been repeatedly frozen and thawed gave a much dirtier background at the start of the chromatographic run, which at times could interfere with the eprinomectin peak. Freeze-thaw cycles should, therefore, be kept to a minimum, both for validation or control purposes and for samples taken in the field. A second clean-up step with alumina, as described by Danaher *et al.*[2], may be applied

when it is necessary to analyse samples that produce such a background. This procedure will be investigated in the future.

Table 5. Repeatability of the method for bovine liver.

Spike (µg/kg)		EPR	MOX	ABA	DOR	IVR
50	rec (%)	67.5	74.9	73.7	75.8	78.5
	RSD (%)	11.0	7.2	6.7	6.0	5.4
	n	6.0	6.0	6.0	6.0	6.0
100	rec (%)	79.0	81.1	77.5	79.4	82.3
	RSD (%)	9.8	9.7	10.0	8.8	7.3
	n	6.0	6.0	6.0	6.0	6.0
150	rec (%)	84.1	92.8	86.6	87.2	88.4
	RSD (%)	3.6	2.6	2.9	2.5	1.9
	n	6.0	6.0	6.0	6.0	6.0
overall	rec (%)	76.9	82.9	79.3	80.8	83.1
	RSD (%)	12.2	11.2	9.6	8.4	7.0
	n	18	18	18	18	18

Conclusions

The method has undergone a preliminary validation on a single day and with a single matrix. The results demonstrate good chromatographic resolution of the compounds and acceptable recovery values.

Two Fellows were trained in the method during its development and initial validation in 2005.

The method requires further validation to estimate the applicability, precision and robustness for other matrices, but the preliminary data indicate that this would be a useful method for regulatory and food control laboratories in developing countries.

2.3. Quality control of trypanocidal drugs

African trypanosomiasis is a severe animal disease and is fatal if left untreated. The conventional and most prominent method to combat trypanosomiasis is by chemotherapy. Every year some 35 million doses of trypanocides are administered to domestic ruminants. Several reports indicate the widespread phenomenon of counterfeit and poor quality drugs based on the phenanthridinium trypanocide, isometamidium chloride (ISM) in sub-Saharan Africa. The use of poor quality trypanocides has severe implications for both animal health and food safety, posing problems with unspecified, unwanted chemicals and their metabolites and residues in the food chain. In addition, the use of poor quality trypanocides induces trypanosome resistance, an already widespread phenomenon.

There are currently no internationally agreed standards for the quality of trypanocides. Documented specifications and pharmacopoeial monographies for veterinary trypanocides are either lacking or inaccurate, and there are no references to recommended method(s) of analysis for ISM or for residues of the drug in foods.

In 2003, the Animal Health Service of the FAO and the International Federation for Animal Health (IFAH) developed a joint concept note on QA/QC of trypanocides, with the main objective to pursue internationally and scientifically agreed standards and protocols for QA/QC of trypanocides. The specific objectives include definition of the requirements of analytical quality assurance, establishment of good laboratory practices for chemical analysis, and transfer of the methodologies and technology to laboratories in Africa. Initially, it is proposed to support two regional reference laboratories, one in west Africa and one in the east. The Agrochemicals Unit and the Department of Pharmaceutical Sciences, Strathclyde Institute for Biomedical Sciences were selected as partners for the technical aspects of the project. The laboratory work for this project commenced in the fourth quarter of 2005 in the Agrochemicals Unit.

An accurate, specific and well-characterized method for the determination of ISM in the presence of its manufacturing and degradation products is required both for quality control of the manufacturing process and for authenticity testing of products on the market. In addition, the development of a specific analytical method would form the basis of a method for the analysis of residues of ISM in animal tissues and would facilitate the interpretation of pharmacological and biological data.

A simple, fast and inexpensive HPLC method for the separation and quantification of ISM in the presence of manufacturing and degradation impurities by reverse-phase-HPLC has been described by Tettey *et al*.[4] The objectives of this study were to modify and validate the analytical method to enable fast, reproducible assays.

Experimental

A reference ISM standard containing the following isomers and related compounds was supplied by Merial, France:

M&B 4180 (ISM)	58.6 %
M&B 4250	13.7 %
M&B 38897	13.3 %
M&B 4596	8.1 %
Homidium	0.26 %

A stock standard solution (500 µg/ml of M&B 4180) and dilute standard solutions (20 µg/ml, 50 µg/ml and 100 µg/ml of M&B 4180) were prepared by dilution of the reference standard in acetonitrile:water (25+75).

Samples of ISM products were diluted to give solutions within the reference standard range, according to the stated ISM content of the product. Stock sample solutions were prepared by dissolution of sample powder in Milli Q water. Dilute solutions for analysis were prepared by dilution in acetonitrile:water (25+75).

Chromatography was performed using a Phenomenex Gemini C18 column (150 x 4.6 mm, 5 µm particle size) with a mobile phase of 100 mM ammonium acetate buffer, pH 4.0 : acetonitrile; (75+25). The injection volume was 20 µl and detection was by UV at 320 nm. No temperature control was used.

[4] Tettey, J.N.A., Skellern, G.G., Midgley, J.M. and Grant, M.H. (1998). HPTLC and HPLC determination of isometamidium in the presence of its manufacturing and degradation impurities. Journal of Pharmaceutical and Biomedical Analysis, **17,** 713-718.

Results

Chromatography

Four of the 5 compounds in the reference standard were detected by the method. M&B 4596, stated to be present at 8.1% of the total, was not detected.

The organic component of the mobile phase was increased from 20% to 25% and the previously reported flow rate on a 4.6 mm, 5 µm column of 1 ml/min was increased to 1.5 ml/min to increase the efficiency of the assay. Using these conditions, the run time was decreased from 20 minutes to 9 minutes, whilst maintaining baseline separation of the compounds of interest, as shown in Figure 8.

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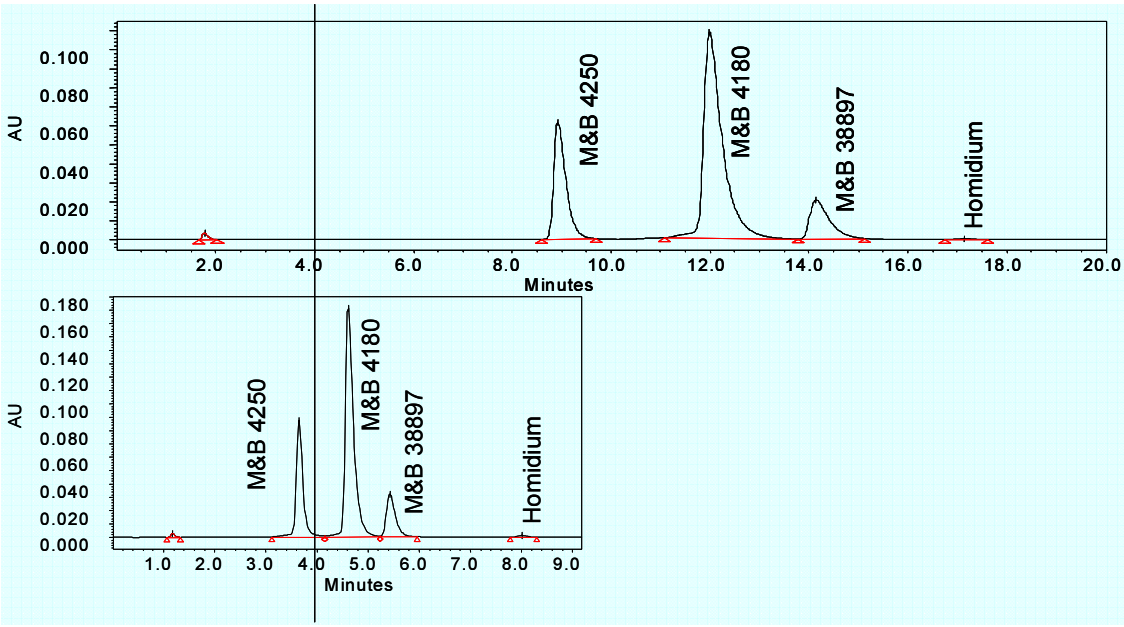


FIGURE 8. Increase of efficiency of the method by increasing the flow rate and organic content of mobile phase. Top chromatogram: mobile phase - acetonitrile/buffer (20+80), flow 1 ml/min, bottom chromatogram: mobile phase - acetonitrile/buffer (25+75), flow 1.5 ml/min.

