Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Food and Environmental Protection Laboratory

Activities Report

2009

EXECUTIVE SUMMARY

The aims of the Food and Environmental Protection Laboratory (FEPL), as a component of the Food and Environmental Protection Subprogramme, 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 focus of the Laboratory's work is on improving member state laboratory and regulatory practices and methodologies. The main areas of activity in pursuit of these objectives are applied research and development, technology transfer and support for the development of international standards and guidelines.

The Laboratory's work currently centers on food and environmental contaminants such as residues of veterinary drugs and pesticides, and mycotoxins. Control of these hazards requires a holistic approach addressing the entire food production chain, which depends on the application of guidelines to minimize risks and incorporates feedback mechanisms to ensure the effectiveness of the controls. An essential element of this holistic approach to food safety is the ability to trace food products to their source in order to facilitate corrective actions when contamination is detected. Traceability and authenticity will become a major focus of the Subprogramme in the 2010-11 biennium and beyond, and several activities and collaborations were initiated in 2009 to facilitate future research on product and contaminant traceability using stable isotope techniques.

Support for counterpart laboratories through the development, validation and transfer of analytical methods and procedures included a multiresidue isotope dilution assay for residues of anthelmintics in meat, developed under the EU 6th Framework Project "ProSafeBeef"and transferred to a laboratory in Brazil as a training hub for Latin America, methods for organochlorines and pyrethroids in meat and natural alkaloid toxins in cereals to support regulatory programmes in member states, and methods for contaminants in soil and water to support risk assessment and provide linkages between environmental contaminants and practices used in food production. The Laboratory also collaborated with the Insect Pest Control Laboratoryt in the development of an analytical method for antiviral drugs to support research into mass rearing of tsetse flies for the sterile insect technique (SIT).

The laboratory interacted with a range of collaborators in its research and development activities, including with the EU projects BioCop, CONffIDENCE and ProSafeBeef and with the International Association for Cereal Science and Technology (ICC). Work was continued in a project with the World Health Organization (WHO) and the World Organization for Animal Health (OIE) to compile a global survey of laboratory quality systems. A project on the quality control of trypanocidal drugs in sub-Saharan Africa with FAO, the International Federation for Animal Health (IFAH), Strathclyde University, UNIDO and the EU was also continued. FEPL staff gave presentations at five major international conferences on food safety research and at two symposia on food safety and traceability.

A train-the-trainers workshop on screening/post-screening techniques for veterinary drug residues was held at Seibersdorf, with assistance from the Austrian Agency for Food Safety and Health (AGES) and TC supported training courses were held in the IAEA Collaborating Centre in Costa Rica, in Panama, and in the United Arab Emirates. Five TC fellows, one scientific visitor and two interns were also trained in the FEPL during 2009. In all, 28 individuals were trained in the FEPL for a total of approximately 30 man-months. Immediate feedback, continued correspondence with trainees and participants and follow-up workshops, courses and seminars held by participants in their home countries indicated that the training activities were highly successful.

CONTENTS

1. PROGRAMMATIC AND UNIT OBJECTIVES

The overall objective of Subprogramme 2.1.3, Improving Food and Environmental Safety, for the 2008-09 biennium is to enhance member state capabilities in the use of irradiation for sanitary and phytosanitary purposes and to improve food safety and quality, protection of the environment and international trade through the application of nuclear and related analytical techniques, and including preparedness and response to nuclear emergencies. Within this context, the main objective of the Agrochemicals Unit for the biennium is to improve member state laboratory practices and methodologies to enhance food quality and safety.

The use of agrochemicals such as veterinary drugs and pesticides is vital for modern agricultural production. However, the presence of residues of these substances and other contaminants in foods and the environment often presents risks to human health and may create barriers to international trade in agricultural commodities. Governments increasingly rely on testing throughout the food production chain, a "farm-to-fork" approach, to ensure the safety and wholesomeness of foods. This involves the control of contaminants at their source through food safety systems that include good agricultural and production practices, post-harvest or post-slaughter monitoring, traceability mechanisms, and the examination of environmental factors leading to contamination of agricultural commodities. These measures emphasize the application of agrochemicals in amounts and timing appropriate to agronomic, food safety and environmental requirements, the use of veterinary pharmaceuticals according to good veterinary and production practices, and the observation of appropriate withholding periods.

Laboratories and trained staff capable of establishing reliable sampling and analytical regimes for quantifying potential hazards within specific production practices or in products are indispensable for informing decision making and risk management and for the improvement of food safety and environmental protection. Analytical capabilities and quality assurance and quality control procedures must be strengthened to facilitate the important role of the laboratory in providing indicators of the effectiveness of food safety controls in production and processing practices within farm-to-fork food safety systems, in improving those systems, and in import/export certification.

In order to achieve the Subprogramme and Unit objectives, close linkages are fostered with other Subprogramme areas, especially those dealing with insect pest management and crop and animal production. This holistic approach will help governments to ensure the safety and quality of foods throughout the food production chain, based on the use of appropriate production technologies, feedback mechanisms and procedures, including those developed through international standardization bodies.

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3. RESEARCH AND DEVELOPMENT ACTIVITIES

3.1. A multi-residue isotope-dilution liquid chromatography-tandem mass spectrometry method to support risk assessment for anthelmintic drug residues in beef

Drug use in food-producing animals can lead to residues in the edible products that are harmful to consumers. Other problems can also arise from the use of veterinary drugs in animal production. For example, the uncontrolled, or insufficiently controlled, use of antiparasitic veterinary drugs (anthelmintics) is associated with emergence of drug resistance worldwide. In countries like Brazil, resistance to multiple anthelmintics such as macrocyclic lactones, benzimidazoles and imidazothiazoles has become a problem. While these drugs are necessary to control parasitic disease burdens and loss of productivity, care must be taken in their use to safeguard public health. Regular monitoring of drug levels in animal products provides an effective warning and feedback mechanism with respect to the application of good practices in the use of these drugs, allowing competent authorities and regulators to maintain and continually improve measures to protect public health. These monitoring schemes require the use of efficient and sensitive analytical tools such as liquid chromatography-tandem mass spectrometry (LC-MSMS). Whilst LC-MSMS remains one of the best tools for quantifying and confirming the presence of such residues in foods, it is also an extremely powerful screening tool for various veterinary drugs. The quantification of residues and contaminants by LC-MSMS, like other analytical techniques, can be hampered by various random and systematic errors. This is exacerbated when the technique is applied for multi-reside analysis, since extraction and clean-up procedures are compromised because the target compounds will have different physico-chemical characteristics. The use of stable isotope labeled compounds as internal standards (ISTD) in LC-MSMS helps in correcting for such errors during the entire method from sample preparation to detection. Sample preparation is a critical component of residues analysis but should be effective and economical in order to be relevant. The application of a QuEChERS-type sample preparation method for the analysis of veterinary drug residues is becoming popular due to its economy and its applicability with analytes with varying physico-chemical properties.

The objectives of this study, undertaken by the Agrochemicals Unit as a partner in the EU 6th Framework Integrated Project "ProSafeBeef", were to collaborate in the development of a multiresidue method for anthelmintics to help ensure food safety and support international trade, to adapt the methodology for application in developing countries, and to implement technology transfer to a developing country partner in the project.

3.1.1. Experimental

Stock solutions of the 38 anthelmintics and the 11 internal standards were prepared in dimethylsulphoxide (DMSO), methanol or acetonitrile, as appropriate. All solutions were stored at –20 °C. Mixed intermediate and working standard solutions were prepared by dilution of the stock solutions in methanol and stored at –20 °C.

Previously minced meat samples (10 ± 0.01 g) were weighed into 50 mL Sarstedt polypropylene tubes. For recovery experiments, these were spiked with a mixture of the 38 anthelmintics and with the mixed internal standard solution. The samples were left to stand for 15 min then extracted with acetonitrile for 30 sec using an Ultraturax homogeniser. The

samples were then extracted by vigorous hand-shaking for 1 min with a mixture of $MgSO_4$ and NaCl, centrifuged, and the supernatants vortex-mixed with the clean-up mixture ($MgSO_4$:C18, 1.5:0.5, w/w). The tubes were centrifuged and an aliquot of each supernatant (600µL) was transferred into a 25 mL calibrated glass tube containing 0.5 mL of DMSO and vortex-mixed for 30 s. This solution was then evaporated under a stream of nitrogen to 0.5 mL using a TurboVap evaporator set at 50 °C. Negative control samples (blanks) were prepared as above but without spiking. Matrix matched calibrators containing both standards and ISs were also prepared. All samples were then pressed through a 0.2µm filter directly into HPLC vials and stored at –20 °C if not being analyzed immediately.

Samples were analysed by gradient reversed-phase chromatography using a C18 Atlantis analytical column at 45 (±5) ^{o}C with a flow rate of 0.25 mL/min. Mobile phase (A) consisted of water:acetonitrile (90:10, v/v) and mobile phase (B) was 5mM ammonium formate in methanol:acetonitrile (75:25, v/v). The gradient profile was: 50% A: 50% B for 2 min, increasing linearly to 100 % B over 8.1 min (2 to 10.1 min) and held for 6 min (10.1 to 16.1 min) before returning to initial conditions for 5.8 min (16.2 to 22 min). The autosampler temperature was set at 22 (±2) ^{o}C and the injection volume was 10µL. Detection was by electrospray ionization (ESI) triple quadrupole mass spectrometry with polarity switching. Multiple Reaction Monitoring (MRM) was employed, with the instrument optimized to acquire a quantitative transition ion and at least one qualifier transition for each analyte in either positive or negative mode. Twenty seven compounds were analyzed in positive ESI mode and 11 in negative mode.

Each analyte concentration was determined using the ratio of the peak area of the analyte MRM transition to that of the internal standard, quantified against a standard curve prepared using the analyte/internal standard ratios of the matrix matched calibrators.

Locally purchased beef samples were used to optimize and validate the method. The validation was carried out to accommodate, as much as possible, maximum recommended residue limits of the 38 anthelmintics and at concentrations as low as 1µg/kg in order to enable analysis of trace anthelmintic residue levels for risk analysis surveys. The recovery and precision of the method were elaborated by spiking blank meat samples. This was done by 2 independent operators on alternate days for 5 days to determine both repeatability and within laboratory reproducibility.

For specificity and selectivity, the occurrence of interfering compounds was investigated by analyzing blank samples to ensure non-interference at the respective analyte retention times. The limit of detection limit (LOD) and limit of quantification (LOQ) were estimated by injecting decreasing concentrations of matrix-matched standards and measuring the response at signal-to-noise ratio (S/N) of ≥ 3 and ≥ 10 for the LOD and LOQ, respectively. Analytes were typically identified on the basis of ion ratios (precursor, quantification and qualifier ions) and their retention times.

3.1.2. Results

The acetonitrile extraction and dispersive solid phase clean up produced clean extracts with no obvious interferences in the chromatograms. Representative MRM chromatograms of the quantifier ion for 15 of the 38 compounds covered by the method are shown in **Figure 1**. Chromatograms for the other analytes were similar.

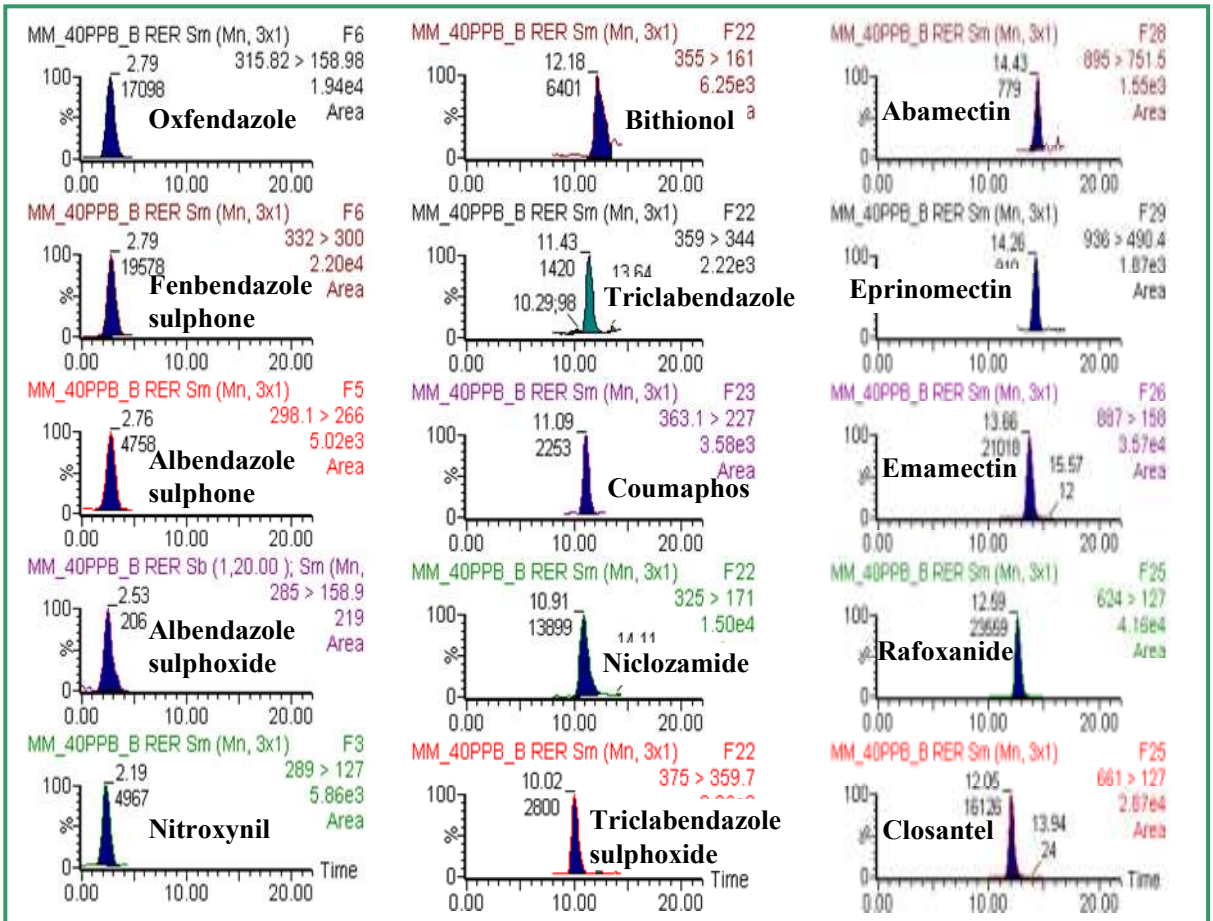

Figure 1. Representative MRM chromatograms of the quantifier ion for 15 of the 38 compounds covered by the method. Chromatograms for the other analytes were similar.

Thirty eight veterinary anthelmintics could be detected and quantified simultaneously. The compounds, the transitions monitored for each, and the internal standards used are summarized in **Table 1**. No suitable internal standard was identified for clorsulon, which was quantified using an external standard calibration curve.

The precursor ions for all the compounds analyzed in positive mode were $[M+H]^+$ except for the macrocyclic lactones, where more intense signals were seen when the $[M+Na]^+$ adducts were used. The precursor ions for all except two of the compounds in negative mode were $[M-H]^-$. For triclabendazole sulfone the ion at m/z 330, which possibly results from loss of an $NHSO_2$ moiety, was the precursor ion used while for albendazole 2-amino sulfone, m/z 274, possibly an anion adduct $[M+Cl]^-$ was preferred to the ion at m/z 238.

While ESI provides high sensitivity, one challenge faced in this mode is ion suppression. The dual challenges of ion suppression and losses during sample preparation were overcome in this method by using a number of stable isotope-labeled internal standards.

Limits of quantification were 10 µg/kg or better for 37 of the 38 analytes. Several of the compounds could be reliably quantified down to 1 µg/kg.

Recovery values, calculated using the internal standards, for all the drugs analyzed at all 6 spiking levels typically ranged from 75 % (amino-mebendazole) to 122 % (oxibendazole).

The repeatability of the method, evaluated by calculating the relative standard deviation (RSD) of 5 series (5 days) of six replicates of beef samples fortified at 40, 20, 10, 5, 2.5 and 1µg/kg ranged from 2.3 to 21.5% while the reproducibility values ranged from 1.37 to 16.7%. Most RSD values were below 10%, with a few higher values for certain compounds at low spiking levels.

Linearity of response was acceptable for all analytes, with correlation coefficients typically ≥0.98 using matrix matched standard curves at concentrations ranging from 0.5 to 40µg/kg.

3.1.3. Conclusions

An isotope dilution LC-MSMS method for the analysis of multiple anthelmintics in beef was developed and validated as described briefly above. An economical but effective QuEChERS-type sample preparation protocol was used. Ten deuterated compounds were used to improve the precision and accuracy of the method to meet the performance criteria set out in international guidelines, underlining the importance of stable isotope dilution methodology in residues analysis. The ability to simultaneously screen for 38 anthelmintics from different chemical classes, rather than using individual or class-based screening methods, makes the method extremely cost-effective. The protocol and standard operating procedure for the method were transferred to the project partner, Microbioticos Laboratories in Brazil, and a technician from that laboratory was trained on the method and contributed to the method validation during a two-month internship in the Agrochemicals Unit. The method is being used in Brazil to provide risk assessment data under the ProSafeBeef project, and Microbioticos will be used as a training hub to further transfer the method to a number of laboratories in Latin America under the regional TC project, RLA/5/055.

The method is suitable for use in other developing and developed countries wishing to collect risk assessment data or for regulatory monitoring to safeguard public health, facilitate international trade and improve food production practices.

Table 1. Summary of the 38 anthelmintics, retention times (t_R) and ions acquired.

Analyte	t_R (min)	Quantification transition	Qualif. ion	Internal Standard (ISTD)	ISTD transition	mode
Fenbendazole	8.12	300.0 > 159.0	268.0	Fenbendazole-D3	303.1 > 158.7	+
Cambendazole	3.41	303.0 > 217.0	261.0	Fenbendazole-D3	303.1 > 158.7	+
Flubendazole	4.84	314.0 > 123.0	282.0	Fenbendazole-D3	303.1 > 158.7	+
Amino-flubendazole	3.01	256.0 > 95.0	123.0	Fenbendazole-D3	303.1 > 158.7	+
Hydroxy-flubendazole	3.03	316.0 > 97.0	125.0	Fenbendazole-D3	303.1 > 158.7	+
Mebendazole	4.13	296.0 > 264.0	105.0	Fenbendazole-D3	303.1 > 158.7	+
Hydroxy-mebendazole	2.81	297.9 > 266.0	224.0	Fenbendazole-D3	303.1 > 158.7	+
Amino-mebendazole	2.81	238.0 > 105.0	133.0	Fenbendazole-D3	303.1 > 158.7	+
Oxibendazole	4.37	250.0 > 176.0	218.0	Fenbendazole-D3	303.1 > 158.7	+
Albendazole	6.35	266.1 > 191.0	233.8	Albendazole-D3	269.1 > 191.1	+
Albendazole-sulphoxide	2.53	282.0 > 158.0	208.0	Albendazole-SO-D3	285.0 > 158.9	+
Albendazole-sulphone	2.76	298.1 > 266.0	219.8	Albendazole-SO_2-D3	301.0 > 159.2	+
Albendazole-2-amino-sulphone	1.86	274.0 > 238.2	131.0	Albendazole-2-amino-SO_2-D2	240.0 > 131.0	-
Fenbendazole-sulphoxide	2.79	315.8 > 158.9	191.1	Fenbendazole-SO-D3	319.1 > 159.0	+
Fenbendazole-sulphone	2.79	332.0 > 300.0	159.0	Fenbendazole-SO_2-D3	335.0 > 299.9	+
Thiabendazole	2.83	202.0 > 131.0	175.0	Thiabendazole NH-D6	280.2 > 180.0	+
5-Hydroxy-thiabendazole	1.96	218.0 > 147.0	191.0	Thiabendazole NH-D6	280.2 > 180.0	+
Morantel	2.26	221.0 > 111.0	164.0	Thiabendazole NH-D6	280.2 > 180.0	+
Levamisole	2.07	205.0 > 178.0	122.9	Levamisole-D5	210.1 > 183.1	+
Haloxon	7.71	415.0 > 211.1	273.0	Levamisole-D5	210.1 > 183.1	+
Coumaphos	11.09	363.1 > 227.0	307.1	Levamisole-D5	210.1 > 183.1	+
Coumaphos-Oxon	6.82	347.0 > 291.0	211.0	Levamisole-D5	210.1 > 183.1	+
Triclabendazole	11.43	359.0 > 344.0	197.0	Triclabendazole-D3	362.0 > 344.0	-
Triclabendazole-SO	10.11	375.0 > 359.7	181.0	Triclabendazole-D3	362 .0> 344.0	-
Triclabendazole-sulphone	9.51	330.0 > 184.0	118.0	Triclabendazole-D3	362 .0> 344.0	-
Bithionol	12.26	355.0 > 161.0	194.0	Triclabendazole-D3	362 .0> 344.0	-
Closantel	12.05	661.0 > 127.0	345.0	Triclabendazole-D3	362 .0> 344.0	-
Rafoxanide	12.59	624.0 > 127.0	345.0	Triclabendazole-D3	362 .0> 344.0	-
Nitroxynil	2.37	289.0 > 127.0	162.0	Triclabendazole-D3	362 .0> 344.0	-
Niclozamide	10.91	325.0 > 171.0	289.0	Triclabendazole-D3	362 .0> 344.0	-
Oxyclozanide	9.83	400.0 > 176.0	202.0	Triclabendazole-D3	362 .0> 344.0	-
Abamectin	14.43	895.0 > 751.5	327.3	Selamectin	770.5 > 144.8	+
Doramectin	14.90	921.0 > 777.2	183.2	Selamectin	770.5 > 144.8	+
Emamectin	13.66	887.0 > 158.0	126.0	Selamectin	770.5 > 144.8	+
Eprinomectin	14.26	936.0 > 490.4	352.2	Selamectin	770.5 > 144.8	+
Ivermectin	15.40	897.0 > 153.1	329.1	Selamectin	770.5 > 144.8	+
Moxidectin	14.77	662.3 > 240.1	337.0	Selamectin	770.5 > 144.8	+
Clorsulon	2.19	380.0 > 344.0	342.0	NONE	NONE	-

3.2. Multi-class isotope dilution methods for anthelmintics in soil and water using ^{14}C labelling

The use of veterinary drugs, including anthelmintics, has become essential to ensure the sustainable production of livestock animals to meet the growing global demand for food. Since the administered drugs are usually eliminated from the body in the animals' faeces, they can be found in the manure. Animal excrement from the livestock industry is a cost-effective fertilizer frequently applied to agricultural land to regenerate the depleted minerals and organic substances consumed in crop production, especially in the production of organic produce or "bio-crops". The use of animal manure as fertilizer, along with run-off from intensive or semi-intensive farming systems in which such drugs are used and excreta from grazing animals treated with the drugs, may result in significant amounts of these drugs in soil and ground water, and consequently in the plants, including food crops, growing on the affected land. The increasing use of anthelmintics to improve animal production could, therefore, affect the ecological system and the food supply. Various researchers have demonstrated that water sources used for irrigation can contain organic contaminants, often in significant amounts. Re-cycling of the drugs through the use of contaminated crops for animal feeds, or grazing animals on contaminated pastures may also cause residues in food-producing animals and could add to the problem of anthelmintic resistance.

Unlike pesticides, which have been extensively studied in the past, the impact of veterinary drugs on the environment has not been adequately considered. The impact and magnitude of the effects depends upon factors such as the drugs' physico-chemical properties, the amount used and the method of administration, treatment type and dose, animal husbandry practices, manure storage and handling practices, metabolism within the animal, degradation rates in manure and the types of soil to which the manure is applied. Studies are needed to elaborate the risks associated with these issues, and analytical methodology must be developed to facilitate such studies. Determination of the concentrations and characterization of the behaviour of these drugs as trace contaminants in crops, soil and the aquatic system would provide information for future considerations on their impact on the environment, and their possible recycling into food.

The lysimeter facility at AIT used for the study.

A lysimeter study was initiated in collaboration with the University of Natural Resources and Applied Sciences, Vienna (BOKU) and the Austrian Institute of Technology (AIT) to examine the behaviour of anthelmintics in a soil-plant-water system. Both radiotracer and conventional analytical methodologies were used for the investigation. The first phase of the study involved the development of analytical methodology and the application of slurry spiked with selected anthelmintic compounds to soil columns in lysimeters. Carbon-14 labeled levamisole was applied in the lysimeters to study the behaviour of

the anthelmintics in soil. Preliminary experiments were performed on a small, greenhouse scale using the ^{14}C-labelled compound to optimise the design of the lysimeter experiments.

During 2009, multi-class methods were developed for anthelmintics in soil and water.

3.2.1. Experimental

To facilitate the lysimeter study, one representative substance was chosen from each of three main anthelmintic classes. The methods developed covered the following groups: imidazothiazoles (represented by levamisole), benzimidazoles (fenbendazole and its sulpoxide and sulphone metabolites), and macrocyclic lactones (eprinomectin). These represenatative compounds have varying physico-chimical characteristics, such as hydrophobicity.

3.2.1.1. Soil method

To investigate the effect of soil type, the lysimeter study was carried out using two types of soil with different characteristics; a sandy and a loamy soil. The analytical method was validated for both types of soil. The soil samples used for method development and validation were collected using GPS referencing to source the same soil as was used in the lysimeters. A soil depth of approximately 15-20 cm was excavated, mixed, sieved through a 2-mm sieve and well mixed on a rotary blender. Sample portions were transferred into plastic containers, taken to the laboratory and air-dried in a porcelain dish at room temperature (**Figure 2**). They were then mixed and stored in a closed container.

Figure 2. Air-drying a soil sample.

The analytical method employed dispersive solid phase extraction. Portions (10 g) of the air-dried soil sample were weighed into centrifuge tubes. For analyte recovery experiments, samples were spiked by the quantitative addition of analytical standards in methanol and a specific amount/activity of ^{14}C-levamisole, vortexed and allowed to stand at room temperature for at least 20 min. An acetonitrile/methanol mixture (1:1, v/v, 15 mL) was added and samples were shaken for 30 seconds. A mixture of anhydrous magnesium sulphate (4 g), sodium chloride (1 g), sodium citrate dihydrate (1 g) and sodium citrate sesquihydrate (0.5 g) was then added and the tubes were shaken vigorously for 1 minute. The mixture was centrifuged and an aliquot of supernatant (6 mL) was transferred into a 15 mL plastic centrifuge tube and cleaned-up by shaking for 1 minute with primary-secondary amine (PSA, 150 mg) and magnesium sulphate (900 mg) (**Figure 3**). The tubes were centrifuged and a portion (3.0 mL) of each supernatant was transferred into a conical glass test tube and evaporated to dryness using a

Figure 3. Pre-weighed PSA/magnesium sulphate for soil sample clean-up.

Turbo-Vap at 45°C under a stream of nitrogen. Internal standard mixture was added to the evaporated sample. The residue was reconstituted in 500 µL of mobile phase (A:B,1:4, v/v, see **Section 3.2.1.3.** for mobile phase composition) with vigorous vortex mixing. The extract was transferred into an auto-sampler vial for LC-MSMS analysis.

During method development, duplicate aliquots of each sample extract were removed at various stages of the procedure, transferred to plastic vials and mixed with scintillation cocktail for radioactivity detection by liquid scintillation counting.

3.2.1.2. Water method

A simple extraction method using solid phase extraction cartridges was used to adsorb and concentrate the analytes. Tap water was used for method development. One litre of water was collected in a 1L amber glass flask and acidified with hydrochloric acid to pH ~ 3.0. For recovery experiments, the sample was spiked with a known amount of the analytes and mixed thoroughly using a magnetic stirrer. The sample was loaded onto an SPE cartridge (Oasis-HLB 500 mg, 6 mL) previously conditioned with methanol, followed by methanol/water (1:1, v/v) and finally with acidified water (pH ~ 3.0). Using a vacuum manifold, the sample was passed through the SPE cartridges with a flow rate of about 6 mL/minute (**Figure 4**). The SPE cartridges were dried with a stream of nitrogen for about 10 minutes, then eluted with 5 mL methanol-acetonitrile (1:1, v/v) and the eluate was collected in 15 mL glass centrifuge tubes. The eluate was evaporated in a Turbo-Vap under a stream of nitrogen gas at 50 °C, almost to dryness. A mixture of internal standards was added and the residue was dissolved in 500 µL of mobile phase (A:B,1:4, v/v, see Sample Analysis section for mobile phase composition) with vigorous vortex mixing. The dissolved extracts were transferred into auto-sampler vials for LC-MSMS analysis.

Figure 4. Extraction/concentration of water samples on SPE cartridges.

3.2.1.3. Sample analysis

The compounds were separated by HPLC on an Atlantis T-3, 2.1 x 100 mm x 3 µm analytical column, maintained at 45 °C. Gradient elution was employed, with mobile phase (A) comprising water/acetonitrile (90:10, v/v) and (B), methanol/acetonitrile (50:50, v/v), both containing 10 mM ammonium formate. The gradient was initially 30% B ramping to 100% B from 0.5 – 2.1 minutes, remaining at 100% B from 2.1 - 5.5 min, returning to 30% B between 5.5 and 5.6 min and equilibrating at this composition from 5.6 – 10 min. The flow rate was 350 µL/min and a 10 µL injection volume was used. Detection and quantitation was by triple-quadrupole mass analyzer in positive electrospray ionization mode. Multiple reaction monitoring was used; the ion transitions selected are listed in **Table 2**.

Quantitation was based on matrix-matched calibration standards for soil or solvent standards for water. Stable isotope internal standards (d5-tetramisole, d3-fenbendazole sulphoxide, d3-fenbendazole sulphone, and d3-fenbendazole) were used to compensate for run-to-run variation in instrument response, and to improve the precision of the results.

The method was validated for the 5 target compounds on 3 days at three concentration levels, 10.0 µg/kg, 20.0 µg/kg and 40.0 µg/kg for soil and 0.25 µg/L, 0.5 µg/L and 1.0 µg/L for water.

3.2.2. Results

3.2.2.1. Soil method

Figure 5 shows typical multiple reaction monitoring (MRM) chromatograms for a negative soil matrix, a soil sample spiked with the analytes at 10 µg/kg, analyte peaks at the limit of quantitation/determination (LOD) of the method, and a matrix-matched calibrator equivalent to the spiking level. All peaks for selected quantification ions were chromatographically separated. All compounds eluted on a reversed phase column within 6.5 minutes. The limit of quantitation was 5 ug/kg for all analytes except for eprinomectin, for which it was 10 ug/kg.

The trueness (recovery) and precision of the method are summarized in **Table 2.** There was no significant difference observed between the performance of the method on the two types of soils for most of the studied compounds. The typical recovery of the method in sandy soil was 89.4% (CV 4.2%) and in loamy soil was 83.6% (CV 3.8%).

Table 2. Trueness (% recovery) and precision (% relative standard deviation) of the method in sandy and loamy soil performed at 3 fortification levels on three different days.

		Spiking Levels		20.0 µg/kg		40.0 µg/kg		**Overall**	
Analytes	Quantifier ion transition (m/z)	Rec.	RSD	Rec.	RSD	Rec.	RSD	**Rec.**	**RSD**
		Sandy soil							
LEV	205>178	80.1	3.2	77.9	5.7	81.7	3.8	**79.9**	**4.3**
FBZSO	316.1>191	91.1	2.7	91.3	4.2	88.4	2.4	**90.3**	**3.1**
$FBZSO_2$	332.1>300	94.1	3.7	91.3	4.0	93.8	4.6	**93.1**	**4.1**
FBZ	300.2>159	98.9	4.3	94.1	6.3	97.6	7.1	**96.9**	**5.9**
EPR	914.4>186.1	85.5	5.2	90.4	2.0	84.5	4.2	**86.8**	**3.8**
		Loamy soil							
LEV	205>178	77.9	3.3	74.8	4.9	77.0	3.5	**76.5**	**3.9**
FBZ-SO	316.1>191	88.0	7.3	85.3	2.3	82.9	2.4	**85.4**	**4.0**
$FBZSO_2$	332.1>300	91.1	3.8	89.9	2.9	89.8	3.2	**90.2**	**3.3**
FBZ	300.2>159	83.2	2.6	79.9	1.8	84.1	6.0	**82.4**	**3.5**
EPR	914.4>186.1	83.4	4.0	82.2	4.4	85.7	4.6	**83.8**	**4.3**

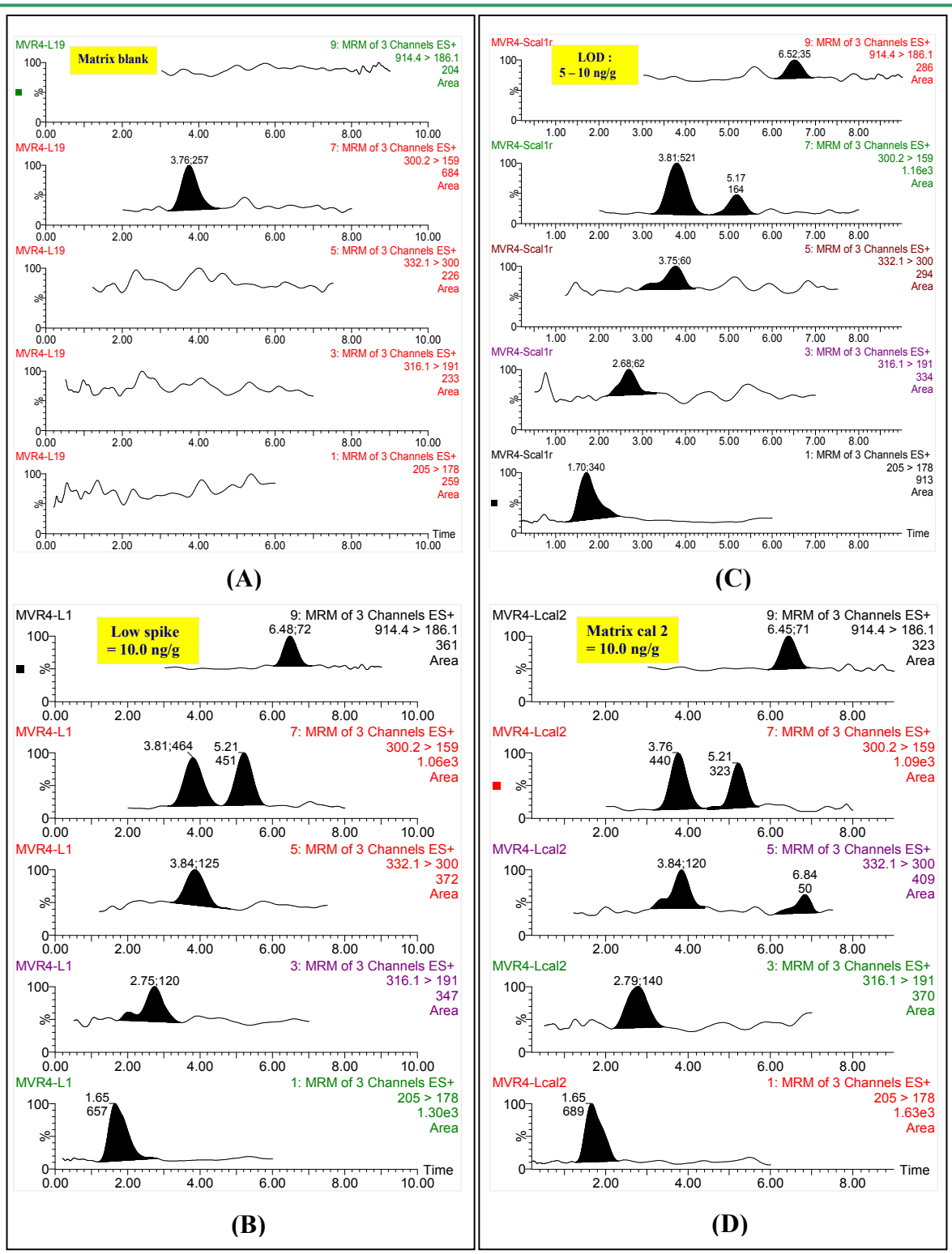

Figure 5. MRM chromatograms of precursor ions from (A) a blank soil extract; (B) a soil sample spiked at 10 µg/kg; (C) matrix std. calibrator and spiked sample.

The inclusion of ^{14}C-levamisole during method development facilitated easy and quick identification of critical steps for loss of recovery or increased variance contributing to greater overall measurement uncertainty, and allowed optimisation of those steps to improve method performance. The ^{14}C-levamisole data collected during method validation will be used for estimation of the measurement uncertainty of the method (not presented here).

3.2.2.2. Water method

Pre-treatment of the water sample with acid to pH~3.0 was necessary to adjust the ionization state of the analytes to facilitate extraction and concentration. When normal tap water without acid treatment was analyzed, poor recoveries were achieved for all analytes, especially fenbendazole and levamisole. At pH lower than 3.0, a shift of retention time was observed, especially with levamisole, the first compound eluted.

The recovery and precision of the method for the 5 target analytes in water are presented in **Table 3.**

Table 3. Recovery and precision of 5 anthelmintics in water analyzed at 3 spiking levels on three different days

Analytes	Quantifier Transition (m/z)	ACCURACY			PRECISION		
		0.25 µg/L	0.5 µg/L	0.1 µg/L	0.25 µg/L	0.5 µg/L	0.1 µg/L
		% Recovery			% RSD		
LEV	205>178	66.2	63.8	62.5	5.5	5.6	6.1
FBZ-SO	316.1>191	112.2	117.4	130.7	10	8.1	7.3
FBZ-SO2	332.1>300	104.0	130.1	141.2	14.4	8.1	8.6
FBZ	300.2> 159	107.0	98.9	116.6	8.2	10.6	11.9
EPR	914.4>186.1	27.8	38.6	39.8	22.8	24.0	18.9

3.2.3. Conclusions

The methods described were developed and validated using the comparative advantages of both radio-labelling, during method development and for estimating uncertainty, and stable isotope-labelling, for internal standards to improve method performance. The methods are currently being applied alongside direct carbon-14 measurements of labelled compounds in lysimeter studies to elaborate and characterise the behaviour of anthelmintics in a soil-water-plant system. The results of that study will be published in 2010-11 and will provide information for risk assessment bodies for eventual use in the development of international standards.