Linearity of the method

The linearity of detector response for M&B 4250, M&B 4180, M&B 38897 and homidium was determined by evaluation of the correlation coefficient of the calibration curves acquired from replicate (n = 5) analysis of a set of calibration solutions of analytes at concentration levels of M&B 4180 of 20, 40, 60, 80 and 100 µg/ml. The linearity parameters are presented in Table 6.

Table 6. Linearity of the analytical method for isometamidium and related substances

Analyte	Range (µg/ml)	*n*	Slope	Intercept	Correlation coefficient (r^2)
M&B 4250	5 – 25	5	60655 ± 1196	11473 ± 12615	0.9998 ± 0.0002
M&B 4180	20 – 100	5	36895 ± 546	32283 ± 38210	0.9999 ± 0.0002
M&B 38897	5 – 25	5	33801 ± 609	9995 ± 7658	0.9999 ± 0.0002
Homidium	0.1 - 0.5	4	38426 ± 5522	625 ± 289	0.9908 ± 0.0151

Precision of the method

The repeatability of the method was determined by analysis of five replicates of the same sample. Intra-day precision was established by assays of three separately prepared sample solutions from the same testing material, a commercial sample of isometamidium (Veridium; Ceva Sante Animale, France). The four compounds of interest were quantified using a calibration curve acquired from replicate (n = 4) analyses of standard solutions with concentrations of M&B 4180 of 20, 50 and 100 µg/ml.

To determine inter-day precision, replicates (n = 5) of freshly prepared sample solutions were analysed on three different days, using freshly prepared standard solutions for quantification as described. The relative standard deviations (RSDs) of the analyte concentrations were determined. The precision of the method is summarised in Table 7.

Table 7. Repeatability, intra- and inter-day assay precision for isometamidium and related substances

Compound	precision (RSD)		
	Repeatability	Intra-day	Inter-day
M&B 4250	0.68 %	2.16%	3.14 %
M&B 4180	0.19 %	1.47 %	2.48 %
M&B 38897	0.44 %	1.74 %	3.39 %

Limits of detection and quantitation

The limit of detection, using the dilution protocol described above, was established by determining the concentration of a dilute solution of M&B 4180 that gave a signal to noise ratio of 3:1, while limit of quantitation was established by determining the concentration of a dilute solution of M&B 4180 that gave a signal to noise ratio of 9:1 and a RSD of peak areas obtained of < 5 %.

The limit of detection of M&B 4180 was 60 ng/ml, while the limit of quantitation was 120 ng/ml.

Specificity and ruggedness

The specificity of the method was confirmed by comparing the peak retention values of chromatographic peaks of M&B 4250, M&B 4180, M&B 38897 and homidium acquired from injections of single standard solutions with those obtained from injections of a standard solution of a compound mixture and injections of commercial samples. The chromatographic peaks of the four compounds were clearly resolved with M&B 4180 and M&B 38897 as the critical peak pair (Rs > 2.5).

Ruggedness was assessed by determining the resolution between the critical peak pair M&B 4180 and M&B 38897 when different system parameters were slightly modified. With a small change in molarity and pH of the CH_3COONH_4 buffer solution used to prepare the mobile phase, some shift in retention times was observed, as shown in Figure 9, but the resolution obtained remained > 2.0.

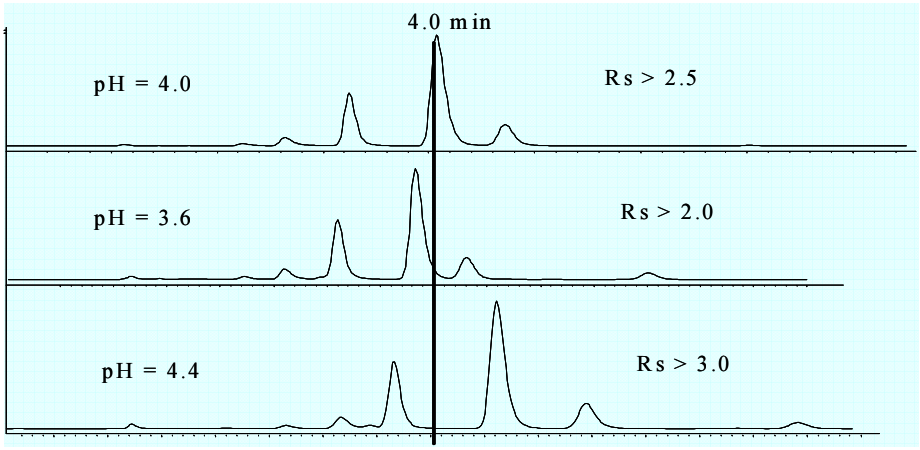


FIGURE 9. Determination of ruggedness with modified mobile phases

Analysis of commercial samples

In this study all standard and sample solutions were freshly prepared prior to analysis as described above. Different brands of products containing ISM were analysed, the principal component (M&B 4180) was quantified and the relative amounts of related substances were determined. The results obtained are shown in Table 8. Figure 10 illustrates the power of the method to compare samples with the same declared amount of ISM.

Table 8. Analysis of commercial samples of isometamidium

Sample	content (% w/w)			
	M&B 4250	M&B 4180	M&B 38897	Homidium
Product 1	10.4	62.9	10.2	-
Product 2	7.3	19.6	7.7	0.2
Product 3	13.4	58.4	9.5	0.4
Product 4	2.1	30.7	2.7	-
Product 5	14	58.1	9.1	-
Product 6	11.0	71.4	12.6	0.4

Conclusions

Successful resolution of the isomers of isometamidium chloride M&B 4180, M&B 4250, M&B 38897 and homidium was achieved. Since the concentration of the ISM-related substance, homidium, in the analytical reference standard as well as in the sample substances was below the limit of quantification, the validation does not cover the quantification of this component. If quantification of homidium is required, it is expected that the method would be applicable using a lower dilution factor. This would require further validation. Similarly, further work would be required to quantify the M&B 4596 isomer of ISM. However, the important requirements in terms of quality control of ISM-based trypanocidal drugs are quantitation of ISM (M&B 4180) and resolution of ISM and its isomer, M&B 38897.

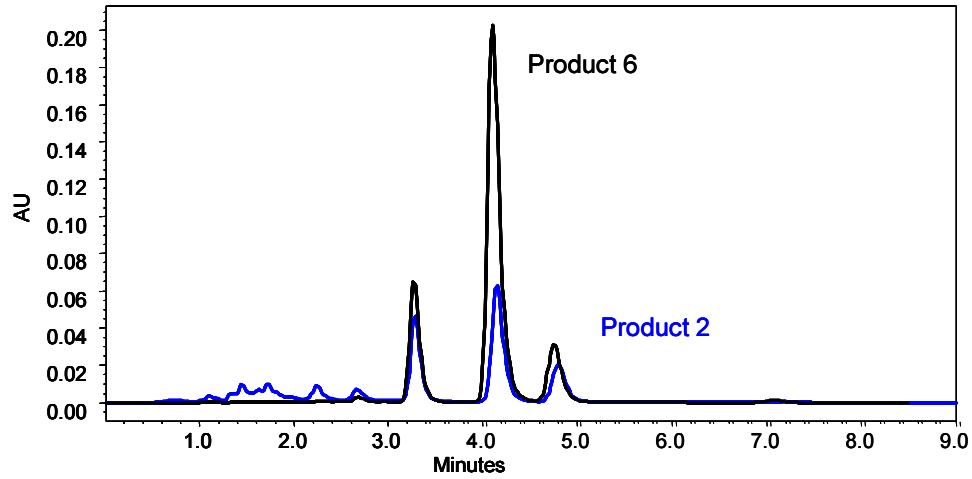


FIGURE 10. Sample chromatograms from products with the same declared amount of ISM

The developed method is specific, precise, robust and rugged and a linear relationship between concentration and the detector signal for the compounds of interest has been demonstrated. The method was successfully employed for the quality evaluation of several generic formulations of isometamidium chloride.