3.3. A multi-residue method for the analysis of organochlorine contaminants in meat using a modified QuEChERS clean-up and GC-ECD and GC-MS detection

Brazil has rapidly emerged as the world's leading meat exporter. Over the last ten years, Brazilian meat exports have increased by an average of nearly 21% a year, with Brazilian beef exports in 2009 amounting to about 930,000 tonnes. Food safety for Brazil is a strategic topic entailing not only national public health aspects but also competitiveness in international trade. An important component of any food safety program is the control and monitoring of residues posed by certain substances encountered as residues in food products. One aspect is the contamination of foods of animal origin with pesticides used on the farm as veterinary medicines, and through the indirect contamination of animals through grazing on pasture that is contaminated with chemicals. Through a bio-magnification process, lipophilic contaminants from pasture may be concentrated in the fatty tissues of the animal. Organochlorine pesticides are widely used by farmers due to their effectiveness and broad-spectrum activity. They have low volatility, high stability and lipophilic affinity. Their degradation creates metabolites which are also persistent and can have harmful effects. Once ingested, chemicals with high lipid solubility tend to concentrate in fat tissues, such as adipose tissue, brain, liver, kidney, and in the case of lactating animals, in the milk. The presence of a chemical in tissues and milk is also affected by its degree of biotransformation and its elimination rate from the body. Because these chemicals are toxic to living organisms, increased accumulation in the food chain is a serious health risk to the general population. In order to manage this risk, it is crucial to establish mechanisms for monitoring residues levels in the food chain.

The aim of this study was to adapt and validate a quick, rapid and cheap method for the control of meat quality, in particular for the determination of organochlorine compounds in meat tissues by gas chromatography coupled to either an electron capture detector (GC-ECD) or to mass spectrometry (GC-MS). The method was adapted from that published by Anastassiades et al. (2003)[1]. The method was studied within the framework of an internship from Brazil, with the objective to enhance the analytical capacity in Brazil through a train-the-trainers approach and to validate a method that would be widely applicable in developing countries in general.

3.3.1. Experimental

A mixture, mainly composed of organochlorine compounds, as shown in **Figure 6**, was used to fortify individual aliquots of meat tissue at three different fortification levels; 10, 100 and 1000 µg/kg. Tissue samples were finely chopped in a homogenizer and 5 g aliquots were weighted into glass tubes and individually fortified. Ten mL of acetonitrile and 1 mL of water were added to the glass tubes, together with a buffer-salt mixture (4 g of anhydrous magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogencitrate sesquihydrate). The tubes were immediately shaken for a few seconds and finely homogenised for 1 min using a dispersive homogeniser. The tubes were centrifuged and aliquots of the acetonitrile phase (upper layer) were transferred into screw capped vials containing a mixture of clean-up salts (25 mg of primary-secondary amine [PSA]

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

and C18, and 150 mg of $MgSO_4$ per mL of extract). The vials were shaken vigorously for 1 minute using a shaker and centrifuged. The supernatant was transferred into glass vials and evaporated to dryness in a nitrogen evaporator. The extracts were then transferred into auto-samplers vials for analysis. Calibration curves were prepared in matrix.

The method was validated on three different days at three concentrations levels. The within laboratory repeatability was established by the analysis of five replicate samples at each level. Quantitation was based on a matrix matched calibration, and according to the quality control procedures the standard deviation of the relative residuals (Srr) of the weighted regression calibration was less than 0.1 for all analytes and the correlation coefficient (R^2) greater than 0.99.

3.3.2. Results

All compounds included in the study eluted from a HP5MS-UI GC column in less than 32 minutes. **Figure 6** shows the overall recoveries obtained by GC-ECD and GC-MS detection systems. With a few exceptions, the recoveries obtained using the two detection systems were not significantly different at the 95% confidence level. All recoveries were within the range 86-116 %, which is in accordance with the Codex acceptable range (70-120%) for accuracy. Precision is expressed in terms of repeatability and reproducibility.

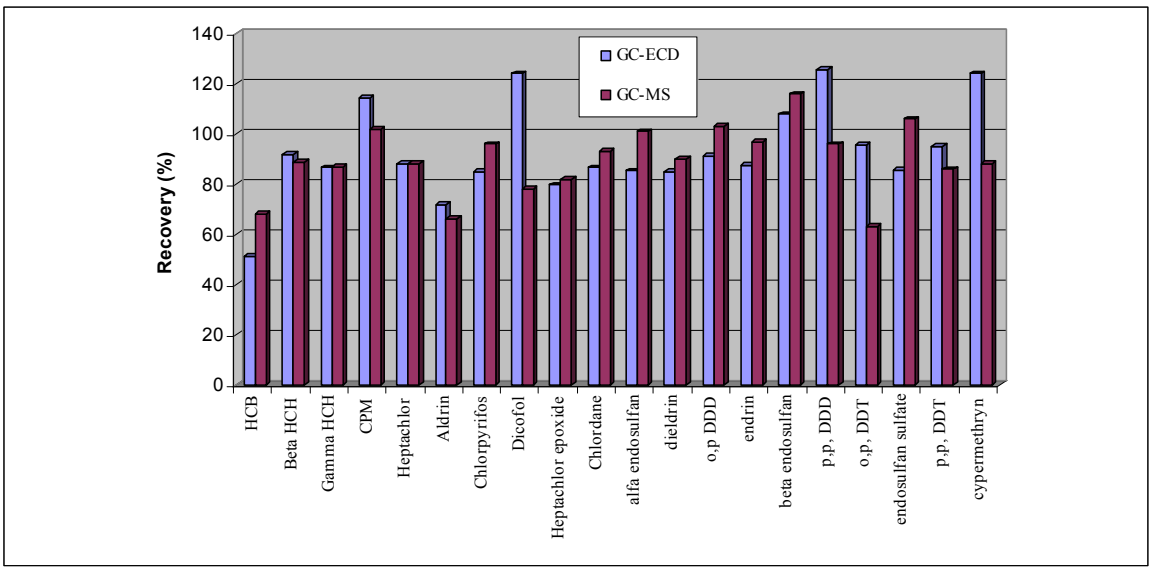

Figure 6. Comparison of overall recoveries obtained by GC-ECD and GC-MS.

Figure 7 gives an indication of the repeatability values for each individual compound as measured on three different occasions and with the two different detection sytems. Although the repeatability values were higher overall for both detection systems on day 2, they remained within the Codex acceptable limits on both days. The reproducibility values at three fortification levels were within the limits established by the Codex guidelines, at 32, 32 and 23% for the levels 10, 100 and 1000 µg/kg respectively.

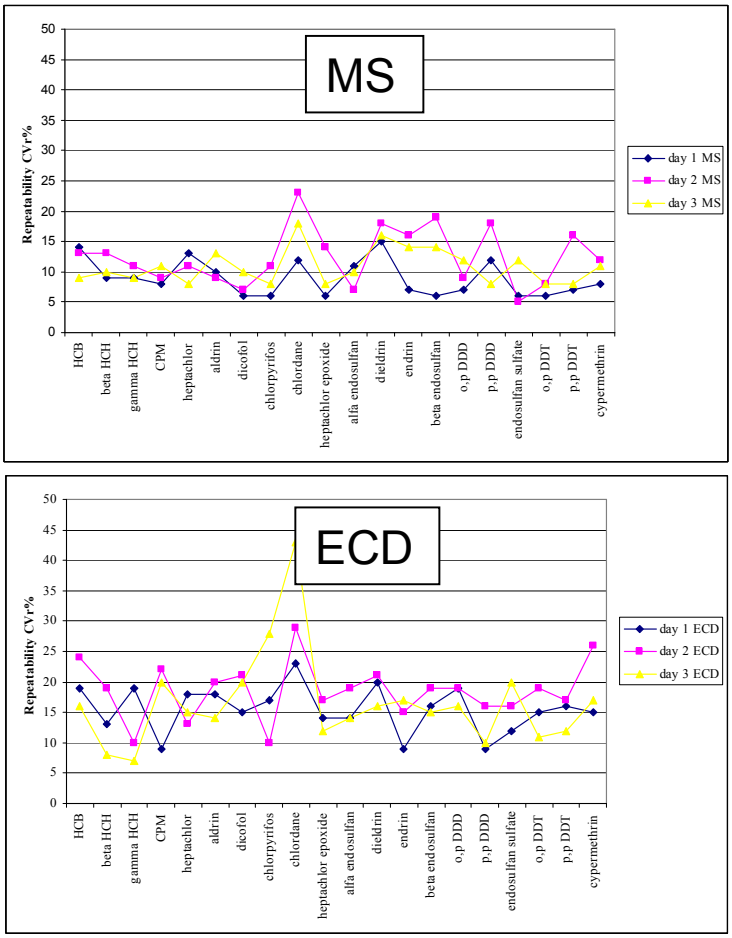

Figure 7. Repeatability values for the MS detection (upper figure) and the ECD detection (lower figure).

The limit of detection (LOD) of the method was estimated using matrix matched weighted calibration curves (see **Figure 8**). Limits of detection for the analytes for GC-MS and GC-ECD analysis were within the ranges of LODs of contemporary published methods for organochlorines in animal matrices, such as meat and fish tissues.

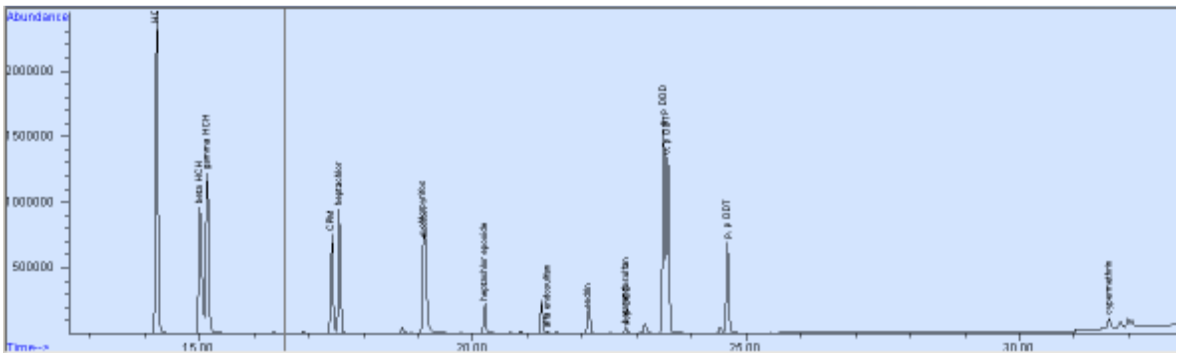

Figure 8. MS total ion chromatogram of a matrix-matched calibration standard mixture.

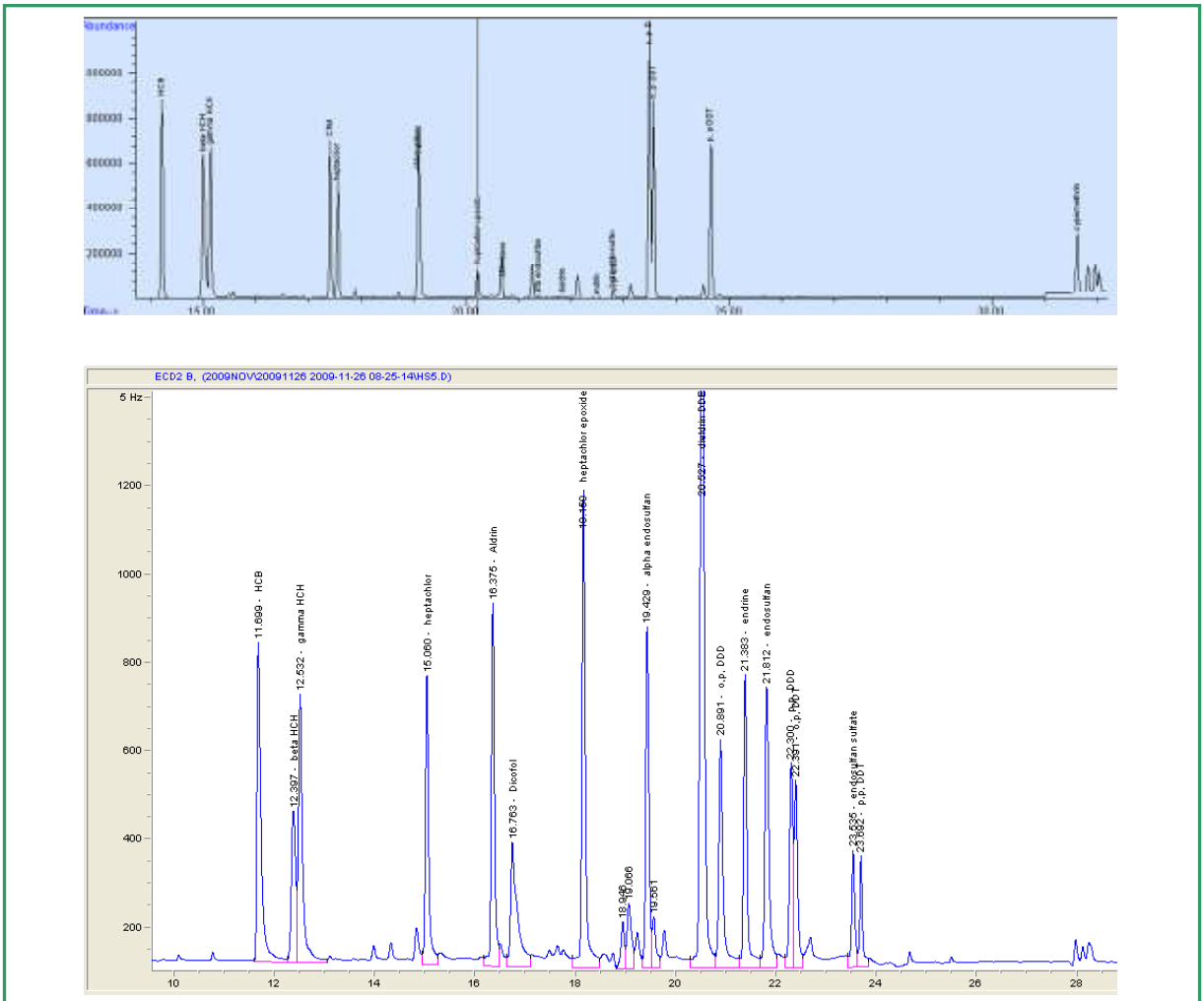

Figure 9. The MS total ion chromatogram (top) and ECD chromatogram (bottom) corresponding to the highest fortification level.

Figure 9 shows the MS total ion chromatogram and the ECD chromatogram corresponding to the highest fortification level. In the GC-MS system some of the compounds co-elute. The advantage of the mass spectrometric detection over the GC-ECD is the possibility to selectively monitor different ions for each co-eluting compound, thus using the resolving power of the mass spectrometer to accurately quantify each co-eluting peak.

3.3.3. Conclusions

The method adapted and validated in the Agrochemicals Unit is quick, relatively cheap, and applicable in a wide range of laboratories. The precision of the method is good for all analytes and accurate quantitation was achieved by using matrix matched calibrators. It can be applied to the analysis of meat tissue for the determination of organochlorine compounds in both developed and developing countries regulatory laboratories that are equipped with GC-ECD and/or GC-MS. Further in-house validation studies are needed when the method is applied in member state laboratories to expand the range of matrices to include, for example, kidney, liver and milk, which are the target tissues for lipophilic compounds.

3.4. Development of a liquid chromatography-tandem mass spectrometric method for determination of tropane alkaloids and glycoalkaloids in grains

As a regular part of their biochemical activity, plants produce alkaloids, the primary function of which is to help protect the plants from insects. The terms tropane alkaloids and glycoalkaloids refer to the family *Solanaceae or Nightshade*, comprising over 100 genera and 3000 plant species found worldwide. High concentrations of nightshade alkaloids have been found particularly in the genera *Datura* (*Datura stramonium, Datura ferox, Datura innoxia), Atropa (*beladonna), *Hyoscymus* (henbane), *Brugmansia* (angels trumpet), *Solanum tuberosum and Solanum lycoperiscum*. All of these contain variable amounts of tropane alkaloids and glycoalkaloids and all parts of the plant, particularly the seeds, are potentially poisonous. These plants are weeds of cultivated fields, waste places, barnyards, and other disturbed habitats. Bulk commercial grains, such as wheat, rye, soybeans, linseed, corn and solanaceous crops may be contaminated by non-grain impurities that coexist with the crop to be harvested. The most important tropane alkaloids are: (-)-hyoscyamine and (-)-scopolamine, atropine (the racemic mixture of (-) and (+)-hyoscyamine), homatropine and anisodamine. The plant glycoalkaloids are toxic steroidal glycosides and the commonest types found in food plants are α-solanine and α-chaconine.

Humans, horses, cattle, sheep, goats, pigs and poultry are all known to be affected by the alkaloids found in nightshades. Nightshade alkaloids have the ability to block the activity of the nerve-cell enzyme cholintesterase, which is involved in neurotransmission. If the activity of cholinesterase is strongly blocked, nervous system control of muscle movement becomes disrupted, and muscle twitching, trembling, breathing paralysis or convulsions can result. A second type of problem potentially related to the nightshade alkaloids involves damage to the joints caused by inflammation and altered mineral status and can contribute to excessive loss of calcium from bone and excessive deposition of calcium in soft tissue. The alkaloids also have a direct irritant effect on the digestive system causing nausea and vomiting, diarrhoea and stomach cramps.

Currently, there are no standard methods for the determination of nightshade alkaloids in grains. Toxicological studies of these biologically active compounds demonstrate their importance as contaminants of food and feed. It is necessary to develop and validate methods to allow the control of total and individual nightshade alkaloids content in food and feed.

In this study, a multi-residue method is being developed and validated using LC-MS-MS for the determination of tropine, atropine, scopolamine, homatropine, anisodamine, α-solanine and α-chaconine in wheat.

3.4.1. Experimental

Cereal samples were prepared by dispersive solid phase extraction using 1% formic acid in water/acetonitrile mixed with magnesium sulphate, sodium chloride, sodium hydrogen citrate and sodium citrate. After vortex-mixing and centrifugation, the supernatant was evaporated and the residue dissolved in mobile phase B (below). Chromatographic separation was achieved using a Chirobiotic V column and guard column. The mobile phase comprised 10 mM ammonium formate in water/acetonitrile (80:20, v/v) (eluent A) and 10 mM ammonium formate in methanol/acetonitrile (50:50, v/v) (eluent B) at a ratio of 20:80 (A:B). The run time was 16 min. Mass spectrometric parameters were optimised to produce at least two measurable transition ions for each analyte, as shown in **Figure 10**.

3.4.2. Results

Because of their similarity, these molecules are difficult to separate by conventional reversed-phase chromatography. Separation was achieved using a chiral separation column and a carefully optimized mobile phase. Typical multi reaction monitoring chromatograms are shown in **Figure 10**. The method is capable of quantifying the target alkaloids down to approximately 10 ng/g (ppb).

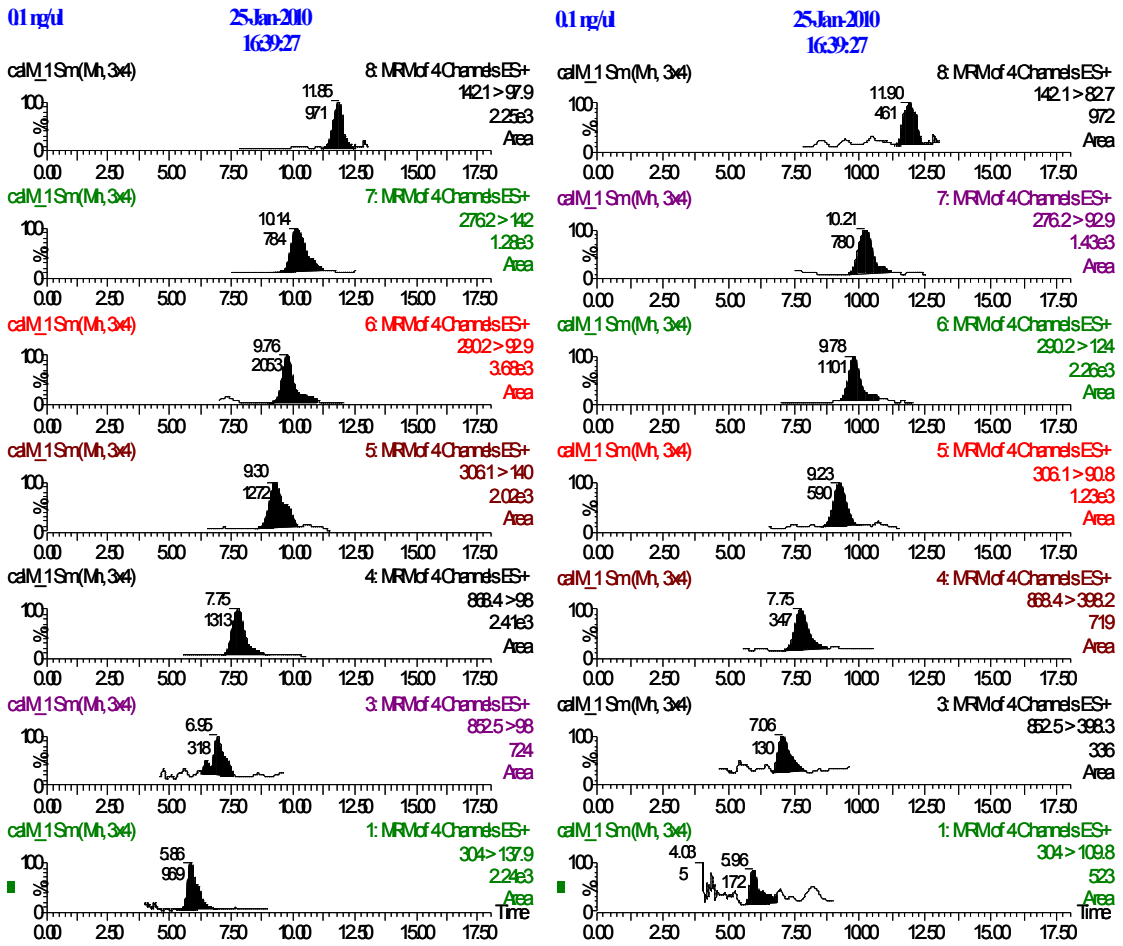

Figure 10. ESI(+) LC-MS-MS SRM chromatogram of 2 daughter ions derived from each of the alkaloids (from top to bottom - tropine, homatropine, atropine, anisodamine, α-solanine, α-chaconine and scopolamine).

3.4.3. Conclusions

Currently, there are no methods available for simultaneously detecting and quantifying the tropane and glycoalkaloid toxins in food and feed. Risk assessment data has been considered by various bodies and regulations for the control of these compounds in food and feedstuffs are currently being drafted in some regions, including the EU. The method developed and currently under validation in the Agrochemicals Unit will, therefore, be applicable in both developed and developing countries to support food safety and trade in food and feedstuffs. The method will be fully validated before being published and made available to support farm to fork food and feed safety systems.

3.5. Analysis of pyrethroids in bovine tissue by gas-liquid chromatography with electron capture detection and uncertainty determination using ^{14}C-deltamethrin

Pyrethroids are synthetic chemical compounds similar to the natural pesticide pyrethrum, which is produced from chrysanthemum flowers. Synthetic pyrethroids are more stable in light and have higher insecticidal activity than products made from chrysanthemum flowers. Pyrethroids have irritant and/or sensitizing properties. Although not easily absorbed through the skin, they are absorbed through the gut and pulmonary membrane. Many pyrethroids have been linked to disruption of the endocrine system with adverse effects on reproduction and sexual development, the immune system and increased risk of breast cancer.

The pyrethroids constitute a major proportion of the synthetic insecticide market and are used to control pest insects in agriculture, homes, communities, restaurants, hospitals and schools, and as a topical head-lice treatment. Pyrethroids registered for use on livestock include cypermethrin, deltamethrin, λ-cyhalothrin and cyfluthrin. These may be applied as pour-on formulations, sprays or dips. Cattle and other livestock are treated to control the transmission of malaria and other diseases through the reduction of vector density (e.g. mosquito, tsetse) and to control ectoparasites. Both beneficial insects and pests are affected by pyrethroids and they are also toxic to fish, aquatic organisms, amphibians and bees. Health concerns have arisen recently due to increased use of this group of chemicals as farmers have shifted from the use of organophosphates. The ability of these compounds to concentrate in mud and other sediments has also fueled concerns. Risks include contamination of animal-derived foods such as milk, meat and honey, as well as the environment.

Rapid, inexpensive methods, applicable in developing country environments, are required in food safety control systems to address these risks. An analytical method based on dispersive solid-phase extraction with analysis by gas chromatography with electron capture detector was adapted and validated in the Unit, using ^{14}C-deltamethrin to evaluate the efficiency of the extraction procedure and estimate the measurement uncertainty of the method.

3.5.1. Experimental

Portions of homogenized beef muscle were weighed into 50 mL plastic centrifuge tubes. For method development and validation, negative tissue samples were spiked with known concentrations of λ-cyhalothrin, cyfluthrin, cypermethrin, and deltamethrin and 30000 dpm ^{14}C-labeled deltamethrin, vortex-mixed and left to equilibrate for 15 minutes. The samples were extracted with acetonitrile (10 mL) by shaking the tube vigorously for 1 minute. Magnesium sulphate (4 g) and sodium chloride (1 g) were added and the tubes were shaken for another minute, then centrifuged. Three replicate aliquots of the crude extract were transferred from each sample to plastic vials and mixed with scintillation cocktail for analysis by liquid scintillation counting (LSC). The remainder of the supernatant was transferred into a centrifuge tube and cleaned-up with $MgSO_4$ (1.5 g) and C18 (0.5 g) by vortex-mixing for 30 seconds. A further three replicate portions of the cleaned-up extract were taken for LSC determination. Aliquot of the extracts were transferred into a glass centrifuge tubes and evaporated almost to dryness using a Turbo-Vap at 40 °C under a stream of nitrogen. The residue was reconstituted in 500 µL of acetone/iso-octane (15:85, v/v). Two replicate portions of each extract were taken and analysed by LSC. The remainder was transferred to auto-sampler vials and injected into a gas chromatograph equipped with electron capture detector (ECD).

3.5.2. Results

The method was validated for the four target analytes (at three spiking levels, approximately 15, 30, and 45 µg/kg. The analysis was repeated on 3 occasions under repeatability and reproducibility conditions, using dieldrin as an internal standard.

Typical chromatograms are shown in **Figure 11** for a negative bovine muscle, a matrix-matched calibrator at 14.4 µg/kg and an extract of a blank muscle sample spiked at 14.4 µg/kg. The limit of quantification (LOQ) was 7.2 µg/kg. Both cyfluthrin and cypermethrin consist of 4 isomers, which were chromatographically separated, as can be seen in **Figure 11**, and individually quantifiable.

Recovery values were in the range 86.5 – 102.4% with relative standard deviations (RSD) of 9.3 – 17.1% (**Table 4**). The typical recovery of the method for all 4 analytes was 97.3% with typical RSD of 14.5%.

Table 4. Recoveries and RSDs of 4 pyrethroids in bovine muscle by GC-ECD analysis.

Spiking levels	**14.4 ug/kg**		**28.5 ug/kg**		**43.2 ug/kg**	
	Trueness	RSD	Trueness	RSD	Trueness	RSD
λ-Cyhalothrin	95.3%	9.3%	102.4%	15.6%	100.8%	15.3%
Cyfluthrin	99.6%	10.8%	98.4%	15.0%	97.7%	16.0%
Cypermethrin	86.5%	11.4%	90.7%	17.1%	11.4%	16.0%
Deltamethrin	93.8%	12.2%	98.4%	15.9%	88.7%	16.6%

The overall measurement uncertainty associated with the method, estimated using the validation data in a "top-down" approach, was 14.3%. The quadratic mean, representing the combined uncertainty of each analyte, was estimated using **equation 1.** In this study, the estimation was based on the average values of the three fortification levels.

$$u_{cAv}(\%) = \sqrt{\frac{u_c^2 o_1 + u_c^2 o_2 + u_c^2 o_3}{3}} \qquad \text{equation 1}$$

Deltamethrin labeled with ^{14}C was used to evaluate the performance of each step of the method and to provide a "bottom-up" estimate of the measurement uncertainty. Three replicates of supernatant were analysed by LSC after each sample preparation step (extraction, clean-up, and evaporation). As can be seen in **Table 5**, the evaporation step showed the greatest contribution to the overall uncertainty, possibly attributable in part to the error inherent in pipetting low volumes of reconstituted extract as compared to the higher volumes pipetted at the extraction and clean-up steps, and was therefore identified as a critical step for quality control of the method. The combined uncertainty contributed by the systematic (uR) and the random (r) error components was estimated using **equation 2.** The systematic error

component was estimated based on a rectangular distribution, while random error component was determined as the relative standard deviation of replicated measurements.

$$u_c = \sqrt{r^2 + uR^2} \qquad \text{equation 2}$$

Table 5. Uncertainty contribution of individual analytical step by LSC using ^{14}C-deltamethrin.

	Extraction	Clean-up	Evaporation
Uncertainty of recovery (uR)	2.5%	1.2%	1.7%
Uncertainty of repeatability (r)	5.9%	6.9%	7.7%
$\text{Uncertainty}_{\text{Combined}}$	6.4%	7.0%	7.9%

From the data in **Table 5,** the overall uncertainty was derived as the square root of the sum of the squares of the combined uncertainties of each analytical step. The overall uncertainty of the combined analytical steps was 12.3% and the overall recovery was 105.7% with overall RSD of 7.8%.

There was no significant difference in the estimates of uncertainty using the two different approaches. However, the "bottom-up" approach, using ^{14}C-delatmethrin, provided additional information on the critical step of the method to be targeted to minimize uncertainty.

3.5.3. Conclusions

This method addresses a need for the monitoring of pyrethroid residues for food safety control systems. The method is simple and rapid and uses equipment available in many developing country residues laboratories and is, therefore, widely applicable. A TC fellow from the Tanzania Food and Drugs Authority (FDA) worked with Unit staff on this study, gaining valuable experience and intensive training on the operation of gas chromatograph (GC) instrumentation, method development and validation, and statistical evaluation of results. The method protocol has been transferred directly to the Tanzanian FDA laboratory and is available to other member state laboratories.

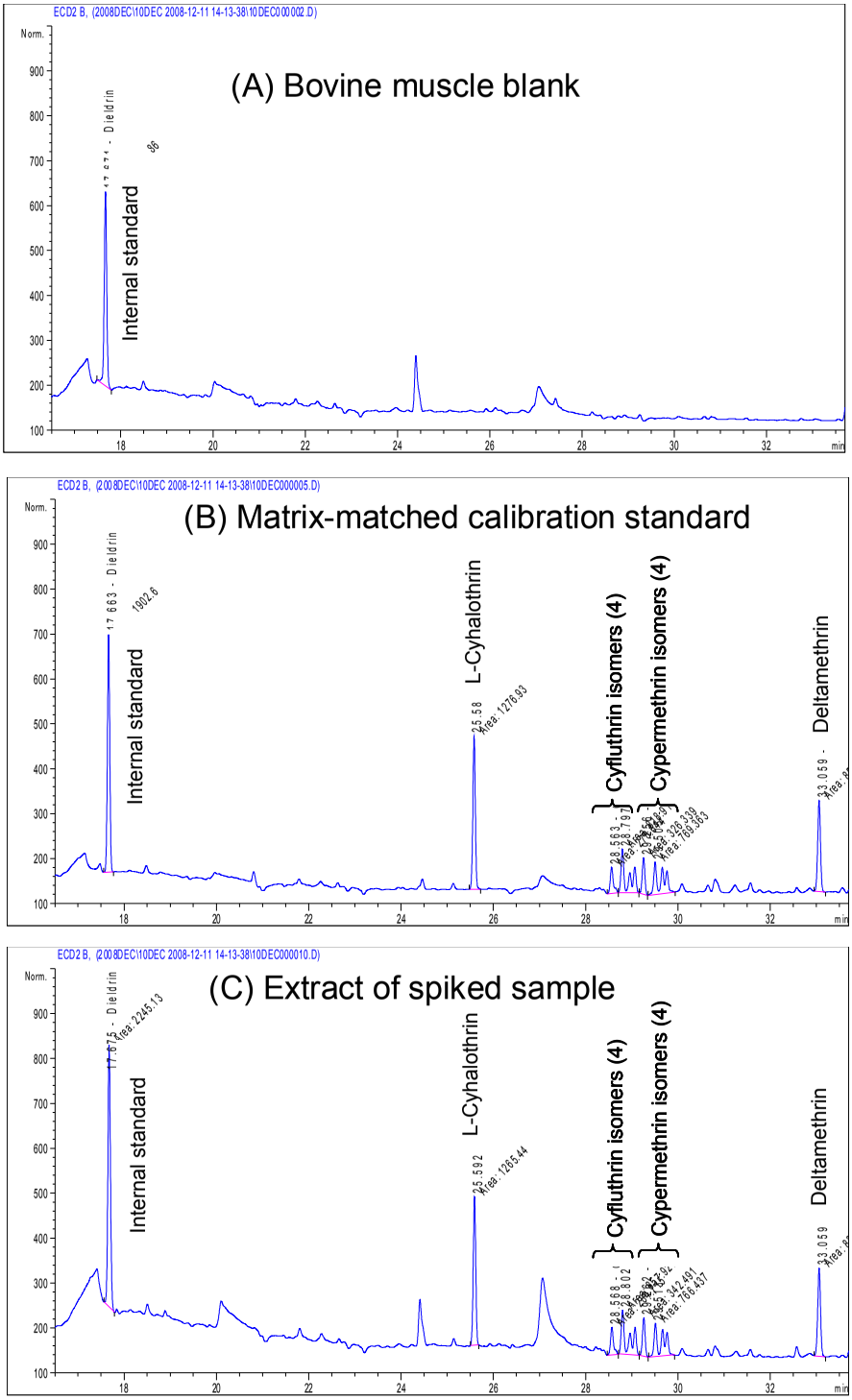

Figure 11. ECD chromatograms of 4 pyrethroids in bovine muscle tissue; (A), blank bovine muscle; (B), matrix matched calibrator equivalent to 14.3 µg/kg of each analyte in muscle, and; (C), an extract of a sample spiked at 14.3 µg/kg with each of the target pyrethroids.

3.6. A multi-residue gas chromatography-mass spectrometry method for the analysis of contaminants in soil

Pesticides have brought considerable benefits to humankind as an invaluable contribution to agriculture, increasing the yield and the quality of crops. However, concerns about the adverse effects of these substances to humans, animals and the environment are growing, especially if they are not used according to the good agricultural practices. The ecological balance could suffer changes through the inappropriate use of pesticides, causing undesirable effects such as the extinction of aquatic invertebrates, fish and some species of birds.

The study presented here was undertaken within the framework of coordinated research project (CRP) D5.20.35, "Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale", one of the objectives of which is to provide analytical tools to monitor the effectiveness of good agricultural practices. The method was demonstrated in the Agrochemicals Unit to participants in the second research coordination meeting of the CRP, held in February 2009. A regional TCP, RLA/5/053, has also adopted the analytical methodology within its network of laboratories in order to effectively monitor the effects of pesticide use in several environmental compartments.

3.6.1. Experimental

Soil samples were finely ground using a laboratory homogenizer. Analytical portions of 5 g were individually fortified at three fortification levels and stored in a freezer for 24 hours. On the day of analysis, the samples were thawed and a small amount of water was added according to the water holding capacity of the soil, which was previously determined.

A practical method to estimate the amount of water to add to a soil sample for analysis was established by one of the CRP laboratories from Brazil.

Soil (5 g) was introduced into a funnel containing a plug made of glass wool. Water (10 mL) was added to the soil, and the amount of water eluted from the system was measured using a cylinder. 50-70% of the water that is retained in the soil is then taken as the amount of water to add to the soil for analysis. It is very important to add water to the soil, as the active sites of the particles need to be prepared for the partitioning effects that will take place between three components, the solvent, the water and the pesticides.

Ethylacetate (10 mL) and water (4 mL) were added to the glass tubes, together with a buffer-salt mixture (anhydrous sodium sulphate and sodium hydrogen carbonate). The tubes were immediately shaken for a few seconds and finely homogenised for 1 min using a dispersive homogeniser. The tubes were centrifuged and aliquots of the ethylacetate phase were transferred into screw-capped vials containing a mixture of clean-up salts (25 mg of PSA and 150 mg of $MgSO_4$ per mL extract). The vials were shaken vigorously for 1 minute using a shaker and centrifuged. The cleaned-up extract was transferred into glass vials and evaporated to dryness in a nitrogen evaporator. The extracts were re-dissolved in isooctane and transferred into auto-samplers vials for GC-MS analysis. Calibration curves were prepared in solvent.

3.6.2. Results

The method was validated for a number of compounds, including organochlorine, organosphosporus, carbamate, urea derivates, chloroacetamide, triazines, strobilurin, anylinopyrimidine, and pyrethroids, as listed in **Table 6**.

The conditions for the GC-MS acquisition in selected ion monitoring mode (SIM) are shown in **Table 6**. One target ion and at least two qualifier ions were selected for each analyte. The method was validated on 3 days at three concentrations; 50, 100 and 250 µg/kg. The total ion chromatograms (TIC), at the 250 µg/kg level, is shown in **Figure 12**. In the TIC some of the compounds are overlapping at the retention time of interest; however quantification is performed in SIM mode, where unique ions are chosen for each analyte, effectively resolving the peaks.

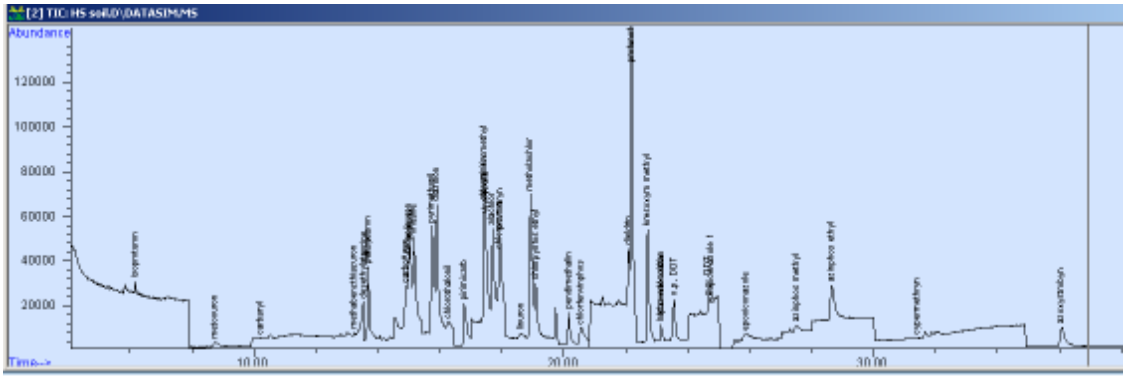

Figure 12. The total ion chromatogram acquired in SIM mode for 250 µg/kg fortification level.