The method employs standard isocratic HPLC instrumentation with UV detection and widely available reagents and chemicals and is suitable for transfer to the proposed reference laboratories in Africa and to regulatory laboratories in other developing countries. A study report will be presented at both the Programme Against African Trypanosomiasis (PAAT) Committee Meeting and the 16th Session of the Codex Committee for Residues of Veterinary Drugs in Foods (CCRVDF) in 2006.

2.4. Estimation of the uncertainty of sample processing for the analysis of fumonisin B_1 in maize

According to the definitions introduced by Hill and Reynolds[5], sample preparation is the procedure used, if required, to convert the laboratory sample into the analytical sample by removal of parts (soil, stones, bones, etc.) not to be analysed. Sample processing is the procedure (e.g. cutting, grinding, mixing) used to make the analytical sample acceptably homogeneous with respect to the analyte distribution, prior to removal of the analytical portion. In the case of maize samples, the processing procedure includes sub-dividing and grinding the maize kernels.

Sample processing is very important in mycotoxins analysis because of the uneven distribution of the toxins. A reliable and representative result can only be achieved if the variations in the analyte levels in the commodity are eliminated through effective homogenization of a properly collected bulk sample. In other words, efforts must be made to ensure both an appropriate/representative sampling step and an efficient processing step.

As an example, the FAO sampling plan for aflatoxins in peanuts[6] recommends taking a 20 kg bulk sample and analysing a representative 100 g portion. This size of bulk

[5] Hill, A.R.C. and Reynolds, S.L. (1999). Guidelines for in-house validation of analytical methods for pesticide residues in food and animal feeds. Analyst, **124**, 953-958

[6] FAO (1993), FAO Food and nutrition paper, 55.

sample, however, is difficult to process at the laboratory. The aim of this study was to estimate the uncertainty of sub-sampling for fumonisin B_1 determination in maize samples. Fumonisin B_1 is the most common of the fumonisin mycotoxins and has recognised adverse health effects on animals and suspected carcinogenic potential in humans. Maize is the major commodity affected by this group of toxins.

Experimental

Grinding a bulk sample of 20 kg is very impractical in the laboratory, so the applicability and efficiency of a multi stage procedure was tested as an alternative.

The procedure consisted of:

- thorough mixing of 20 kg naturally contaminated maize grains in a concrete mixer,
- subdivision into 2 x 10 kg portions, using a sample divider
- further mixing and sub-division into 5 x 2 kg portions
- further mixing and sub-division into 2 x 1 kg portions
- grinding of 1 kg portion
- thorough mixing and sub-dividing into 25 g and 150 g test portions with a sample divider
- extraction of the test portion and HPLC analysis of the maize extract

The multistage procedure is presented in Figure 11.

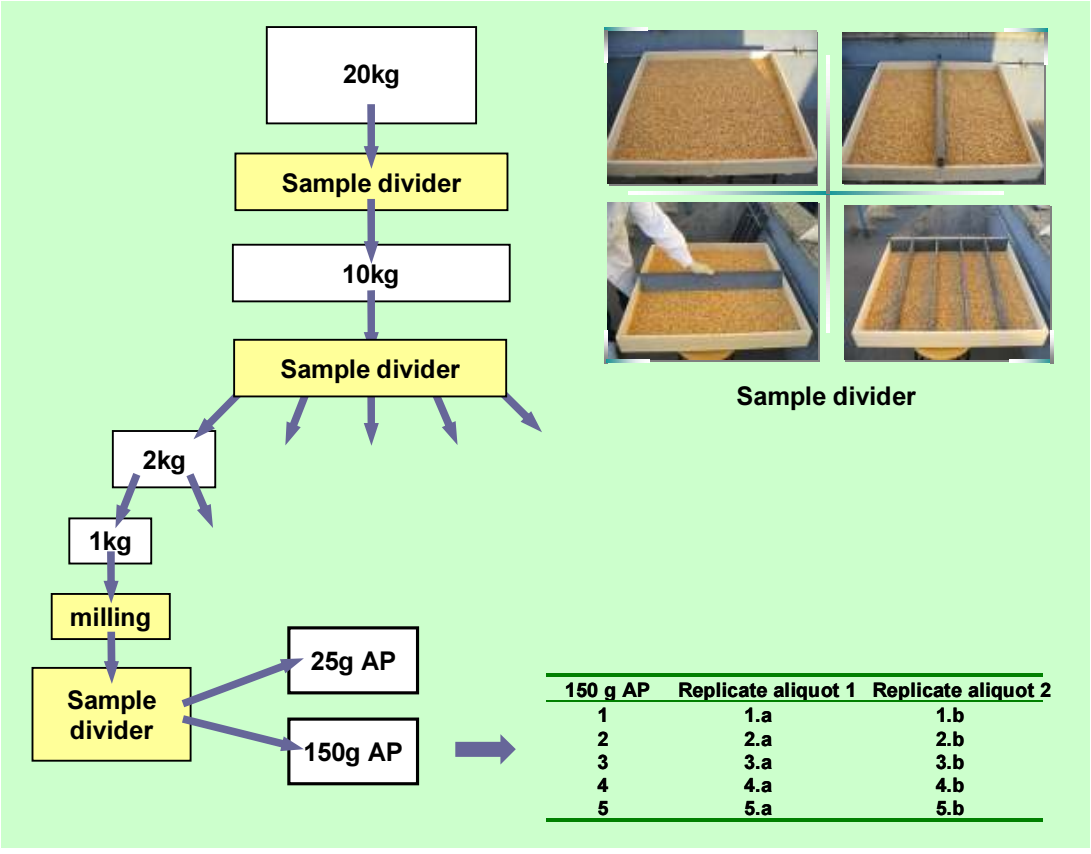


FIGURE 11: Scheme of sample processing and withdrawal of analytical portions

The analysis of fumonisin B_1 in the naturally contaminated maize sample was carried out by the analytical procedure described by Visconti[7] based on extraction with methanol/ water 3:1 v/v, cleanup on strong anion exchange (SAX) cartridges and reversed-phase HPLC analysis with fluorescence detection of the fumonisin B_1 after derivatization with ortho-phthalaldehyde (OPA).

Statistical treatment of data

Each step of the analytical procedure contributes to the total uncertainty of the result (expressed as relative standard deviation or CV) according to the following equation elaborated by Ambrus[8]

$$CV_R = \sqrt{CV_S^2 + CV_{SP}^2 + CV_A^2} \quad \text{(equ. 1)}$$

Where CV_R is the uncertainty of result, CV_S the uncertainty of sampling, CV_{Sp} the uncertainty of sample (preparation) processing and CV_A the uncertainty of analysis. The analytical phase may include, for instance, the extraction, cleanup, evaporation, derivatisation and instrumental determination.

CV_L is the uncertainty of the laboratory phase (combined uncertainty of sample processing and analysis) and is described by equation:

$$CV_L = \sqrt{CV_{SP}^2 + CV_A^2} \quad \text{(equ.2)}$$

From equation (2) the following formula for the uncertainty of sample processing can be derived:

$$CV_{SP} = \sqrt{CV_L^2 - CV_A^2} \quad \text{(equ.3)}$$

Results and conclusions

The results of the analyses were statistically elaborated and used to establish the within laboratory reproducibility CV_A value. In the present study, CV_A was calculated using the following formula (Miller et al.[9]):

$$CV_A = \sqrt{\frac{\sum d^2}{2n}} \quad \text{(equ.4)}$$

where *d* is the relative difference between replicates, (d= (R1-R2)/ mean residue), and *n* is the number of duplicate test portions measured. CV_A includes the contributions of the operations performed from the cleanup to the final HPLC determination, and it provides information on the average reproducibility of these procedures for the concentration range covered.

From the results of the study, CV_A=10.2%. This is the best estimate available for the reproducibility of the measurements and can be used for the estimation of the combined uncertainty of the results.

[7] Visconti, A. and Doko, B. (1994). Survey of fumonisin production by Fusarium isolated from cereals in Europe. Journal of the . AOAC International, **77**, 546-550.

[8] Ambrus, A. (2004). Reliability of measurement of pesticide residues in food. Accreditation and Quality Assurance, **9,** 288-304.