The overall recovery and the reproducibility values for all the compounds, obtained during method validation, are summarised in **Figure 13**. The yellow bar indicates the Codex acceptable values for the method precision expressed in terms of within-laboratory reproducibility. The Codex acceptable range for accuracy at the level tested in this method is 60-120 % recovery. Good reproducibility and recovery values were obtained for all compounds, except for pirimicarb. The low recovery value obtained for this compound could indicate a partitioning effect into the water phase as pirimicarb is a very idrophilic compound, belonging to the carbamates family, which has a water solubility value of about 3000 mg/L.

The use of a stable isotope such as ^{13}C-DDT as internal standard improved the precision of GC-MS results.

Quantitation was based on calibrations in solvent. The calibration was acceptable according to the quality control procedures established in the laboratory, the standard deviation of the relative residuals (Srr) of the weighted regression calibration being less than 0.1 for all analytes and the correlation coefficient (R^2) being greater than 0.99.

The limit of detection (LOD) of the method was estimated using calibration curves in solvent. Limits of detection for all the analytes were within the ranges of 0.5 µg/kg (kresoxym methyl) to 30 µg/kg (metoxuron).

3.6.3. Conclusions

Member state laboratories in CRP D3.20.35 and in regional TCPs in Latin America have successfully adopted the method and expanded the range of compounds depending on the priority analytes, as identified using first tier risk assessment tools such as the pesticide impact rating index (PIRI). The method has proven to be quick and relatively cheap. Individual method deviations and adaptations will be published elsewhere.

Table 6. List of compounds, their retention time and the target and qualifier ions (m/z).

Compound	Class of compound	Retention time (min)	T.I.	Q1	Q2	Q3
Isoproturon	Urea	6.0	146	128	161	91
Carbofuran	Carbamate	7.0	164	149	122	131
Diuron	Urea	7.1	187	124	189	159
Metoxuron	Urea	8.7	168	183	140	
Desisopropylatrazine	Triazine	13.1	173	158	145	68
Methabenzthiazuron	Urea	13.3	164	136	135	
Desethylatrazine	Triazine	13.3	172	174	187	
Pencycuron	Urea	13.6	125	180	127	
Trifluralin	Dinitroaniline	13.7	306	264	290	
Monolinuron	Bypirydilium	14.9	61	126	153	214
Atrazine	Triazine	14.9	200	202	215	173
Lindane	Organochlorine	15.1	181	183	219	217
Pyrimethanil	Anylinopirimidine	15.7	198	199	77	
Diazinon	Organophosphorus	15.9	137	179	199	304
Chlorothalonil	Chloronitrile	16.2	266	264	268	267
Pirimicarb	Carbamate	16.8	166	72	238	167
Parathion-Methyl	Organophosphorus	17.4	109	263	125	79
Chlorpyrifos methyl (RTL)	Organophosphorus	17.4	286	125	288	290
Vinclozolin	Dicarboximide	17.5	198	212	187	
Carbaryl	Carbamate	17.7	144	115	116	
Alachlor	Chloroacetamide	17.7	160	188	146	
Prometryn	Triazine	17.9	241	184	226	
Chloroxuron	Urea	17.9	245	247	182	
Linuron	Urea	18.5	61	187	124	
Metolachlor	Chloroacetamide	18.9	162	238	240	146
Chlorpyrifos	Organophosphorus	19.1	197	199	314	
Pendimethalin	Dinitroanline	20.1	252	162	253	191
Chlorfenvinphos	Organophosphorus	20.5	267	269	323	
Endosulfan, alpha-	Organochlorine	21.2	195	241	237	239
Profenofos	Organophosphorus	22.0	337	339	139	97
Dieldrin	Organochlorine	22.1	79	81	263	
Kresoxim-Methyl	Strobilurin	22.7	116	131	206	132
Endosulfan, beta-	Organochlorine	23.1	195	241	237	239
o,p, DDT	Organochlorine	23.5	235	237	165	199
p,p, DDT	Organochlorine	24.6	235	237	165	199
Propiconazole 2	Triazole	24.7	173	259	175	261
Epoxiconazole	Triazole	25.6	192	165	138	194
Cypermethrin 1	Pyrethroid	31.6	163	165	181	
Azoxystrobin	Strobilurin	35.8	344	388	345	

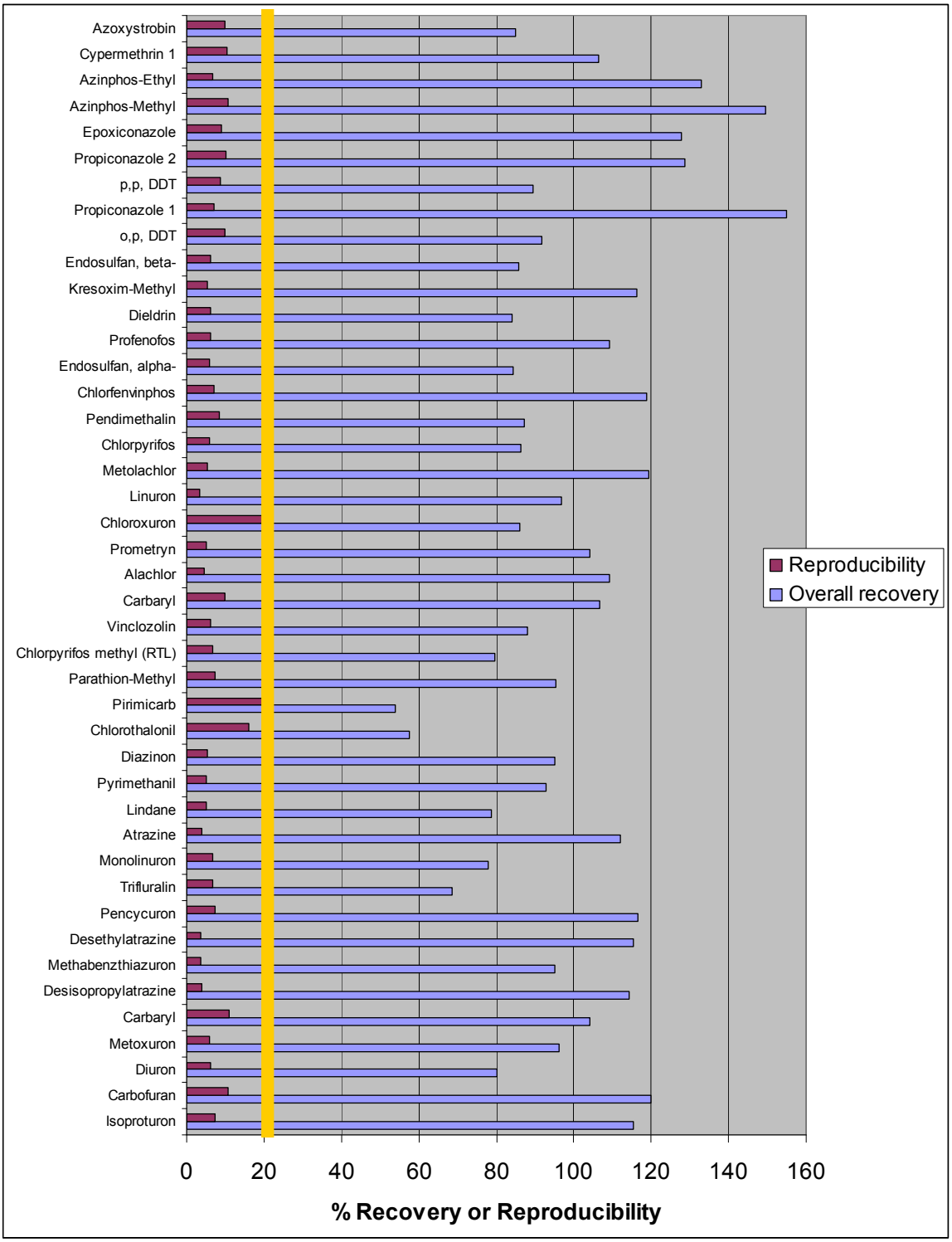

Figure 13: Method recoveries and the reproducibility values reported as percentages. The Codex reproducibility limit is indicated as a yellow line.

3.7. Analysis of the Antiviral Drugs Acyclovir and Valacyclovir-Hydrochloride in Tsetse Flies (*Glossina pallidipes*) Using LC-MSMS

Tsetse flies (Diptera: *Glossinidae*) are vectors for trypanosomes, the etiological agents for two major zoonoses, human African trypanosomosis (sleeping sickness) and animal African trypanosomosis (nagana). Human infection rates are estimated at 300,000 to 500,000 people with up to 60 million others at risk in 36 African countries. The disease has been ranked 7th in sub-Saharan Africa in terms of disability-adjusted life years, and is the second globally ranked parasitic disease after malaria. Besides human suffering, sleeping sickness causes an estimated economic loss of US$4.5 billion per annum. Thus, tsetse flies and the trypanosomal infections they transmit are one of the greatest reasons for poverty and perpetual underdevelopment in Africa. Fortunately, tools exist to combat the disease through vector control, such as sterile insect technique (SIT), an important component of an integrated pest management strategy. SIT involves mass production and release of sterile male tsetse flies.

However, the syndrome *Glossina pallidipes* Salivary Gland Hypertrophy, which is associated with reduced fecundity and sterility in infected flies, presents a major obstacle to the mass rearing of tsetse flies and therefore detrimentally affects SIT as a measure for eradication or control of trypanosomosis, with immense health and economic implications.

One possibility being investigated by the Entomolgy Unit for the mitigation of this problem is to use the antiviral drugs acyclovir (9-[2-hydroxyethoxy]-methylguanine) or its L-valyl ester pro-drug valacyclovir (L-valine-2-[(2-amino-1,6-dihydro-6-oxo-9H-prin-9yl) mehoxy] ethyl ester). The pharmacokinetics/bioavailability of these drugs in vertebrates is well documented. For example valacyclovir, a known pro-drug of acyclovir, is almost completely converted to acyclovir when administered orally. However, the pharmacokinetics of these drugs in invertebrates such as tsetse flies is not known.

The Agrochemicals Unit developed an analytical method to assess the profiles of these drugs in treated tsetse flies to assist in the research being undertaken by the Entomology Unit. Liquid chromatography – tandem mass spectrometry (LC-MSMS) was the technique of choice because of the sensitivity and specificity/selectivity of the technique.

3.7.1. Experimental

Tsetse fly samples (~220 mg) were weighed into 25 mL glass conical test tubes and ground using a glass rod until a homogeneous paste was formed. The samples used for recovery studies were fortified at this point. At least two blank controls were prepared with each batch for use to prepare matrix-matched calibrators. The samples were left to stand for 15 min before adding 5 mL of the extraction solvent (methanol:acetonitrile; 70:30, v/v containing 0.1% acetic acid). Capped test tubes containing the homogenized samples were then placed in 500 mL beakers containing water (approximately 1/3 full) in an ultrasonicator for 25 min.

After the ultrasonic extraction, the extracts were vortex-mixed for 1 min. Where necessary the solid material was disturbed/broken with a pasteur pipette. The contents were then transferred into 15 mL Sarstedt tubes and centrifuged. The supernatants were transferred into new 15 mL Sarstedt tubes containing 1 g of a mixture of $MgSO_4$ and MSPD C18 (1.5:0.5, w/w), vortex-mixed for 1 min and centrifuged. The supernatants were filtered, with washings, into 25 mL conical glass test tubes containing either 0.5 mL (samples & recoveries) or 0.4 mL (for matrix mathched calibrators) of dimethylsulphoxide (DMSO). The extraction solvent was evaporated from the filtrates under a stream of nitrogen using a TurboVap evaporator set at 50 °C,

leaving the extracts in DMSO. Matrix-matched standards ranging from 0.45 to 4.5 µg/g were prepared. All samples were filtered through 0.45 µm filters prior to analysis by LC-MSMS.

For sample analysis, the target analytes were separated using isocratic reversed-phase chromatography on a C_{18} Gemini analytical column fitted with a guard cartridge. The mobile phase consisted of methanol: acetonitrile: water (60:30:10, v/v/v) plus formic acid (0.1%) at a flow rate of 0.25 mL/min and the column temperature was set at 45 (±5) °C. The run time was 8 min and the injection volume was 10 µL. The autosampler temperature was set at 22 °C (±2 °C). Detection and quantification were achieved using a triple quadrupole mass spectrometer with electrospray ionization (ESI) in positive mode. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode and optimized to measure a precursor ion and two daughter ions for each analyte.

The method was validated using blank tsetse matrix, spiked blank matrix and incurred samples prepared by feeding tsetse flies blood meal treated with acyclovir and valacylovir.

3.7.2. Results

The extraction technique was optimized in terms of simplicity and speed. The simple solid phase dispersion clean-up using $MgSO_4$ and C_{18} material was adequate for this potentially difficult matrix. Initial problems with loss of recovery at the evaporation stage were overcome by using dimethylsulphoxide (DMSO) as a "keeper" rather than allowing the extract to evaporate to dryness.

Positive electrospray mass spectra for the two drugs are shown in **Figure 14**.

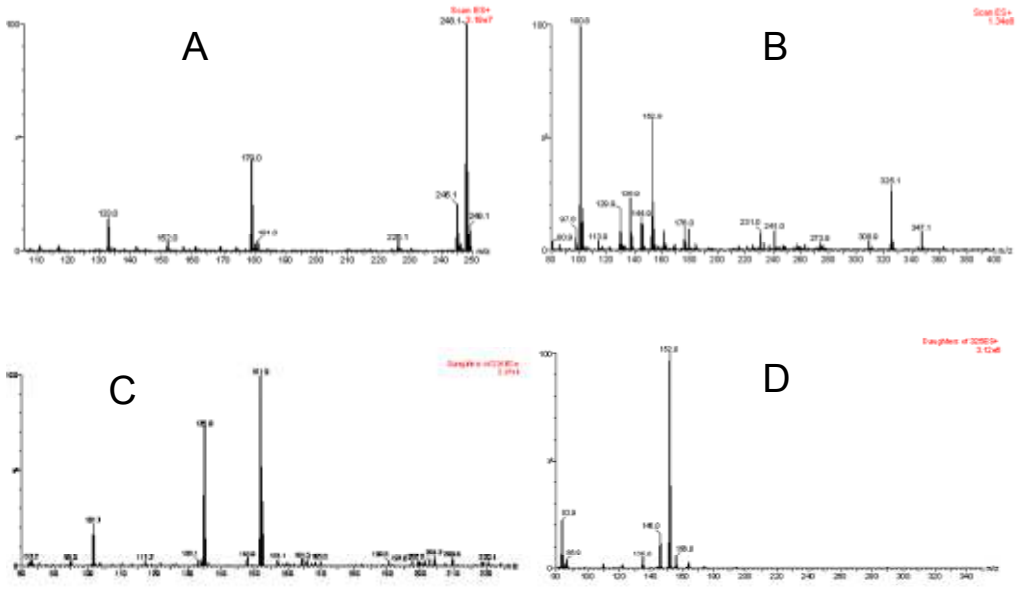

Figure 14. Positive ion electrospray mass spectra of (A) acyclovir precursor ion at m/z 226.1; (B) acyclovir product ions at m/zs 151.9 and 135; (C) valacyclovir precursor ion at m/z 325; (D) valacyclovir product ions at m/zs 152 and 83.9

The pseudo-molecular ions at m/z 226.1 and 325.1 were determined as the protonated precursor ions $[M+H]^+$ for acyclovir and valacyclovir-HCl, respectively, with the most abundant daughter ion at m/z 152 for each drug, possibly representing a protonated guanine species.

Given that acyclovir and valacyclovir are analogues and had closely related MSMS fragmentation patterns, chromatographic separation was essential. Typical chromatograms for blank and spiked tsetse matrix are shown in **Figure 15** (A & B). No interfering matrix peaks were obtained at the retention times of the target analytes.

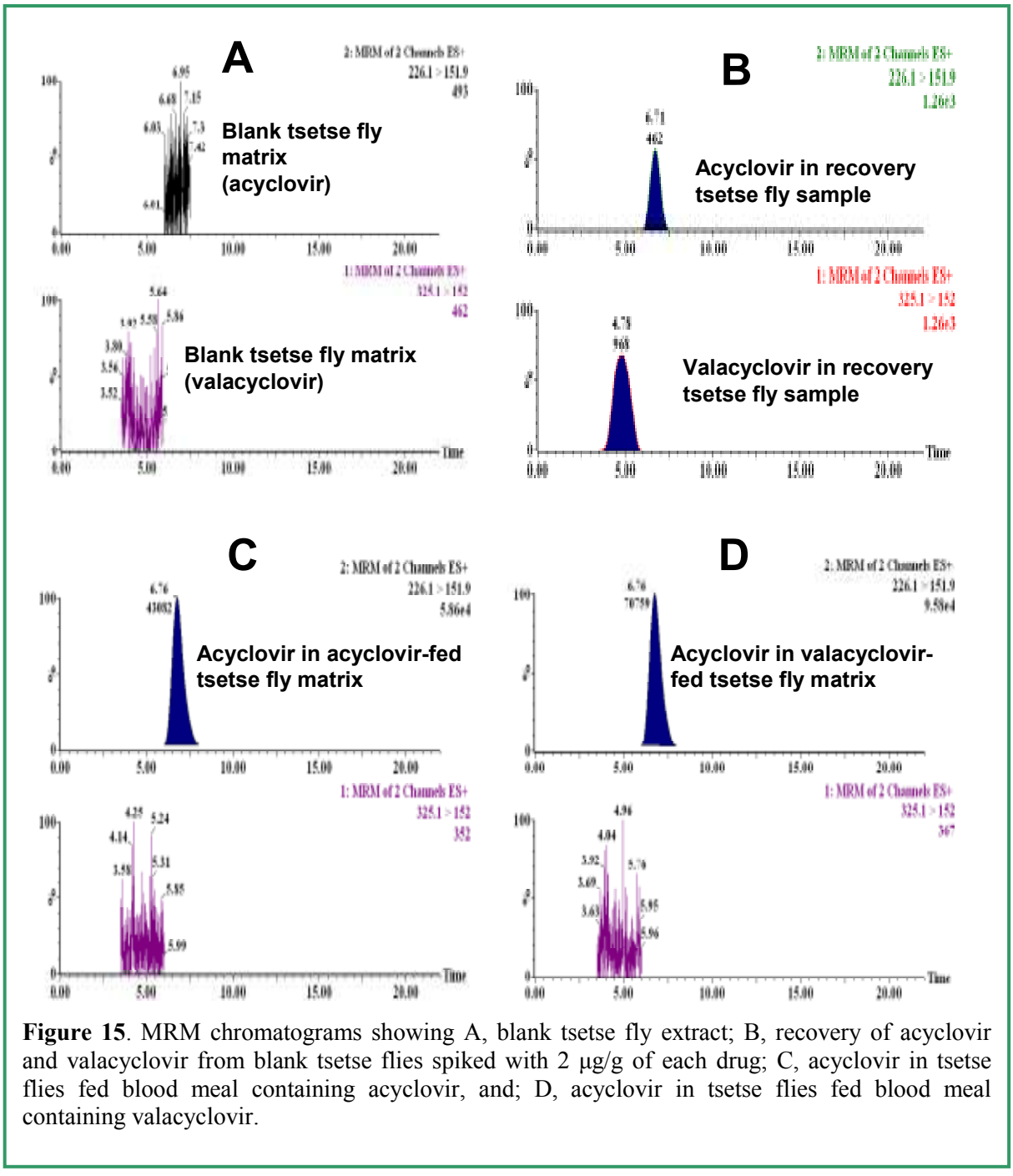

Figure 15. MRM chromatograms showing A, blank tsetse fly extract; B, recovery of acyclovir and valacyclovir from blank tsetse flies spiked with 2 µg/g of each drug; C, acyclovir in tsetse flies fed blood meal containing acyclovir, and; D, acyclovir in tsetse flies fed blood meal containing valacyclovir.