[9] Miller, J.N. and Ambrus, A. (2002). Manual on basic statistics. FAO/IAEA Training and Reference Centre for Food and Pesticide Control

The combined variability of the laboratory phase (CV_L) of the average fumonisin B_1 content of the 150 g test portions withdrawn from 1 kg sub-samples was calculated to be 21.5%.

The uncertainty of sample processing (CV_{SP}) for the 150 g analytical portions, estimated using equation (3), was 20.3% and is representative of the sample processing uncertainty at the 1 kg level.

Since 25 g analytical portions represent the usual amount analysed for mycotoxins in the analytical laboratory, the homogeneity of the milled sample at the 25 g level was tested. Ten analytical portions of 25 g were withdrawn from each 1 kg portion and analysed. The uncertainty of sample processing (CV_{SP}) of 25 g test portions, was estimated by applying equation (3) to the replicate fumonisin B_1 measurement of 25 g test portions, where CV_L= 0.37 and CV_A=0.102. CV_{SP} was calculated to be 0.36 (36%). This represents the estimate of the combined uncertainty of sample processing of 25 g analytical portion withdrawn from 1 kg of milled maize sub-sampled from 2 kg whole grain maize, which in turn is obtained by subdividing 10 kg maize into 2 kg portions using a sample divider.

The average residue content of 150 g analytical portion was compared to the fumonisin B_1 content of the corresponding 25 g analytical portion using a paired t-test. The test revealed no significant difference between the two sets of data. However the average fumonisin B_1 content for the 150 g APs was a better estimate for the 1 kg portion as it was derived from five duplicate (5 x 2) measurements.

For a given commodity, different mills will provide different degrees of particle size. The smaller the particle size, the more comminuted the sample material and the more homogenous the distribution of the mycotoxin. Since the efficiency of sample processing may vary from sample to sample, and from equipment to equipment, its regular control should be included in the internal quality control programme of the laboratory.

In conclusion, the results of this study provide a strong warning signal that the grinding and mixing of 1 kg sub-samples and the subsequent sub-dividing and analysis of 25 g analytical portion should be carried out with utmost care. The efficiency of sample processing may be improved by employing strategies such as double processing and use of the slurry technique.

The results of this study were presented at the BCPC International Congress and Exhibition, Glasgow, Scotland, 31 Oct -2 Nov 2005

2.5. A lysimeter experiment to investigate the influence of climate change on the environmental behaviour of s-metolachlor in a soil-plant-water-system

This is a collaborative project between the Agrochemicals Unit and the Department of Environmental Research of the Austrian Research Centre (ARC) at Seibersdorf. The lysimeter facility of the ARC is a useful tool which can be used to investigate the behaviour of pesticides within the soil-plant-water-system. By using undisturbed soil monoliths, lysimeter experiments yield valuable data for site-specific hydrological models and the leaching behaviour of substances under conditions closely approximating those in the field. The current study aims to investigate how the

climatic changes anticipated by many scientists, in particular an increase of heavy rain events and a slight increase of average temperatures, will affect the environmental behaviour of the pesticide s-metolachlor. The study will provide information on whether such climatic changes will have a significant influence on the leaching behaviour, degradation velocity and plant uptake of the selected substance. Possible effects on the soil water balance will also be investigated.

In May 2005, ARC staff implemented an experimental setup designed to simulate climate change on the monoliths of the ARC lysimeter facility. Soy was planted and sprayed with s-metolachlor. Leachate water and interim harvests of non-mature plants were collected. The main harvest of plant material took place in September and the plant material was prepared for chemical analysis. From November 2005 the lysimeter soil was sampled to investigate the depth distribution of s-metolachlor in the soil profile. In the Agrochemicals Unit, analytical methods for quantifying the target analyte in the required matrices were selected, adapted and tested. The methods will be validated and analyses performed by a student employed by ARC under the supervision of Unit staff in 2006.

2.6. Liquid chromatography – tandem mass spectrometry

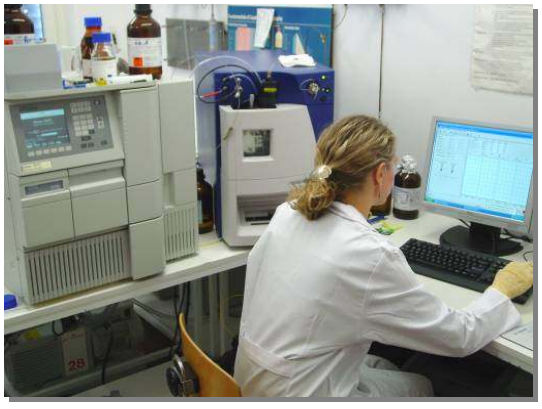
To meet the requirements for the international trade of food products, specific quality assurance measures must be applied with regard to the identification and quantitation of chemical residues (veterinary drugs, growth promoters and pesticides). This is reflected in the current EU legislation on the performance of analytical methods and the interpretation of results (Commission Decision 2002/657/EC), which specifies the use of mass-spectrometry for confirmatory analytical methods. The Codex guidelines on the establishment of a regulatory programme for veterinary drug residues (CAC/GL/16/1993) are also under revision and current drafts also indicate the need for mass spectrometry for confirmatory purposes. The Agrochemicals Unit has already provided input to Codex on the elaboration of identification criteria for mass spectrometric analysis of pesticide residues (see 3.1).

The technique most relevant to these issues is liquid chromatography-mass spectrometry (LC-MS) and, more specifically, -tandem mass spectrometry (LC-MSMS). This affords the sensitivity required to unequivocally identify and quantify analytes at the very low Maximum Residue Levels (MRL) for legitimately used drugs or Minimum Required Performance Levels (MRPL) for illegally used drugs required in regulatory residue analysis. Although LC-MSMS is a relatively recent development, it is now a standard technique in laboratories in the developed world and is becoming common also in developing country laboratories. For example, data from a questionnaire circulated to the six developing countries represented at the FAO/WHO Workshop on residues of veterinary drugs without ADI/MRL, Bangkok, 24-26 August 2004, indicated that mass spectrometry was being used for residues analysis in all six countries (Argentina, Vietnam, Indonesia, Malaysia, Philippines, Thailand) and LC-MS or -MSMS in all except the Philippines, which had plans install the equipment. Some of these countries are equipped with many such instruments; Thailand, for example, reported that >10 instruments were present in Government laboratories and 8 in private laboratories. Many of the instruments in these countries were installed as a direct response to trade difficulties encountered due to the detection in food products of residues of antimicrobial substances such as nitrofurans

and chloramphenicol, which are banned for use in food-producing animals in the major trading blocks due to their potentially harmful effects on human health. LC-MSMS is currently the only technique available with the required sensitivity and specificity/selectivity to detect these compounds at the levels required. Measures put in place by the countries and blocks such as the EU, USA, Canada, Australia and Japan due to the detection of such residues and/or deficiencies in residues monitoring programmes include mandatory testing of every export shipment at the expense of the exporting country and rejection or disposal of suspect shipments. These measures have recently been applied to Brazil, Thailand, India and various other East Asian countries. In 2002 the EU imposed a complete ban on imports of animal derived foods from China – a market worth approximately US$400 million in the year 2000.

In addition to the application of LC-MSMS to address these problems with veterinary drug residues, numerous methods using LC-MSMS have also been published for pesticide residues, including the benzimidazoles, carbamates, *N*-methylcarbamates and organophosphorous compounds. A recent example is a multiresidue method for phenyl-*N*-methylcarbamates in surface water in Tunisia. The technique is also applicable to a wide range of other contaminants and is a powerful research tool.

Feedback from the three FAO/IAEA regional training courses on screening and confirmatory methodologies for veterinary drug residues held in 2003-2004 indicated a real need for training in the application of LC-MS and LC-MSMS to residue analysis and the transfer of methods using these techniques. There have also been many direct enquiries to the Agency regarding training in this field, both for veterinary drug residues and pesticide residues. The requests fall into two broad categories; (i), countries who have invested in the instrumentation but have had insufficient training in its use or have been trained only in one method by the manufacturers and who wish to expand their expertise to fulfil the potential of this powerful analytical platform (for example the countries listed above), and (ii), countries that wish to invest in the technology to protect their export markets but have little knowledge of the techniques and no experienced personnel to provide guidance (for example, South Africa, Botswana, Uruguay, Panama, Chile, China).



To meet these needs and enhance the training and research capabilities of the Agrochemicals Unit, a Waters Quattro Micro triple-quadrupole LC-MSMS instrument was installed in September 2005. Unit staff are currently familiarising themselves with the equipment and will undergo training in its application to residues and contaminants analysis in 2006. The instrument will be used for:

- Training courses on the operation, maintenance and application of this powerful analytical platform to the problems relevant to Member States
- Development and validation of methods for transfer to Member States
- Applied research and sample analysis
- Elaboration of protocols and guidelines for QA/QC using this technology.

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)

Project Officer A. Cannavan

The third RCM for the CRP on the development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries was held in Natal, Brazil, from 11-15 April 2005. The meeting was attended by ten Research Contract Holders, a second representative of the research group of the host country, two Research Agreement Holders, two Technical Contract Holders and the Scientific Secretary.

The objectives of this CRP are to identify, adapt or develop and validate screening and confirmatory methods for the control of veterinary drug residues applicable in developing countries and to elaborate quality assurance and quality control procedures and sampling plans. Presentations on the objectives and progress of the project and on the INFOCRIS database and the e-learning modules and courses available and under development by the Joint FAO/IAEA Programme were presented by the Scientific Secretary, Dr. Andrew Cannavan. Background and review papers were presented by Agreement and Technical Contract holders.

The progress of each research group was presented and the results were discussed and used to formulate individual work plans for the final phase of the CRP. The main focus of the project to date has been on method development or adaptation and evaluation. Progress has again been made in this phase of the project on the development of immunoassay methods for chloramphenicol. Many of the problems encountered in the first phase of the CRP with reagent production have been overcome and good quality antibodies and conjugates are now being produced in several laboratories. Some stability problems have been addressed, for example by a change of format from direct to indirect ELISA. Antibody production and maturation has been studied in various species. The overall objectives for the final phase of the project for those groups working on ELISA (Indonesia, Kenya, Korea, Sri Lanka, Barbados, Malta, Cyprus) are to develop test protocols using the reagents produced, validate the methods and compare results with test kits and confirmatory methods. This will require further investigation into methods to stabilize reagents, further antibody purification and characterization, optimization and validation of test protocols, and transfer of methods for comparison in partner laboratories and against commercially available kits.

The scope of the RIA method employed in Brazil has been successfully broadened to include a second beta-agonist, mabuterol. Full validation data were presented at the RCM. Future work aims to extend the scope further to include a range of beta-agonistic compounds.

Confirmatory LC-MSMS methods for the nitrofuran metabolites and chloramphenicol in meat have been developed by the research group in Argentina. Confirmatory methods are, therefore, now available in laboratories in South America and in Asia (Thailand). The work plan for the final phase includes optimization of extraction procedures for different matrices and full validation of the methods.

A full set of reagents and protocols for their optimisation in a novel ^{125}I-radioimmunoassay (RIA) for chloramphenicol was developed in the first phase of the project by the technical contract holders and transferred to a contract holder. No progress has been made on the further elaboration of this promising method and it has been decided to transfer this work to the research group in Brazil.

Progress was also reported on the development of HPLC methods for nitrofuran metabolites. Nitro-phenyl derivatives of the metabolites of the four main nitrofuran drugs have been produced in Namibia and applied in an HPLC-UV method. The derivatives will be characterized in the Agrochemicals Unit, Seibersdorf. A suitable fluorescent derivative of AOZ (furazolidone metabolite) has been selected by the researchers in South Africa. Further work for both groups will include elaboration of sample extraction and clean up protocols and validation of the methods.

A number of investigations into the possible natural occurrence of chloramphenicol in poultry litter have been carried out by the researchers in Thailand. The results presented were interesting and further experiments were included in the work plan to complete this work.

The objectives of the CRP also include the elaboration of Quality Assurance and Quality Control procedures and the sharing of practical advice on the implementation of sampling plans. To help address these objectives, a detailed presentation on the preparation of a "QA Handbook for the Implementation of the German National Residue Control Plan in Bavaria" was presented by Dr. Lange. The practical steps outlined in this presentation can be adapted and used by the CRP participants in their respective countries. Dr. Elliott also presented a lecture on method validation for immunoassays. It was agreed to use the protocol provided by Dr. Elliott, with necessary adaptations to suit local conditions, as the standard for all research groups. Dr. Montes Niño presented a lecture on Conformity Assessment, which outlined the history and development of various standards and explained the role of certification and accreditation bodies.

Conclusions of the meeting

The work in the second phase of this CRP has built upon the progress reported from the first phase, resulting in good quality immunoassay reagents, confirmatory methods and a number of validated methods. The final phase of the project will include adoption of a harmonized validation protocol for screening assays, completion of method development and validation and transfer of methods between partners.

The final RCM will be held in approximately 18 months. The venue has yet to be selected.

A summary of the results of this project to date was presented by the Unit Head as a poster at the 2nd International Symposium on Recent Advances in Food Analysis, 2-4 November 2005, Prague, Czech Republic.

2.7.2. Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale (D5.20.35)

Project Officer B.M. Maestroni

A new CRP on "Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale". (D52035) was approved in 2005, with Ms. Britt Maestroni as the Project Officer.

The specific objective of this CRP is to establish laboratory capacity and indicators to assess the effectiveness of good agricultural practices at catchment scale.

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. To ensure and sustain high crop yields, fertilizers and pesticides are widely applied and their use has steadily increased over the years. Inappropriate use of pesticides and other agricultural inputs has caused discharges of pollutants (pesticides, fertilizers, etc.) to surface and/or groundwater. These can have adverse effects on food safety, human health and the environment and consequently also affect countries' economies and trade.

This CRP integrates risk assessment tools and targeted analytical monitoring as a cost-effective option for developing countries to identify specific water pollutants, their sources and occurrences. Nuclear and related techniques will assist in generating CRP 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.

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). The TRC was established in 1998 and 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, veterinary drug residues analysis and sample preparation. 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 programme gain experience which they can use to improve conditions in their home countries, 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. Since the inception of the TRC, several such courses have been held.

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, 12 Sept – 7 Oct 2005

This workshop was designed to provide a basic understanding of the principles of laboratory quality management systems and the quality control procedures necessary to apply such systems, and comprised lectures, discussion and feedback sessions, and practical exercises in the laboratory. Twenty three participants were selected from well over one hundred applications received, and with

the inclusion of three additional scientists who were undergoing Fellowship training in the Agrochemicals Unit, there were a total of twenty six participants from twenty four developing countries. The participants, although varying to some degree in experience and background, proved to be well informed and enthusiastic. An initiative employed for this workshop was the inclusion during the first few days of some team-building and presentation skills presentations and exercises. This approach proved to be very successful and resulted in good interaction and information exchange between the participants from the start of the workshop.

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. 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 Uruguay, Brazil, USA, Hungary, OECD and FAO. Participants also gave individual presentations on their laboratories and group presentations on food safety issues and on group exercises undertaken during the workshop. The practical sessions included demonstrations of sample preparation, extraction and clean-up techniques and group sessions on TLC, HPLC, GC and GC-MS methods. The emphasis during the practical sessions was on identifying, discussing and demonstrating quality control issues such as system suitability checks, recovery samples and control charts.

The workshop also included presentations on HPLC and GC troubleshooting, provided by Agilent personnel, and a visit to the AGES laboratories in Vienna, where workshop participants viewed the procedures in place for sample reception, processing and analysis in an accredited national laboratory.

The final morning of the workshop was taken up by a presentation and round-table discussion session on the role of the analytical laboratory in the implementation of good agricultural practices and food safety and trade, which included representatives of FAO, USDA, AgroVet and ILAU GmbH.

Feedback on the workshop 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 2006. The “train the trainers” approach has proved successful in that courses on QA/QC and GAP have already been held in Cambodia, Thailand and Uganda by participants in the workshop, using material provided at the workshop.