The limit of detection (S/N ≥ 3) and limit of quantification (S/N ≥ 10) for both compounds were estimated as 0.0625 and 0.2µg/g, respectively. Both acyclovir and valacyclovir-HCl gave a linear response over a calibration range of 0.45 to 4.5µg/g of the drugs in matrix.

The recovery and precision of the method are summarized in **Table 7**. The overall mean recoveries were 94.7 and 91.7% for acyclovir and valacyclovir, respectively, with within-laboratory reproducibility values of 3.4 and 4.4%, respectively.

Table 7. Recovery and precision of the method for tsetse fly matrix. The experiment was done on 3 different occasions, on the third occasion by a different operator.

Occasion	Fortification concentration (µg/g)	Recovery and precision (CV, %)	
		Acyclovir	*Valacyclovir - HCl*
Day 1(op1)	0.5	99 (1.9)	91 (1.5)
	1	95 (3.8)	93(1.4)
	2	97 (2.4)	96 (2.5)
Overall Day1	***All three levels***	***97(2.7)***	***93 (1.8)***
Day 2 (op1)	0.5	96 (0.9)	97 (1.1)
	1	95 (1.1)	94 (0.8)
	2	98 (1.2)	94 (0.8)
Overall Day 2	***All three levels***	***96 (1.1)***	***95 (0.9)***
Day 3 (op2)	0.5	95.4 (0.7)	90.2 (6.1)
	1	95.8(2.4)	89.1 (4.4)
	2	81.8 (2.0)	82.2 (5.6)
Overall Day 3	***All three levels***	***91 (1.7)***	***87.2 (5.4)***
Overall Days 1-3	**All three levels**	**94.7 (3.4)**	**91.7 (4.4)**

Application of the method to detect the two drugs in flies fed blood meal containing either acyclovir or valacylovir showed that acyclovir was detected in both groups, whereas valacyclovir was not detected in either set of flies at the method performance limits, as shown in **Figure 15** (C & D). This is consistent with reports in the literature that valacyclovir is a pro-drug for acyclovir and that, in vertebrates, almost all of the valacyclovir is converted to acyclovir. These results suggest that the same transformation occurs when the drugs are used in tsetse flies.

3.7.3. Conclusions

A new simple, sensitive and precise LC-MSMS method was developed and validated for the determination of valacyclovir-HCl and acyclovir in tsetse flies. The method is being applied in current and future applied research, in collaboration with the Entomology Unit at Seibersdorf, into mass rearing strategies for tsetse flies to support the sterile insect technique as a method to control human and animal trypanosomosis in sub-Saharan Africa. The method would also be applicable, with appropriate validation, to the detection of valacyclovir-HCl and acyclovir in other biological matrices.

3.8. Food Safety, Traceability and Authenticity

3.8.1. Background

A combination of the increasingly global food market, involving complex production and distribution patterns, and the growing number of food related health incidents has lead to a need for greater transparency of food supply chains. Creating this transparency requires the ability to trace food products and ingredients rapidly and precisely. The main drivers for this are food safety, certification requirements, compliance management, production control and rationalisation, supply chain communication and competitive advantage.

A main focus of the work of the Food and Environmental Protection subprogramme in the next biennium and beyond will be the application of stable isotope measurement techniques for food traceability, authentication and the detection of food adulteration. Stable isotope analysis provides a powerful investigative and regulatory tool to address these issues. Research and capacity building in this field is in line with current and past subprogramme activities in the application of nuclear and related techniques for the development of holistic farm-to-fork food safety systems through the development of laboratory capabilities and regulatory systems. The Agency is in a unique position to coordinate and lead the integration of isotope measurement methods with complimentary techniques to provide feasible systems for food traceability, verification of authenticity and detection of adulteration on a global basis.

In 2009, the Agrochemicals Unit acquired an isotopic water liquid analyser operating on the principle of wavelength-scanned cavity ring-down spectroscopy. This instrument has the potential to perform isotope ratio measurements for deuterium/hydrogen and $^{18}O/^{16}O$ in water to support traceability applications at a fraction of the cost of isotope ratio mass spectrometry, and is also portable, with potential field applications. Future applied research in the Unit will focus on this and similar techniques.

A consultants' meeting will be convened in Vienna in March 2010 to plan a coordinated research project in the field of food traceability and authenticity, with isotope ratio measurement as the central theme. The project is planned to commence in 2011.

3.8.2. International collaboration on traceability/authenticity

During 2009, the Agrochemicals Unit Head participated in two symposia in the field of food traceability/authenticity in 2009; the CSL/JIFSAN (Central Science Laboratory, UK/Joint FDA-University of Maryland Institute for Food Safety and Applied Nutrition, USA) Joint Symposium on Food Safety and Nutrition: Methods and Systems for tracking, Tracing and Verifying Foods, 13-15 May 2009, Greenbelt, Maryland, USA, and the Symposium on Food Safety, Traceability and Authenticity: Opportunities and Challenges for the Agri-Food Industries, held in Belfast, UK, 29 September 2009. The Unit Head also accepted an invitation to join the Scientific Committee to organize an international symposium on "Food Integrity and Traceability" to be held in the UK in March 2011.

The focus of the 10th annual CSL/JIFSAN symposium was on tracking, tracing and verifying food throughout the supply chain. The symposium provided an overview of advances in research related to the traceability of foods and ingredients and tools for electronic exchange of product information within the food supply chain. The meeting had approximately 100

participants. Presentations were given by speakers from regulatory agencies, public interest groups, universities and research institutions in Europe and North America and researchers involved with the EU TRACE project. Symposium sessions include discussions on advances in analytical technologies to characterise the origin of food products, application of technologies for tracking food through the supply chain, IT tools for electronic exchange of information, and barriers to adopting these technologies.

The Unit Head presented a poster entitled "Food traceability and authenticity in developing countries through isotope ratio techniques". The poster outlined the need, in the context of farm-to-fork food safety systems, for techniques to trace food products to their source in order to facilitate corrective actions when contamination is detected, emphasised the related issues and problems in less-developed countries, including traceability, authenticity and food adulteration, and presented a planned IAEA coordinated research project to address these issues through the development and application of stable isotope measurement techniques. The outcomes of the project are expected to be enhanced food safety and consumer protection and compliance with standards for food composition and identity, resulting in the ability to meet regulatory requirements and the expansion of international trade in foodstuffs.

Data collected during the EU-funded TRACE project formed the basis for discussions on methods for model development and verification using a holistic approach, with emphasis on the translation of laboratory studies into the marketplace and on the provision of easily understood measurement indicators of relevance to the international consumer.

The symposium "Food Safety, Traceability and Authenticity: Opportunities and Challenges for the Agri-Food Industries" was held to mark the opening of a new research centre, the Centre for Food, ASsured SafE and Traceable (Food ASSET), within the Institute of Agri-Food and Land Use, at Queen's University Belfast, UK. The objective of the centre is to harness scientific knowledge and develop evidence based approaches for assessing risks and hazards associated with agri-food supply chains. The Centre will use this knowledge to develop innovative ways to enhance food safety and traceability that will support the future economic development of the agri-food sector. A key focus of ASSET is the formation of strategic linkages with an array of agri-food industries, multi-national retailers and international research groups to ensure sustainability and recognition as a global centre of excellence in food safety and traceability.

The symposium had approximately 90 participants from academia, the private sector and regulatory authorities, mainly from UK and Ireland.

The Agrochemicals Unit Head presented a keynote lecture entitled "Food Traceability and Authenticity – Global Issues". The presentation introduced the new and planned work of the Joint FAO/IAEA Programme in food traceability and gave an overview of the need for effective traceability systems on an international scale, with examples of recent food safety incidents and issues world-wide due to lack of effective traceability systems. The key focus was on the development of analytical technologies for the verification of paper-trail traceability systems and to address the traceability of food commodities and contaminants internationally where records are insufficient or where accidental or fraudulent misrepresentation of goods is a problem. The measurement of stable isotopes provides a unique and powerful tool for this purpose, facilitating direct traceability of the actual product regardless of the labeling or record trail.

A second presentation by Mr. Paul Brereton, Head of FERA International, focused on food authenticity issues, and led to discussions on collaborations between the Joint Programme and the partners in an EU 6th Framework integrated project, "TRACE". To follow this up, a meeting was arranged between Joint Division staff and Mr. Brereton in Vienna to further discuss collaborative activities in this field.

Discussions were held with Prof. Chris Elliott, Director of the Institute of Agri-Food and Land Use and Dr. Luc Rock, Head of the isotope ratio mass spectrometry laboratory in the ASSET Centre. The Centre is well financed, well equipped, addresses various technical and supply chain management aspects of food traceability and safety and has good support from the private sector. Prof. Elliott expressed his keen interest in working with the Agency in some form of partnership and affirmed the commitment of the centre to contribute to traceability research and capacity building projects on an international basis. In this regard, he also offered to host research coordination meetings or consultants' meetings related to the Agency's projects at the ASSET Centre. The ASSET centre is a well equipped, dynamic research centre with strong links to industry and to other research centres world wide and is an excellent potential Collaborating Centre for work in the field of traceability for food safety.

A number of other research institutes have also volunteered to collaborate with the Agency in this field, including the Institute for the Application of Atomic Energy, Chinese Academy of Agricultural Sciences (CAAS); the Institute of Agro-Food Science and Technology, CAAS; the Nuclear Chemistry Research Division, Korea Atomic Energy Research Institute; the Institute of Chemical Technology, Prague, Czech Republic; the European Commission Joint Research Centre; the Federal Institute for Risk Assessment, Berlin, Germany; the Institute of Food Research, Norwich, UK; Teagasc (Ireland's Agriculture and Food Development Agency) and the Austrian Institute of Technology (AIT Seibersdorf GmbH).

The international issues with respect to food traceability raised a lot of interest with the participants in both symposia, reflecting the global nature of the food supply chain in today's market and the recent development of international regulations and guidelines, both governmental and industry-driven, for traceability systems to facilitate trade. It is clear that food traceability and authenticity has become an important issue worldwide. Most of the effort to establish traceability systems has focused on developing electronic "paper-trail" and labeling strategies. Research on the development of technologies to verify these systems, or to provide direct information on the geographical origin of food commodities to combat fraud, is currently fractionated at best. The Agency is well recognized in the field of stable isotope measurements, largely due to the use of reference materials produced by the Isotope Hydrology Laboratory, and amongst the researchers at the symposia it was both expected and welcomed that the Agency would take a lead role in the coordination of activities to develop stable isotope techniques for food traceability applications.

3.9. Coordinated Research projects

3.9.1. Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale (D5.20.35)

The Second Research Coordination Meeting (RCM) of the Coordinated Research Project (CRP) on Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale was held at IAEA Headquarters in Vienna, Austria, from 9-13 February 2009. The meeting was attended by research contract/agreement holders from Argentina, Australia, Brazil, Bulgaria, Chile, China, Costa Rica, Cyprus, Ecuador, Germany, Kenya, India and the Philippines, as well as observers from Costa Rica and Slovakia.

The objectives of the meeting were to share and disseminate the results of the first two years of the programme, to agree on a work plan for the next two years of the project and to strengthen the role of participating laboratories in the assessment of the implementation of good agricultural practices (GAP). Specifically, to:

- consolidate the network of laboratories to assess indicators of pesticide management practices in water and soil/sediment samples;
- disseminate information about the results obtained from the first two years of work;
- revise individual work plans for the next two years of the project;
- fine tune the risk assessment results using the pesticide impact rating index (PIRI);
- update skills in the analysis of pesticide residues in water/soil/sediments;
- disseminate information about bioassays relevant to the CRP, and;
- familiarize participants with the use of flow meters, GPS and GIS and new LIMS developments.

Discussions at the meeting included a reminder of the specific objectives of the CRP; a review of related regional initiatives (RLA/5/050 and RLA/5/053); a lecture on pesticides and their degradation products as emerging pollutants in the context of risk assessment in European river basins; an insight into the processes of agricultural non point-source contamination and pesticide monitoring based on first tier risk assessment; an introduction to bioassays and to GIS; an update of current LIMS resources as well as a compendium of analytical methods, and; a lecture on the use of stable isotopes.

One day was spent at the Agrochemicals Unit laboratory at Seibersdorf, where two analytical protocols were demonstrated and the participants were trained on the use and deployment of a flow meter. All research contract holders presented their results to date under the CRP. The discussions among Research Con-tract/Agreement Holders during the RCM aided the revision of individual work plans.

The following conclusions and recommendations were agreed to by the meeting.

The Meeting, while noting the importance of:

- Networking, training-the-trainers, and raising awareness of case studies on catchment monitoring, and the opportunities provided by the 12th IUPAC Pesticide Congress of pesticide chemistry;
- Revising 2009 work plans to achieve preliminary outputs from the 2009 sampling campaigns and sharing findings with stakeholders;
- Value-adding by harmonizing hazard identification, sampling approaches, analytical approaches and compliance with water quality standards achieved under the EU Water Framework Directive (WFD) and Groundwater Directive;
- Sampling limitations and a concomitant risk of missing contamination events and the value of estimating pesticide loads;
- Developing a better understanding of the tools and the initiatives by the FAO/IAEA Soil and Water Management & Crop Nutrition subprogramme to characterize processes contributing to contaminant transport at a landscape scale, and;
- Value-adding through inter-laboratory comparisons and the use, where appropriate, of stable isotopes and radio-labelled pesticides for trouble-shooting, facilitating the establishment of method performance parameters, and to understand the transport pathways;

agreed to the following recommendations:

- To participate and interact as a group with international experts at the 12th IUPAC International Congress of Pesticide Chemistry (Melbourne, Australia; 4-9 July 2010), present preliminary results from monitoring indicators of GAP and to hold the 3rd RCM in Melbourne, Australia, immediately before or after the IUPAC Congress.
- To disseminate preliminary results to stakeholders and the scientific community from case studies showing the benefits of monitoring indicators of GAP, and the value of a research and development levy to facilitate work that will result in prevention of contamination at source.
- To apply, where possible, harmonized approaches such as that of the EU Water Information System for Europe (WISE) project
- To implement, where possible, sampling approaches to characterize pesticide loads from representative land management units by use of passive samplers, bioassays (in situ/laboratory), bioindicators, automated sampling techniques including Doppler flow meters for estimating stream flow.
- To participate and contribute to working groups established under RLA5050 on updating first-tier risk assessment, analytical methodology (including QuEChERS for sediments/suspended particles), bioassays, mass spectrometry, GIS, LIMS, as well as the creation of a new working group on data quality.
- To participate in further inter-laboratory comparisons and, where appropriate, use stable isotopes and radio-labelled pesticides to improve analytical skills, the establishment of method performance parameters and to investigate pesticide parameters.

3.9.2. Development of Radiometric and Allied Analytical Methods to Strengthen National Residue Control Programs for Antibiotic and Anthelmintic Veterinary Drug Residues (D5.20.36)

There has been rapid growth in livestock and aquaculture sectors in many developing countries as a result of growing economies, resulting in changing production practices and an increase in international trade in food products of animal origin. However this growth has been accompanied by a rise in disease outbreaks and the use of veterinary medicines, in particular antibiotics and anthelmintics. Although many countries encourage responsible use of these medicines, there are significant constraints, including availability of suitable analytical methods to detect the presence of residues resulting from their use.

A coordinated research project (CRP) was developed after extensive consultation with stakeholders, including participants in previous CRPs, regulatory and scientific experts of authorities in member states and consultants to identify areas of concern to less developed countries. The main purpose of the CRP is to assist National Reference Laboratories of FAO and IAEA member states in meeting the need for effective and appropriate monitoring methods for residues of selected antibiotic and anthelmintic veterinary medicines through the development and application of screening methods that exploit the advantages (robustness, sensitivity, transferability) of radiotracer detection methods, in conjunction with confirmatory techniques using stable-isotope labelled molecules.

In order to promote effective control policies to prevent/minimize drug resistance, emphasis will be placed on anti-parasitic drugs widely used in developing countries, and compounds highlighted by the Joint FAO/WHO/OIE Expert Meeting on Critically Important Antimicrobials. The project will establish a harmonised network of expertise able to share knowledge and transfer technology to strengthen national residue control programs of Member States to improve food safety, combat drug resistance and comply with harmonized Codex standards.

Applications to participate were received from 22 countries and after a rigorous selection process 11 Research Contracts were awarded in May 2009 to participants from Brazil (2), China (2), Kenya, Korea, Mongolia, Peru, Sri Lanka, Thailand and Tunisia. Additionally 4 Agreement Holders from Austria, Belgium, Germany and UK and 2 Consultants from the Netherlands and USA were invited to join the project.

The 1st RCM for the project was held in Vienna, 19-23 October 2009. The RCM brought together the contract and agreement holders, consultants and staff of the Food and Environmental Protection Subprogram to review plans and work done to date by the participants. Based on this, existing plans were refined in view of priority areas identified by the Consultant's meeting. Two days were spent in formal presentations, followed by discussions over three days to formulate priorities and research plans. One afternoon consisted of a joint session with the FAO/IAEA Train-the-Trainers Workshop on Screening/Post-screening Techniques for Veterinary Drug Residues held at the Agency's Laboratories, Seibersdorf, Austria, from 12 to 23 October 2009. During this session, presentations were made by the Agreement Holders in their areas of expertise.

The RCM agreed that the main purpose of the CRP was to assist National Reference Laboratories of FAO and IAEA member states in meeting the need for effective and appropriate monitoring methods for residues of selected antibiotic and anthelmintic veterinary

medicines through the development and application of screening methods that exploit the advantages (robustness, sensitivity, transferability) of radiotracer detection methods, in conjunction with confirmatory techniques using stable-isotope labelled molecules, with research outputs providing knowledge on:

- pharmacokinetics of veterinary drugs in aquaculture fish species previously not investigated;
- improved screening and confirmatory methods fit for use in National Residue Control Programmes;
- sources of natural antimicrobial compounds likely to impact the regulatory framework for veterinary drug residues;

and providing new tools to understand and assess the environmental impact of the use of veterinary medicines.