3.2. Workshop on Food Safety Requirements for the International Market: Strategies for Residues Programmes

This workshop, which was conducted in Spanish, was held in Santiago, Chile, from 18-22 October 2004. The aim was to build awareness of the major elements involved in the implementation of residue control programmes. The workshop was attended by 51 delegates drawn from academia, residue control laboratories, and regulatory and administrative bodies (25 direct participants representing 15 Latin American countries, 19 observers, 3 special attendees and 4 foreign experts). A series of presentations was given by invited speakers from Latin America, the EU and the USA. Topics included the design of residue control programmes, strategies for the prevention and control of veterinary drug residues in food, licensing of veterinary

medicines, pharmacokinetic models for the assessment of withdrawal periods, the role of reference laboratories in the EU, validation of analytical methods, quality control in analytical laboratories, the production and use of reference materials, proficiency testing protocols, interpretation of relevant legislation and guidelines, EU requirements for third countries and the role of the EU Food and veterinary Office in evaluating residue control systems. The attendees actively participated through questions and contributions during plenary and discussion sessions. It was apparent that there is currently a wide spectrum of competence in the areas of residue control and veterinary medicine regulatory systems in South and Central America and it was concluded that this workshop would help address the major issues.

Workshop on Food Safety Requirements for the International Market

3.3. Fellows and Scientific Visitors

Two Fellows completed their time with the Agrochemicals Unit at the end of October 2005, Lawal Shitta-Bey (Nigeria), who spent 5 months in the Unit and was trained in maintenance and troubleshooting of laboratory instrumentation and Jasna Dokic (Serbia/Montenegro), who trained in HPLC methods for veterinary drug residues and participated in method development and validation studies for sulphonamides and avermectins. Another Fellow, Ms. Ana Topolovic (Serbia/Montenegro) left the Unit at the end of November, having trained for 3 months on the application of HPLC, with veterinary drug and pesticide residue methods as examples. In addition to this, seven of the participants in the training workshop held at Seibersdorf in September/October (see 3.1) were supported through TC Fellowships. The Unit also accommodated one Scientific Visitor, Ms. Siriphan Sukmak (Thailand), 10-14 October. Contact has been maintained with the Fellows and the Scientific Visitor and they have indicated that their time in the Unit was useful and enjoyable and they were satisfied with the training provided.

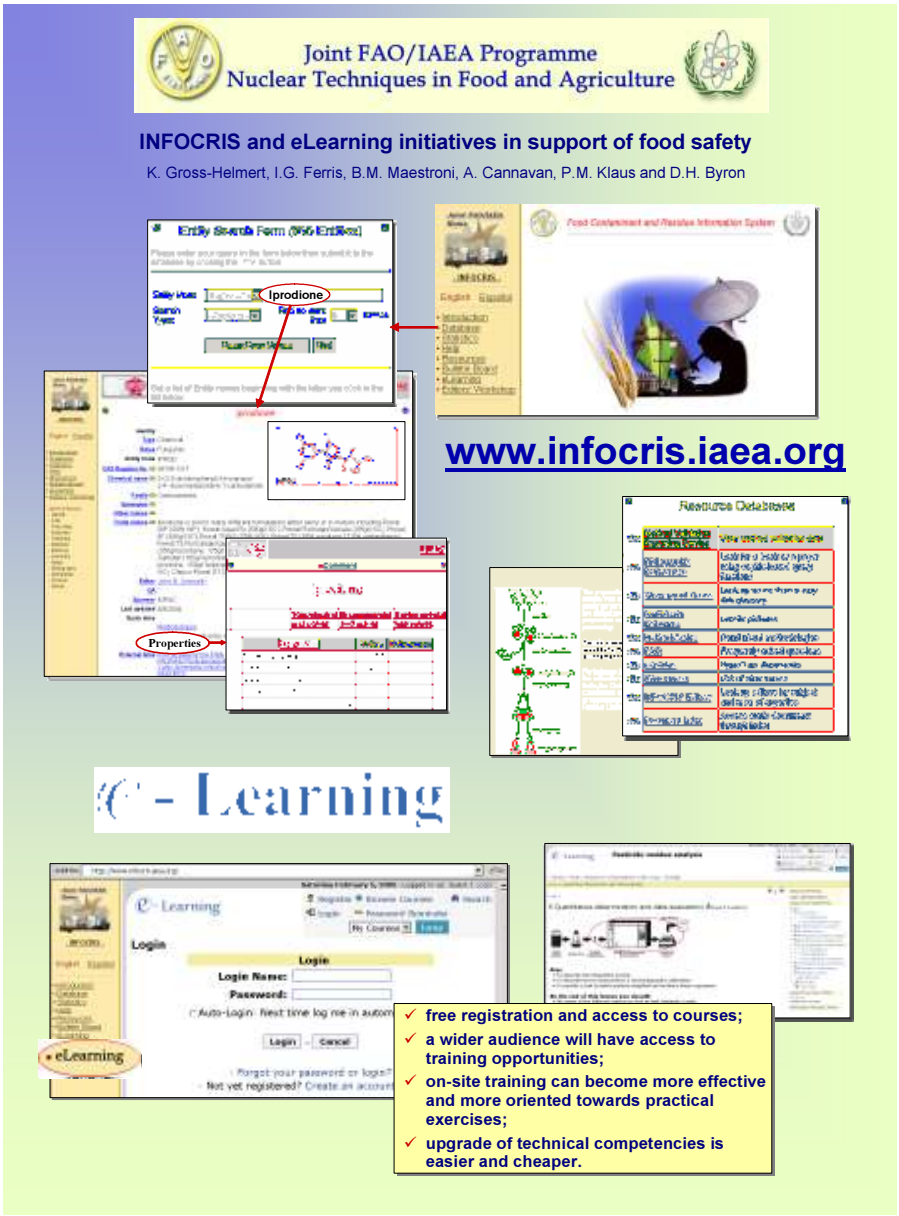
3.4. eLearning

Agrochemicals Unit staff continued to work in collaboration with the Food and Environmental Section to support the Sub-programme's database and eLearning initiatives. Work began on updating the TRC training material CD, which contains

material developed and collected since 1997. This also required peer-review in some cases to ensure the quality and relevance of the material. 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, was commenced.

Agrochemicals Unit staff provided a major input to the development of the "Laboratory Prerequisites 1" course which became available in 2005. This course was a prerequisite for participation in the workshop described above (3.1) and this and other courses and modules will ensure that workshop/training course participants have the basic knowledge to benefit from the training provided in the laboratory.

A poster on the Food Contaminant and Residue Information System (INFOCRIS, www.infocris.iaea.org) and the eLearning system was presented by the Unit Head at the 2nd International Symposium on Recent Advances in Food Analysis, 2-4 November 2005, Prague, Czech Republic.



3.5. Agrochemicals Unit staff training

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

- Training on Waters "Empower" software for HPLC by Waters personnel, ACU, 21 March 2005- (M. Schweikert, B. Maestroni, N. Rathor)
- Training on Dionex HPLC system and Chromeleon software by Dionex personnel, 23 March 2005 (M. Dabalus, N. Rathor, B. Maestroni, P. Aysal, M. Schweikert, P. Klaus)
- Refresher course on Radiation Protection, Seibersdorf, 19 April 2005 (N. Rathor)
- Sigma-Aldrich- seminar on food contaminants analysis by GC and GC/MS, Vienna, 17 June 2005 (M. Schweikert)
- Basic training on LC-MS/MS operation and hardware by Waters personnel, ACU, 2 September 2005 (M. Dabalus, N. Rathor, B. Maestroni, P. Klaus)
- Waters Technologies Symposium, Vienna, 10 November 2005 (N. Rathor)
- Laboratory Lead Auditor training course for ISO/IEC 17025, Excel Partnership, Seibersdorf 14-18 November 2005, (A. Cannavan)
- LC/MS seminar by Brückner, Vienna, 6 December 2005 (N. Rathor)

4. GUIDELINES AND STANDARDS

4.1. Adoption of Guidelines on the use of mass spectrometry (MS) for the identification, confirmation and quantitative determination of residues

Working papers were drafted by the Agrochemicals Unit following a request by the Codex Committee on Pesticide Residues (CCPR) and the Guidelines were further elaborated in a consultants' meeting in Vienna, 22-26 March 2004 and submitted to CCPR. The proposed draft guidelines were forwarded by CCPR (37th Session) and were adopted (Alinorm 05/28/24; para.228 and Appendix X) by the Codex Alimentarius Commission at its Twenty Eighth Session at FAO Headquarters in Rome, Italy, 4-9July 2005.