The meeting recognised the importance of exchange and sharing of research materials and technology. To facilitate this, Agreement Holder Prof. Hubert De Brabander has set up a dedicated section for the CRP on the website of the Faculty of Veterinary Medicine, University of Gent, Belgium.

Participants in the 1st RCM at IAEA.

The meeting concluded that the use of radionuclides is vital to generate new knowledge, especially in areas such as the pharmacokinetics of veterinary drugs used in aquaculture, to underpin effective control programs and that the project results should be disseminated through scientific publications and presentation to a wide range of stake holders, mainly scientific and regulatory communities. The need for additional agreement holders to facilitate the research activities has also been recognised.

The Agrochemicals Unit will play an important role in this CRP, providing coordination, technical advice, applied research and technology transfer. This CRP forms a unique and global network of scientific expertise addressing complex and important food safety challenges and its successful implementation will result in improved food and feed quality and safety in FAO/IAEA Member States and further help developing countries to access major global food markets. Results of the selected projects will assist regulators in the development of new guidelines and regulations pertaining to food safety and the environmental impact of veterinary drugs.

3.10. EU Projects

3.10.1. Improving the Quality and Safety of Beef and Beef Products for the Consumer in Production and Processing (ProSafeBeef)

The EU Framework 6 Integrated Project "Improving the quality and safety of beef and beef products for the consumer in production and processing" (ProSafeBeef), is a €19 million project, with €10.87 million contributed by the EU, involving 42 multidisciplinary partners from 19 countries. A major objective of the project is to assist the EU, national policy makers, regional authorities (including developing countries involved in the programme for international scientific cooperation, INCO) and the beef industry to reduce the burden of microbial and chemical related illnesses due to the consumption of beef and beef products.

The Agrochemicals Unit is a partner in work package 4, 'chemical residues', of Pillar 1 (WP1.4), 'Quantitative risk assessment for microbial and chemical contaminants,' of the project. The Unit works closely with the other partners in WP1.4; Microbioticos Laboratories (Brazil), RIKILT Institute of Food Safety (The Netherlands), the Agri-Food and Biosciences Institute (UK), Ashtown Food Research Centre/Teagasc (Ireland), and OCU - Organización de Consumidores y Usuarios (Spain).

The Agrochemicals Unit Head participated in an international conference "Advancing beef safety through research and innovation", which was held under the ProSafeBeef project by the Teagasc Ashtown Food Research Centre (AFRC), Dublin, Ireland, , 25-26 March 2009.

The Unit Head chaired the conference session on chemical contaminants, which covered various aspects of analytical methodology for screening and determination of contaminants such veterinary drugs, dioxins and dioxin-like PCBs in food, and risk management strategies and case studies.

The Unit Head also presented a poster entitled "A multi-residue isotope dilution liquid chromatography-tandem mass spectrometry (LC-MSMS) method to support risk assessment for anthelmintic drug residues in beef" which summarised development work carried out by the Agrochemicals Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf as a partner in the ProSafeBeef project. The focus of the development work was the adaptation and validation of new methodology for transfer to laboratories in less developed countries, with Microbioticos Laboratories in Brazil as the primary counterpart. Further dissemination of the method will be achieved through training carried out in Microbioticos laboratories, including fellowship training under the regional TCP RLA/5/055 "Establishing a South American Regional Network of National and Reference Laboratories for Pharmacologically Active Substances and Contaminants in Food of Animal Origin through the Implementation of Approved Nuclear & Conventional Analytical Techniques (ARCAL CIV)".

The conference presented various interesting approaches to assuring the quality and safety of beef products, addressing the issue from a holistic "farm-to-fork" angle, with control issues throughout the production, processing, distribution and consumer phases. Many of the concepts introduced will be considered for application in IAEA projects.

The conference was followed by a meeting of the partners involved in workpackage 1.4, "Chemical Residues" of the ProSafeBeef project on 27 March 2009. The Agrochemicals Unit Head represented the Unit as a partner in the project.

The meeting discussed the progress of method development, which is on schedule, and technology transfer, which is the responsibility of the IAEA partner (Agrochemicals Unit, Seibersdorf), and sampling plans and protocols for risk assessment.

The multi-residue isotope dilution method for residues of 38 anthelmintic drugs in meat is already operational in the Agrochemicals Unit (see section 3.1). As a follow-up to the meeting, a member of the Unit staff spent one week at the AFRC laboratory in Ireland to refine the method and harmonise data analysis protocols. A scientist from the Microbioticos laboratory in Brazil will join the Agrochemicals Unit in June for two months, funded by the ProSafeBeef project, to be trained in the method and assist in its validation, thereby facilitating transfer to Microbioticos and to other South American laboratories thereafter.

The isotope dilution method for anthelmintic residues developed under the ProSafeBeef project is an extremely powerful tool both for risk assessment studies and for regulatory control of the residues. The Agency has an important role to play in both the adaptation of the method for its application in developing countries and in transfer of the technology. The method will benefit laboratories not only in Europe, but also in many IAEA TC counterpart laboratories and in countries throughout the world.

3.10.2. New Technologies to Screen Multiple Contamnants in Food (BioCop)

The 4th annual meeting of the EU 6th Framework Integrated Project "BioCop' was hosted by the Laboratoire d'Hormonologie, Centre d'Economie Rurale (CER) Groupe, Marloie, Belgium, 26 -27 November 2009. The Agrochemicals Unit Head participated in the meeting as acting Chair of the project Advisory Board and gave a presentation on Friday 27 November summarising the observations of the Advisory Board as the project enters its final phase.

The project focuses on the development and implementation of new methods to monitor and control the occurrence of multiple chemical contaminants in foods through the use of advanced sample preparation techniques & emerging biotechnological screening approaches. The main project objectives include the development of novel screening methods to detect multiple chemical contaminants in foods, training of scientists in the developed technologies, and widespread dissemination of project results and information.

A wide range of techniques have been developed for application to the detection of food contaminants, utilizing technologies such as transcriptomics, proteomics, molecular immunology, microarrays, biosensor technology, bioinformatics and mass spectrometry, and resulting in a number of rapid tests that can detect and identify many types of toxins in foods. The results include techniques suitable for routine regulatory monitoring and also those that are more suited to research applications to underpin food safety policy development.

A significant development in the final phase of the project is the dissemination of methods and techniques to developing countries through the inclusion of a new project partner for training and technology transfer in Thailand. This was in response to recommendations from the Advisory Board at an early stage of the project that the technologies developed should be made available to countries outside Europe, and especially to developing countries, to

enhance their food safety and help to establish equivalence of food safety standards with those of the EU and other major trading blocks, thereby enhancing international trade in food commodities. The first training activity for countries in the East Asia/Pacific region will take place in June 2010.

The project partner in Thailand, Dr. Sasitorn Kanarat, has interacted in IAEA projects in the past and is currently a Research Contract Holder under the CRP "Development of radiometric and allied analytical methods to strengthen national residue control programs for antibiotic and anthelmintic veterinary drug residues" (D5.20.36).

Participation of an Agency representative in an advisory capacity in this type of project helps to facilitate the effective transfer of the technologies developed to a wider customer base, including IAEA and FAO developing country member states that have no chance to undertake the primary development themselves. This adds value to the project outcomes through the enhancement of food safety standards both within and outside the EU, and through increased trade between developing countries and the major trading blocks of the developed world.

Further information on the BioCop project is available at http://www.biocop.org/.

3.10.3. Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure (CONffIDENCE)

The first annual meeting of the EU 7th Framework Project "Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure" (CONffIDENCE) took place in Barcelona on 5-6 March 2009. This 4-year project has 17 partners from 10 countries and a budget of € 7.5 million, € 5.8 million from the EC. The main objective of the project is the development of novel, multiplex screening methods for a wide range of contaminants in high-risk products such as fish and cereal-based food and feed, and vegetables. The validated methods will be applied to provide data for risk assessment and for regulatory systems for food safety. The meeting had approximately 50 participants from the project consortium, management and advisory board.

The Food and Environmental Protection Laboratory Head participated in the meeting as chair of the project advisory board (AB).

At the plenary session on the first day of the meeting, held at Institut d'Estudis Catalans, reports on the progress of the project were given by the project coordinator and by representatives of each of the workpackage clusters. Research and method development is, to a large extent, progressing as planned. Questions, comments and discussion followed each presentation, and many points were raised by the AB members for clarification or consideration by the project consortium. Key points were the applicability of the methods and their suitability to cover a sufficiently broad scope of contaminants to effectively support EU policy and reflect risk assessments and scientific opinions published by the European Food Safety Authority (EFSA) and by bodies such as the Joint FAO/WHO Codex Alimentarius. Such risk assessments are important not only within the EU, but are used by many other countries as a basis for national legislation or guidelines.

The AB meeting was held in IIQAB on 6 March. Agenda items included a discussion on the response of the Project Management Board (PMB) to the first AB report, following the project kick-off meeting, comments on the progress of the research reported on the first day of

the meeting, identification of potential additional members of the AB, and agreement on the timeline for the AB report to the PMB. In addition to the technical recommendations to be forwarded to the PMB, several names were suggested as additional AB members to broaden the scope of experience and expertise of the board. The other AB members agreed with a proposal from the chair that a representative from a developing country, with broad experience in the policy and practicalities of food contaminant monitoring in developing countries in general, would be a useful addition. An individual who has worked with IAEA in many projects in the past was suggested.

The technologies under development in CONffIDENCE will be of importance not only within the EU, but also in many countries world wide, and will be especially relevant to those developing countries that must demonstrate equivalence of their food safety standards with those of the EU in order to establish or maintain trade with the EU in food commodities. The inclusion of a representative of developing countries on the project advisory board, will help to ensure that the technologies and methodology that result from the project will be, where appropriate, of benefit to countries outside the EU, particularly less developed countries. The technologies developed should be directly applicable in a number of current IAEA TCPs and would enhance the analytical and regulatory capabilities built under those projects.

3.11. Supporting Research

In addition to the research and development carried out directly in the laboratories at Seibersdorf, the expertise and experience of the Unit in research and capacity building for food safety, especially with our network of laboratories and institutes in developing countries, is frequently called upon externally. The examples below, from 2009, include keynote presentations at research policy making symposia, collaborations with other UN Agencies, NGOs and others, and the application of research results to build capacity.

3.11.1. Research in Support of Science-Based Regulations: Challenges for Producers and Consumers; Prague, Czech Republic; 21-22 April 2009

This conference, which was held under the Czech Republic's Presidency of the European Union, was organised by the Ministry of Agriculture of the Czech Republic, the Institute of Chemical Technology in Prague and the European Food Safety Authority. The aims of the conference were to support the use of food research for better assessment of risks and drafting of legislation necessary to ensure safe and high-quality food and to strengthen cooperation on both national and international level. The conference had approximately 200 participants from regulatory agencies, scientific institutions, academia and industry, mainly from Europe, but also including representatives of, for example, U.S.A., New Zealand, Australia and Canada.

The conference was opened by the Minister of Agriculture of the Czech Republic, Mr. Petr Gandalovič, who stressed the importance of food research on a global basis. Presentations were given by a number of Czech Governmental and Public Sector representatives and by representatives of European Institutions.

Presentations focused on topical themes, including issues perceived as sensitive by consumers, such as residues, microbiological risks and additives as well as emerging risks. Plenary sessions were held on the EU position in the food safety area and international collaborations, food quality and safety control, consumers and food safety issues, and new technologies for food production. Parallel sessions, including round-table discussions, were held on food safety regulation and research challenges and perspectives of the food industry.

Mr. Cannavan (left) with the session panel.

The Agrochemicals Unit Head was invited to give a presentation on "Research and capacity building to meet food safety regulations – a global perspective", focusing on the research activities in the field of food safety and quality undertaken in less developed countries through the Joint FAO/IAEA Programme, with emphasis on the objectives, modalities and problems of implementation, outputs, outcomes and contributions to sustainable capacity building. The need for networking and collaboration with developed country research institutes was stressed.

Discussions with the conference organizers and various participants outside the plenary sessions revealed that the role played by FAO/IAEA in helping developing countries to attain

equivalence of their food safety systems with those of the EU is well recognized and the impacts appreciated.

3.11.2. European Food Science Day: bringing results back to consumers, Brussels, Belgium, 18h November 2009

Managers responsible for dissemination and/or communication in EU framework (FP6) projects focused on food quality and safety formed the network "CommNet" (http://www.commnet.eu) in 2005, to improve their communication skills. It was realised that results of EU research projects are not properly reaching Europe's policy makers, consumers and the food industry, and that there is a need for a platform to provide European citizens, via the relevant authorities and the media, with reliable food advice. As a joint effort, ten research networks within CommNet; CASCADE, CHILL-ON, EARNEST, EURRECA, EPIZONE, MoniQA, PathogenCombat, PHIME, SABRE, and SME-RECEPTOR, organized an event called the "European Food Science Day", held in Brussels on the 18th of November, 2009.

The goal of this one day event was to further spread the important scientific knowledge generated under the EU projects to a wider audience, including communication experts, responsible policy makers, European Parliamentarians, consumer representatives and EU scientists.

Presentations were given by Mr Antonio Di Giulio, Head of the Food, Health and Well-being Unit in the European Commission, Directorate-General for Research, Ms. Lena Ek, EU member of Parliament, Mr Roland Poms, MoniQa coordinator, Ms Catherine Geslain Laneelle, Executive Director of The European Food Safety Authority, Ms. Beate Kettlitz, Director of Scientific and Regulatory Affairs at the Confederation of the Food and Drinks Industries of the EU (CIAA), Ms. Rhonda Smith, Director of Minerva, and Ms. Britt Maestroni, from the Agrochemicals Unit.

The morning panel (from left): Mr. A. di Giulio, Ms. L. Ek, Mr. R. Poms, Ms. C. Geslain Laneelle, Ms. B. Maestroni, Ms. R. Smith.

Ms. Maestroni addressed food safety research from the perspective of developing countries that are food producers. She highlighted the support that international organizations (FAO and IAEA) are providing to developing countries to strengthen national food control systems and promote international trade. Ms. Maestroni also urged the EU Commission to ensure that future legislation is science-based rather than technology driven, in order to avoid creating barriers to trade.

Following discussion sessions, Mr. Ingemar Pongratz, vice coordinator of Cascade, concluded the day with some final remarks. He commented that good communication to the consumer must be balanced, addressing both risks and benefits, that there is a need to produce more information, and a need to educate scientists on knowledge management and communication.

The issue of improved food traceability with respect to food safety was one of the future challenges identified by the meeting.

3.11.3. Fourth International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 4-6 November 2009

The International Symposium on Recent Advances in Food Analysis is a biennial event, held in Prague, Czech Republic. The 4th symposium, in line with previous events, focused on recent advances in analytical and bioanalytical technologies and emerging food-related applications in various areas. The main areas of interest to the Food and Environmental Protection Subprogramme (FEP) of the Joint FAO/IAEA Programme were: residues and contaminants; authenticity, traceability and fraud; mycotoxins, marine and plant toxins; nanoparticles, and; QA/QC and chemometrics in food analysis. The symposium was attended by 380 participants from 37 countries representing 4 continents.

The Agrochemicals Unit Head gave a keynote presentation on "The current state of analytical methodology for food safety and traceability in developing countries". Key messages in the presentation were that the increasing pace of technological development in the field of analytical instrumentation and methodology in the developed world has made it impossible for developing countries, or those in transition, to keep pace; increased effort must be focused upon optimising the use of existing technologies in developing countries to establish food safety equivalence with trading partners and to protect the consumer; care must be exercised in identifying and evaluating those new technologies that would be applicable and would benefit developing countries in food safety regulatory testing, rather than those that are best utilised as research tools for upstream food safety work, and; because of the pace of technological development, food safety legislation, regulations and guidelines require constant revision, but the texts should always be based on sound risk assessment rather than on available technological capabilities. The presentation was well received and led to extended discussion with several participants both immediately afterwards and in the following break sessions.

The Unit Head took the opportunity to discuss with delegates many issues relevant to the work of the Food and Environmental Protection Subprogramme and also participated as a member of the scientific poster award committee in evaluating more than 400 posters within the various sessions of the symposium.

The information exchange in the field of food analysis and regulations at the symposium was extremely useful. The participants included key figures in the relevant fields, and several individuals who had previous or current interactions with the Agency through TCPs, CRPs, or as participants in training workshops were present. The event provided an opportunity for mutual updating of information and maintenance and extension of contact networks.

3.11.4. ICC Expert Summit on Food Security, Vienna, 29-30 June 2009

The International Association for Cereal Science and Technology (ICC) invited the Joint FAO/IAEA Programme to participate in and contribute to the ICC Expert Summit on Food Security held in Vienna on 29-30 June 2009. The aim of the summit was to bring together a group of experts in grains, foods and feeds, agriculture and socio-economics, scientists and technologists, and governmental representatives from across the world to determine critical research and development project needs and related funding schemes in grain and crop related

areas in response to the world's staple food crisis. The summit was attended by over 60 ICC delegates and invited experts from 28 countries. A key objective was to develop internationally funded research and development projects to address the issue of safe, sustainable and sufficient food and feed supply.

Ms. Maestroni addressing the meeting.

The meeting started with a series of short introductory presentations, including a presentation by Ms. Britt Maestroni on "FAO/IAEA food safety and food security programmes: research and development needs". The delegates then split into three working groups to consider different elements of food security and to develop ideas which could form the basis of suitable research proposals. Each working group dealt with one of the three topics: staple food security for all in an age of climate change; food and feed safety for all; and nutrition and health for all. All working groups discussed the potential impact of climate change in terms of the challenges to the availability and the nutritional quality of staple foods. The participants also recognized that responses to particular food security needs must be tailored and delivered in manners appropriate to the relevant regions and ethnic backgrounds around the world. The group discussions highlighted the use of crop science and technology to improve quality and increase yields of staple foods, for example by identifying mechanisms to reduce both pre- and post-harvest losses, including the development of new, rapid, robust and inexpensive testing methods to identify contaminated crops and therefore help to deliver food safety and security.

At the end of the Summit the various outputs were collated and summarized to form a draft of an agreed 'Declaration' on food security which can be accessed at:
http://www.icc.or.at/ICC-Expert-Summit-Declaration-Final-2009-08-05.pdf

3.11.5. Joint FAO/IAEA/OIE/WHO Global Survey of Laboratory Quality Systems

The Agrochemicals Unit Head represented IAEA and FAO in the Joint FAO/IAEA/ OIE/WHO project on a Global Survey of Laboratory Quality Systems. The objective is to develop a global inventory of laboratory quality systems and external quality assessment schemes (EQAs) in order to help UN and other capacity building organizations to identify gaps and needs in laboratory networks involved in providing a response to prevent the spread of disease through notification of all public health emergencies and issues of international concern, in accordance with the International Health Regulations (2005).

The project commenced in August 2007 and a questionnaire was developed and tested in a preliminary survey with a limited number of laboratories in late 2008 – early 2009. Based on the results, the questionnaire was revised and finalized in 2009. The questionnaire was circulated to a wide range of laboratories, competent authorities and EQA providers in August 2009.

The data from the full survey will be validated, collated and analysed by the WHO office in Lyon in early 2010. A meeting will be held in April or May 2010 to discuss the results and dissemination routes.