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

The proposed guidelines were drafted at the consultants' meeting referred to in 4.1 and submitted to CCPR. At its Twenty Eighth Session, The Commission adopted the proposed draft Guidelines at Step 5 and advanced them to Step 6 with the understanding that some concerns expressed by the Delegation of China would be considered by the next Session of CCPR.

4.3. Residues of veterinary drugs without ADI/MRL

The *Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL* met in Bangkok, Thailand from 24th to 26th August 2004, in order to provide FAO, WHO and Codex with a first analysis of disruptions in food trade that occurred in 2001/2002. The disruptions were caused by the detection of trace amounts of chloramphenicol and nitrofurans in animal products. The experts were asked to identify the scientific, technical and regulatory problems related to these findings and to identify appropriate follow-up steps.

Mr. A. Cannavan presented a working paper on "Capacity Building for Veterinary Drug Residue Monitoring in Developing Countries" and participated in a working group on capacity building. A draft report of the workshop was adopted in the final session. The main conclusions reached were that the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) should identify substances whose residues are known to be highly toxic and develop a policy to prevent their use in food-producing animals; the performance requirements of analytical methods for the detection of such substances should be harmonized; and a harmonized policy for the evaluation of consignments of foods containing such residues should be established. The guidelines produced should in no way condone the use of these substances, for example, through the introduction of tolerance limits. For substances that have been evaluated and are legally used in many countries but which have no Codex MRL, CCRVDF should develop a more comprehensive approach to allow completion of risk assessments by JECFA and the introduction of fixed or temporary MRLs within 10 years.

The main recommendation of relevance to FAO/IAEA was:

"Some developing countries require specific advice and technical assistance on:

> The theory and practice concerning the application of appropriate analytical methods. This may be directed towards screening technologies and/or towards more sophisticated confirmatory technologies, as dictated by the needs of the individual country. This should be addressed by the Joint FAO/IAEA (Programme) at IAEA."

Since the workshop was convened, working papers have been circulated and this matter will be discussed at the 16th Session of the Codex Committee on Residues of Veterinary Drugs in Food in May 2006, in which Mr. Cannavan will participate.

4.4. Development of Sampling Guidelines for Pesticide Residues and Strengthening Capacity to Introduce Certification Systems

Work on this project (PFL /INT/856/PFL – 111740) was completed in 2005 and a technical report was prepared by the former Agrochemicals Unit Head, Mr. A. Ambrus, as a consultant.

A study was designed to evaluate the variability of pesticide residues in crops. Field trials were carried out in 13 countries with 13 commodities including 3 small fruits, 5 large crops, 2 medium/large crops and 3 leafy vegetables. The 25 pesticide active ingredients applied represented the dicarboximide (3), organophosphorus (8), synthetic pyrethroids (5), phthalimides (2), organochlorine (1) and other types of pesticides (6). The crop pesticide combinations amounted to 91, from which 6116 samples were analysed resulting in 11353 residue data. The methods and results presented in section 2.1.2 and 2.1.3 of this report formed part of this study.

The trials represented regular agricultural practice prevailing in different parts of the world. They were performed on commercial fields cultivated by local farmers according to their normal practice. The samples were taken by trained personnel following detailed sampling plans, and analysed with validated methods of known and acceptable performance parameters.

The studies carried out within this project provided sufficient residue data for the estimation of sampling uncertainty. The estimated typical uncertainty values for various types of crops can be used for designing statistically based sampling plans for various purposes.

The data base on residues in crop units and distribution of unit weights can be used to refine the short term exposure assessment methodologies. Further research and detailed analysis of the available vast data base will be required to fully utilise the unique information generated within the project.

From the data collected, the combined uncertainty of residue data, taking into account the probable uncertainties of sample processing and analysis, can be expected to be between 25 and 40% depending on the type of sample. This relatively large uncertainty should be taken into account when decisions are made based on the results of measurements. The results obtained from this study are in good agreement with the typical variability factor of 3 accepted by the JMPR in 2004. They do not support the conclusion drawn by the EFSA indicating the need for larger variability factors, such as 4 or 5.

The specific objectives of the project were to improve reliability of sampling and the capabilities of food control laboratories to work according to international quality

standards; and to facilitate acceptance of Codex MRLs supported with reliable acute intake estimates. The objectives were achieved.

After the publication of the results in peer reviewed scientific journals and dissemination through the Codex Committee on Pesticide Residues and other appropriate channels, these data will also provide a very valuable source of information for the scientific community.

4.5. Sampling manual for fumonisins

In 2005, the analysis of 2000 primary samples of maize for fumonisin B_1 was completed in the Agrochemicals Unit. The aims of the study for which these samples were analysed were to investigate the distribution of fumonisin B_1 in primary maize samples and to determine fumonisin B_1 levels and distribution across agro-ecological zones in Nigeria. Under the experimental design of the study, 20 primary samples of maize of 100g each were taken from 100 lots collected from five different agro-ecological regions in Nigeria. The analysis of the data is ongoing. The results will be collated by a consultant in 2006 and published by The Agency as a sampling manual for fumonisins.

Fusarium infected maize contaminated with fumonisin B_1

5. SELECTED COUNTRY ACHIEVEMENTS

The training and methodologies provided by the Agrochemicals Unit have been put to good use in many countries. The "train the trainers" approach taken for the workshops and training courses organised by the Unit has also had considerable impact on awareness-building and expansion of the knowledge-base in Member States. Some specific examples are summarised below;

Thailand – One of our training workshop participants, Ms. Leepipatboon, planned and implemented training courses on method validation and instrumental analysis, including sample preparation techniques, in Thailand using the experience and material from the FAO/IAEA workshop for the Thai trainees.

Nigeria - During 2003 the agrochemicals unit trained 6 fellows from NAFDAC, Nigeria, for a total period of 9 months. The topics covered included mycotoxins analysis (aflatoxins and ochratoxins by TLC, and fumonisins by HPLC) and quality assurance and quality control measures in analytical laboratories. The fellows also took part in a 2 weeks workshop on "advanced instrumental analytical techniques". The feedback we received after 2-3 years was that the NAFDAC mycotoxins laboratory is implementing a range of testing and is on track to attain accreditation. Participation of the laboratory in the FAPAS proficiency testing scheme was successful.

Dr. S. O. Amuda-Giwa, Deputy Director of the Veterinary Drugs and Pesticides – Drug Registration Division of Nigeria organised and implemented workshops in six different regions of Nigeria following the FAO/IAEA workshop "Strengthening Capacities for Implementing Codex Standards, Guidelines and the Recommended International Codes of Practice for the Control of the Use of Veterinary Drugs". The reported outcome was that awareness of veterinary drug residue control was growing, with a consequent enhancement of health protection for consumers.

Korea - Dr. Seong-Wan Son, Director of the Residue Chemistry and Toxicology Division of the Ministry of Agriculture and Forestry, who also attended the above workshop, has reported that his agency has received ISO 17025 accreditation for veterinary drug and pesticide residues and other contaminants in December 2005.

Sri Lanka – A laboratory participating in TRC training courses and workshops, a TCP and a CRP (D3.20.22) have implemented an antibiotic residue screening programme which is used by the major exporting food processors, testing of raw product being included as a critical control point in their HACCP plans.

Cambodia- Dr. Khy Vibolbotra used the experience gained and training materials provided at the 2005 training workshop at Seibersdorf to present a course on Good Agricultural Practice and the promotion of quality control of agricultural products (total 45 hours lectures) at the Royal Universtiy of Agriculture in Phnom Penh. The course was presented to students employed by the Ministries of Agriculture, Rural Development and Water Resource Management.

Uganda – Department of Agriculture officials were briefed by the participant in the 2005 training workshop and the workshop material is being used to enhance a scheme to build awareness and promote good agricultural practices and quality assurance for producers through interaction with laboratories and extension services.

Turkey – A participant in a Seibersdorf training course has published a paper[10] on the estimation of sample processing uncertainty for residues in cucumber in Turkey using the ^{14}C-chlorpyriphos technique developed in the Agrochemicals Unit.

Participants in FAO/IAEA training courses on veterinary drug residue analysis have used the experience and knowledge gained to implement routine antibiotic screening tests in **Mongolia**, **Sri Lanka**, **Yemen,** the **Seychelles**, the **Philippines, Barbados and Malta** to protect international trade, the tourism industry and the health of local consumers**.**

Following discussions at a TRC workshop, the counterparts in a TCP in **Mongolia** drafted an agreement with a workshop participant and a CRP (D3.20.22) contract holder, both from **South Korea,** resulting in technology transfer for residue monitoring from Korea to Mongolia. The Korea International Cooperation Agency (KOICA) provided training and equipment estimated at $400,000 to the IAEA TCP counterpart laboratory. Cooperation between the institutes involved is ongoing.

Contract holders from **Brazil** and **South Africa** (CRP D3.20.22) have implemented a technology transfer agreement resulting in the enhancement of the capabilities of laboratories and the effectiveness of food safety programmes in both countries.

Malaysia – Personnel from the Veterinary Public Health Laboratory in Kuala Lumpur who were trained on TRC training courses and through a TCP are now training TC Fellows from other projects in East Asia.

Philippines – Personnel trained at IAEA are now implementing pesticide testing in the National Pesticide Analytical Laboratory, which enables the testing and certification of more than 3000 samples per year of mango and okra for export to Japan.

Training provided by TRC training courses and TCPs has assisted regulatory residues laboratories in **Malaysia, South Africa, Namibia** and **Cyprus** to attain, or extend the scope of, accreditation under ISO/IEC 17025.

[10] Tiryaki, O and Baysoyu, D. (2006). Estimation of sample processing uncertainty for chlorpyriphos residue in cucumber. Accreditation and Quality Assurance, 10, 550-553.

6. APPENDICES

6.1. Publications

Gross-Helmert, K., Ferris, I.G., **Maestroni, B.M., Cannavan, A., Klaus, P.M.** and Byron, D.H. International Food Contaminant and Residue Information System and eLearning initiatives in support of food safety. Food Additives and Contaminants, *submitted*.

Tiryaki, O. and **Aysal, P.** (2005). Applicability of TLC in multiresidue methods for the determination of pesticides in wheat grain. Bulletin of Environmental Contamination and Toxicology 75, 1143-1149.

Maestroni, B., Bettaglio, M., Ambrus, A. and **Rathor, N.** (2005). Estimation of the uncertainty of sample processing for the analysis of fumonisin B_1 in maize. Proceedings of the BCPC International Congress and Exhibition - Crop Science and Technology 2005, Glasgow, Scotland, 31 Oct -2 Nov 2005.

Cannavan, A. (2005). The development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries. Book of abstracts of the 2nd International Symposium on Recent Advances in Food Analysis, 2-4 November 2005, Prague, Czech Republic, 170.

Cannavan, A., Ferris, I.G., **Maestroni, B.M.**, Gross-Helmert, K and Byron, D.H. (2005). E-learning and electronic database initiatives in support of food safety. Book of abstracts of the 2nd International Symposium on Recent Advances in Food Analysis, 2-4 November 2005, Prague, Czech Republic, 63.

Doko, M.B., **Maestroni, B.**, **Rathor, N.**, **Cannavan, A**. and Ogunbanwo, B.F. (2005) Performance Study for Mycotoxin Analysis in Agricultural Commodities. Poster presentation at the 119th AOAC Annual Meeting and Exposition, 11-15 Sept 2005. Abstract in final programme.

Ambrus Á, Fuzesi I, Lantos J, Korsos I, Szathmary M and Hatfaludi T. (2005). Application of TLC for confirmation and screening of pesticide residues in fruits, vegetables, and cereal grains: Part 2. Repeatability and reproducibility of Rf and MDQ values. Journal of Environmental Science and Health B 40 (4), 485-511

Ambrus, Á., Füzesi, I., Susán, M., Dobi, D., Lantos, J., Zakar, F., Korsós, I., Oláh, J., Beke B. B. and 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: Pesticides, Food Contaminants, and Agricultural Wastes 40 (2), 297 – 339

Cannavan, A. (2005). Capacity building for veterinary drug residue monitoring programmes in developing countries. In "Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL", Bangkok, 24-26 August 2004, pp 43 – 47. http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/008/y5723e/y5723e00.htm. ISSN: 9251052255

Cannavan, A. and Elliott, C.T. (2004). The implementation of veterinary drug residues monitoring programmes in developing countries. Conference on Residues of Veterinary Drugs in Food, van Ginkel, L.A. and Bergwerff, A.A., eds., Proceedings of the Euroresidue V Conference, Noordwijkerhout, The Netherlands, 2004, 151-158.

6.2. Travel

A. Cannavan

2nd International Symposium on Recent Advances in Food Analysis, 2-4 November 2005, Prague, Czech Republic. Poster presentations:

"The development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries." Cannavan, A.

"E-learning and electronic database initiatives in support of food safety." Cannavan, A., Ferris, I. G., Maestroni, B. M., Gross-Helmert, K and Byron, D. H.

First meeting of the Advisory Board for the EU Framework 6 Integrated Project "New Technologies to Screen Multiple Chemical Contaminants in Foods (Biocop)", 6-8 November 2005, Prague, Czech Republic.

Waters Food Safety Summit, Hainan, China, 17-18 October 2005. Keynote address "Veterinary drug residues – implications in Asia"

Waters Food Safety Summit, Singapore, 20-21 October 2005. Keynote address "Veterinary drug residues – implications in Asia"

Waters Food Safety Summit, Manchester, UK, 19-21 April 2005. Invited participant.

Third RCM of the CRP "The Development of Effective Strategies for Monitoring Veterinary Drug Residues in Animals and Animal Products in Developing Countries", Natal, Brazil, 11-15 April 2005. Scientific Secretary.

Twenty sixth meeting of the Codex Committee on Methods of Analysis and Sampling, Budapest, Hungary, 4-7 April 2005. Represent the Agency and present a conference room document.

B. M. Maestroni

InterAgency Games 2005, Crete, 21-25 Apr 2005. Gold medal in swimming.

The BCPC International Congress and Exhibition - Crop Science and Technology 2005, Glasgow, Scotland, 31 Oct -2 Nov 2005. Presentation of a paper, "Estimation of the uncertainty of sample processing for the analysis of Fumonisin B_1 in maize".

Travel to Panama, TCP PAN5/015 "Quality Assurance in Pesticide Residue Analysis for Agriculture Production", 12-14 Dec 2005. Project status evaluation and work plan revision.

6.3. Fellows/Scientific Visitors

Fellows	**TC code**	**Dates**	Training
SHITTA-BEY, Mr. L.	NIR/03012	16/03/05 – 19/06/05	Instrument troubleshooting and maintenance
SHITTA-BEY, Mr. L.	NIR/03012	05/09/05 – 07/10/05	Laboratory QA/QC
DJUKIC, Ms. J.	SCG/04005	01/09/05 – 31/10/05	Veterinary drug residues
TOPOLOVIC, Ms. A.	SCG/04012	01/09/05 – 30/11/05	HPLC techniques
CHECA ORREGO, Ms. B	PAN/05010	12/09/05 – 07/10/05	Training workshop on QA/QC in residue labs
KUCUKU, Ms. M.	ALB/05009	12/09/05 – 07/10/05	Training workshop on QA/QC in residue labs
LEEPIPATIBOON, Ms. N.	THA/05046	12/09/05 – 07/10/05	Training workshop on QA/QC in residue labs
BAYSOYU, Ms. D.	TUR/05019	12/09/05 – 07/10/05	Training workshop on QA/QC in residue labs
RAHMAN, Mr. M.M.	BGD/05003	12/09/05 – 07/10/05	Training workshop on QA/QC in residue labs
MU MU AYE, Ms. A.	MYA/05009	12/09/05 – 07/10/05	Training workshop on QA/QC in residue labs
PASTOR CHAVEZ, Ms. Y.	ECU/05027P	12/09/05 – 07/10/05	Training workshop on QA/QC in residue labs
Scientific Visitor			
SUKMAK, Ms. S.	THA/04007V	10/10/05 – 14/10/05	Pesticide residue MRLs

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**