3.11.6. Quality Control of Trypanocides in Africa

Trypanosomiasis is arguably the most important animal health constraint to sustainable agriculture and rural development of sub-Saharan Africa. The overall impact of trypanosomiasis on crop-livestock production has been evaluated at US$ 4.75 billion/year. About 50 million cattle are at risk over an area of about 10 million km2. Economic losses in cattle production alone are estimated at US$ 1.2 billion/year. Thirty two of the 36 tsetse affected countries in which the disease occurs are classified as Low Income Food Deficit/Least Developed Countries.

The conventional and by far the most prominent method to combat the disease is by chemotherapy. Every year some 35 million doses of trypanocides are administered to domestic ruminants, corresponding to US$ 35 million. However, this figure underestimates the unofficial trypanocide market of unregistered products. A recent estimation from Sudan estimates a total of-US$ 10 million as the annual expenditure for trypanocidal treatments. Although this figure may be exaggerated, it indicates the possibility of gross underestimation. In West Africa, trypanocides are by far the most important among the available veterinary drugs.

The presence of counterfeit drugs is recognized as a world-wide problem, but more so in the developing countries. Wide spread use of counterfeit and poor quality of drugs is relatively frequent among anthelmintics and diminazene based trypanocidals. This has severe implications for both animal health and food safety. The use of poor quality trypanocides induces trypanosome resistance, and may cause other problems such as unspecified, unwanted chemicals and their residues in the food chain. The number of effective compounds still available is severely restricted and prospects for the development of novel molecules are low.

There are currently no internationally agreed standards for the quality of trypanocides. Documented specifications and pharmacopoeial monographs for veterinary trypanocides are either lacking or inaccurate, and there are no references to recommended methods of analysis for quality control of the drugs or for their residues in foods. This situation, together with the weak pharmaco-vigilance in most countries, means that drug quality is frequently compromised.

An initiative endorsed by the Programme Against African Trypanosomiasis brought together several partners in a project to provide assistance for the quality control of trypanocides in Africa. Under the leadership of Dr. Raffaele Mattioli, of the Animal Health Service of the FAO, the partners include IFAD, UNIDO, UNODC and IAEA in collaboration with the International Federation for Animal Health (IFAH) and Strathclyde University. During 2009, the EU has also become involved in the project with regard to the establishment of reference laboratories in sub-Saharan Africa. The main objective of the project is to pursue internationally and scientifically agreed standards and protocols for QA/QC of trypanocides. The specific objectives include definition of the requirements for analytical quality assurance, establishment of good laboratory practices for chemical analysis, and transfer of the methodology and technology to laboratories in Africa. The Agrochemicals Unit and the Department of Pharmaceutical Sciences, Strathclyde Institute for Biomedical Sciences, provide the technical input to the project.

A meeting of the project partners was held in Vienna in July 2009. An analytical method to support the quality control of isometamidium was previously developed and validated by

Strathclyde in collaboration with the Agrochemicals Unit. At the meeting, a three-year work plan was drafted for further progression of the quality control/quality standard investigation work for other trypanocide molecules. This work will be funded through the IFAH financial contribution, within the framework of a FAO-IFAH Memorandum of Understanding.

During 2009, work was started by the Strathclyde partners and the Agrochemicals Unit on the production of technical and scientific monographs for isometamidium chloride and diminazene aceturate.

HPLC method developed by Strathclyde University and the Agrochemicals Unit for the quality control of isometamidium products.

4. TRAINING ACTIVITIES

A major component of the work of the Food and Environmental Protection Subprogramme, and the Agrochemicals Unit in particular, for a number of years has been training of Member State scientists, technicians and food safety regulators, mainly from developing countries, on various topics to assist in the implementation of holistic farm-to-fork food safety systems. The training is focused primarily on the application of nuclear and related techniques to detect and control chemical contaminants such as veterinary drug and pesticide residues, endocrine disruptors and mycotoxins in foods. This training is an integral part of the Agrochemicals Unit's overall work programme, which includes applied/adaptive research, analytical method development and validation to support technology transfer and the provision of data and expertise to underpin the development of international standards through bodies such as Codex Alimentarius. The overall objective of our activities is to support and assist Member States in improving food safety and meeting the requirements for domestic and international trade in food commodities.

The training approach developed at the Agrochemicals Unit is built on the concept of accelerated capacity building. We define accelerated capacity building as a step-by-step approach to meet the demand for trained people, especially in developing countries. The focus is on "training-the-trainers" and "learning-by-doing". It is assumed that people in the target group have already received an initial professional education (secondary and/or tertiary), and are only lacking specific job- or field-related knowledge and skills/qualifications. The step-by-step approach to training-the-trainers through accelerated capacity building aims to reach a greater number of people than possible through training workshops alone. **Figure 16** shows the three steps of accelerated capacity building.

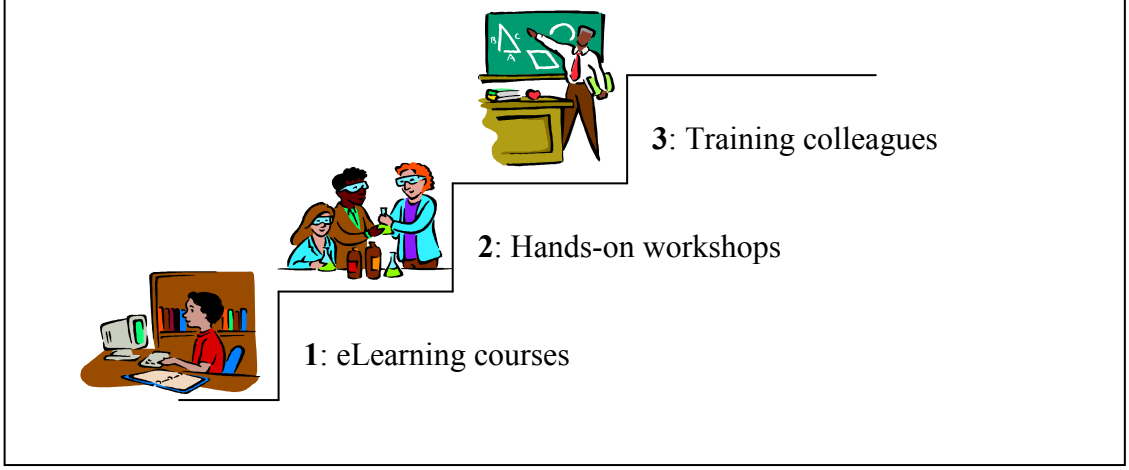

Figure 16. The step-by-step approach of accelerated capacity building for the individual: After updating their knowledge through eLearning (step 1), some participants pass the eLearning exam and continue on to participate in train-the-trainers workshops (step 2). After participating in the hands-on workshops, they pass their enhanced knowledge and skills on to colleagues in their home country (step 3).

The first step in accelerated capacity building is for the participants to take eLearning courses to obtain the necessary background information. This includes both getting technical information and practicing the language required for the workshop. At the end of each e-lecture students have practice questions before they continue with the examination. Any student wishing to go on to the next step, that is to participate in a train-the trainers workshop, must pass the examinations to demonstrate that they have adequate baseline knowledge to gain optimal benefit from the workshop.

In the second step, learners participate in hands-on training workshops. These focus on a "learning-by-doing" approach where the participants apply and expand the acquired knowledge. The focus is on training-the-trainers, and workshop participants work in teams to tackle relevant issues and prepare and deliver presentations in order to gain insight on how to disseminate knowledge.

In the third step, the participants pass their knowledge and skills on to their national and regional colleagues. This can be done through a series of seminars and/or workshops, by mentoring and using training materials which the participants receive during steps one and two. In this third step knowledge and skills can be shared with colleagues through either formal training or informal training. In formal training, the trainers organize one or more workshops in their home country to train colleagues from their own or other institutions. Informal training can be carried out through on-the-job training or through mentoring of individual colleagues. An example of the third step is a presentation given by Ms. Guihua Liu on methodology for veterinary drug residues monitoring at the 20th Academic Conference of the Health Testing Association of Guangdong Province, 11-13 Dec. 2009. Ms. Guihua had previously organized a seminar in China in March 2008 following her participation in a train-the-trainers workshop at Seibersdorf, and gave this second presentation at the conference in 2009 after being provided with updated information and materials from the train-the-trainers workshop held at Seibersdorf in 2009 (**Section 4.1.**).

Step 3: a follow up presentation by Ms. Guihua Liu at the 20th Academic Conference of the Health Testing Association of Guangdong Province, 11-13 December 2009.

Accelerated capacity building provides a mechanism for the sustainable development of adequately trained staff at institutions in Member States. This increases Member States' institutional capacity and thus contributes to the countries' advancement in nuclear science and technology. The concept provides the opportunity for a multiplier or cascade effect. It is to be seen as a fundamental element in life-long learning. Through the expansion of knowledge and expertise, accelerated capacity building can promote sustainable development, especially in developing countries, and foster decentralisation and empowerment of partner institutions.

4.1. Train-the-Trainers Workshop on Screening/Post-screening Techniques for Veterinary Drug Residues, Seibersdorf, Austria, 12-23 October 2009

The issue of veterinary drug residues in foods of animal origin (meat, milk, eggs) has become increasingly important world-wide, including in developing countries. Concerns over veterinary drug usage and residues are primarily related to food safety, human health and the need to meet requirements for international trade. Food safety is impacted by global trends such as climate change, changing demographics and increasing urbanisation, and the consequent changes in agricultural practices throughout the food production chain. Climate change, changes in the structure of livestock production, breeding and husbandry practices, and international trade in animals and animal products are predicted to increase the prevalence and transmission of animal and zoonotic diseases, requiring an increased use of antibiotics and other veterinary drugs.

The control of veterinary drug residues is achieved through the regulation of veterinary pharmaceuticals and the application of good farming/production practices, in combination with effective surveillance systems and follow-up strategies. Analytical laboratories play an important role in the verification of the quality of the food commodity, in feeding back information on the effectiveness of control programmes and in the provision of information services to farmers and producers, either directly or through extension services.

Current international guidelines and regulations require that countries intending to export foods of animal origin must have programmes in place to ensure that food products do not contain residues of banned drugs, or concentrations of legally used drugs exceeding national or international maximum residue limits, and that the laboratories certifying compliance with regulatory levels must implement appropriate quality control and quality assurance systems. These programmes are important not only with regard to international trade, but also to guarantee the safety, quality and security of domestic food supplies. To ensure that domestic or national controls are appropriate and effective, risk assessment programmes must be in place.

Sample preparation practical session.

In order to effectively implement surveillance programmes, laboratory staff must be familiar with the appropriate screening and quantitative techniques, as well as with the principles of ISO Standard 17025.

To assist IAEA and FAO member states in addressing these issues, a "train-the-trainers" workshop on screening and post-screening techniques for veterinary drug residues was held in the FAO/IAEA Training and Reference Centre for Food and Pesticide Control in October 2009. The objectives were to provide information and strengthen the awareness of scientists and laboratory middle-management of the relevant guidelines and regulations and the theoretical and technical aspects of screening and post-screening techniques for the detection of veterinary drug residues; including radio-assay, microbial inhibition, immunological, thin layer chromatographic (TLC) and high-performance

Train the trainers workshop lecture.

liquid chromatographic (HPLC) techniques; to introduce the quality assurance/quality control principles according to ISO Standard 17025 that are relevant to veterinary drug residue analysis; and to discuss the various possible roles of quality assured laboratories in monitoring the effectiveness of good farming practices. Information and training material was provided to facilitate further training by the workshop participants of personnel in their home countries.

The workshop programme comprised lectures and laboratory practical sessions and demonstrations in the following subjects:

- Codex standards, guidelines and recommended international codes of practice for the control of the use of veterinary drugs
- Veterinary drug residue testing in the context of food safety
- Sample preparation
- Screening techniques (radio-assay, microbial inhibition tests, immunoassay)
- Chromatographic theory and practical applications of TLC & HPLC
- Statistical treatment and interpretation of analytical results
- Screening applications of hyphenated mass spectrometric techniques
- Quality assurance systems and QA/QC measures in analytical laboratories
- Laboratory accreditation and mutual recognition
- Method validation and principles of estimation of uncertainty of results
- The role of analytical laboratories related to good farming practices

The workshop had 22 participants, including two TC fellows and one intern, from 20 developing countries. The participants are listed in Appendix 4 to this report. Lectures and practical sessions were given by Agrochemicals Unit staff, staff of the Food and Environmental protection section and several external lecturers. Significant contributions were made by Dr. Iris Lange, from the Bavarian Health and Food Safety Authority, and Ing. Thomas Kuhn and colleagues from the Austrian Health and Food Safety Agency, which provided lectures at Seibersdorf and training demonstration sessions on radio-receptor binding assay and immunoassay procedures in the AGES laboratories.

One afternoon of the workshop was held as a joint session with the participants in a Research Coordination meeting for the CRP “Development of Radiometric and Allied Analytical Methods to Strengthen National Residue Control Programs for Antibiotic and Anthelmintic Veterinary Drug Residues” (D5.20.36). In the joint session, CRP research agreement holders gave presentations for the combined audience of approximately 45 people.

From immediate feedback, the train-the-trainers approach appeared to have been very successful, with various training activities planned by the workshop participants in their home countries. A vital element of all the training activities carried out by the subprogramme is the enhancement and maintenance of networks of institutes, and once again this was judged to be successful. Good working and personal relationships were forged amongst the participants during the workshop and shared problems (and solutions) and experiences were discussed at length.

Training in isotope-dilution screening technique.

Some results extracted from a workshop evaluation questionnaire are presented graphically in **Figure 17**. The responses indicate that the objectives of the workshop were fully achieved. All participants also indicated that the FAO/IAEA Training and Reference Centre was the appropriate location to hold this workshop, and similar workshops in the future.

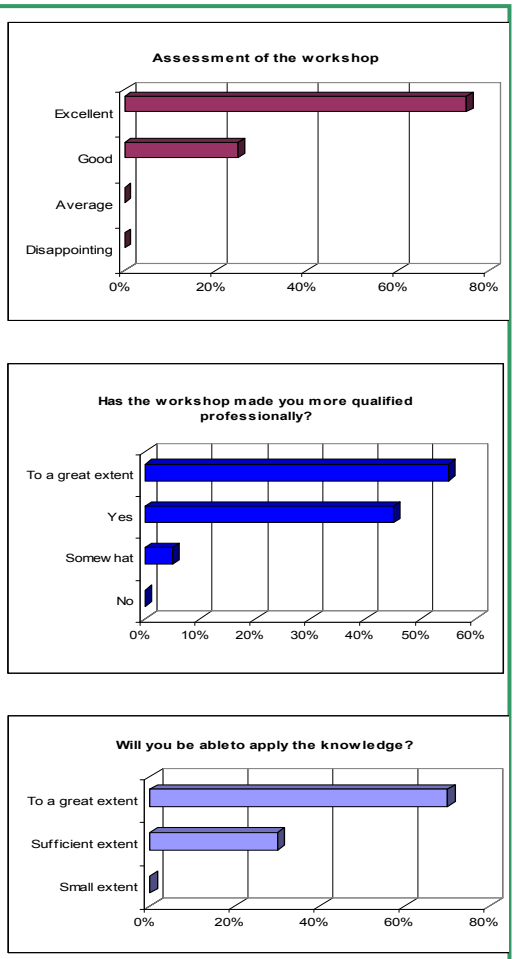

Figure 17. Feedback from the workshop

4.2. Fellows, Scientific Visitors and Interns

Developing analytical methods for transfer to Member state laboratories and training fellows and interns for capacity building are key activities of the Agrochemicals unit as part of its mandate to promote food safety and security among IAEA Member countries. Ms. Siya Assey, a TC fellow from Tanzania, who participated in a training workshop on the quality control/quality assurance measures in pesticide residue analysis from 13 October to 7 November 2008 along with 22 other scientists from various developing countries, continued her fellowship in the Unit until 9 January 2009. During this period, Ms. Assey received more detailed and intensive training on the operation of gas chromatograph (GC) instrumentation, method validation, and statistical evaluation of results. Under the close supervision of the Unit staff, Ms. Assey completed a validation study on a method for the analysis of pyrethroids in beef tissue. The validated method will be implemented in Ms. Assey's home laboratory and is suitable for use in many other member state laboratories.

Ms. Siya Assey and Mr. Khaled El-Hawari training in the Unit.

Mr. Khaled El-Hawari, from Lebanon, who also participated in the above-mentioned QA/QC training workshop, remained as a TC Fellow with the Agrochemicals Unit until 9 April 2009 to train intensively on the use of gas chromatography-mass spectrometry (GC-MS) for residues analysis. Mr El Hawari also participated in a one-month intensive group fellowship training course on GC-MS held in the Unit where he gained additional knowledge on various aspects of GC-MS theory and troubleshooting techniques. During his fellowship Mr. El Hawari worked with ACU staff on the adaptation and validation of a multiresidue isotope-dilution method for the determination of agrochemicals in water which will be applied in CRP D5.20.35. In addition, he also contributed to the Unit's work by performing GC-MS analysis of mosquito net samples submitted by Entomology Unit for pesticide analysis.

Ms. Van Nguyen Thi Thuy from the Centre for Nuclear Techniques, Ho Chi Minh, Vietnam, commenced a 3-month fellowship in the Agrochemicals Unit on 2 November 2009. Ms. Nguyen Thi Thuy was trained in the operation, maintenance and troubleshooting of chromatographic instrumentation, including basic training on hyphenated mass spectrometric techniques for pesticide residue analysis. The training focused on methods for the analysis of pesticide residues in meat and apples and also included laboratory safety, radiotracer techniques and liquid scintillation counting.

Ms. Van Nguyen Thi Thuy being trained in GC-MS by Ms. Zora Jandric.

Under TCP INS/5/055, "Enhancement of quality assurance for the analysis of veterinary drug residues", Mr. Rachmat Firmansyah, from the Department of Toxicology of the Research Institute for Veterinary Science (BALITVET), Bogor, Indonesia participated in the FAO/IAEA train-the-trainers workshop "Screening/post-screening techniques for veterinary drug residues" (**Section 4.1.**) and undertook follow-up fellowship training in the Agrochemicals Unit in the operation, maintenance and troubleshooting and application of analytical instrumentation for the analysis of residues in food. Mr. Muhammed Alamgir Zaman Chowdhury, of the Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commisiion, Dhaka, who was undertaking fellowship training at the Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES) under TCP BGD/5/027, "Establishing a Veterinary Drug Residue Laboratory", spent two weeks of his fellowship with the Agrochemicals Unit as a participant in the train-the-trainers workshop on veterinary drug residues analysis (**Section 4.1.**).

Ms. Carolina Sheng Whei Miaw sampling meat for analysis.

Ms. Amanda Caputti Caleffi, from Microbioticos Analises Laboratoriais Ltda, Campinas, Brazil, undertook a 2-month internship in the Agrochemicals Unit, from 22 June – 21 August 2009. Ms. Caleffi was trained in a liquid chromatographic-tandem mass spectrometric (LC-MSMS) isotope dilution method for the simultaneous detection and quantitation of 38 anthelmintic compounds in meat (**Section 3.1.**). The training included the set-up, calibration and optimisation of the LC-MSMS instrument, the use of stable isotope-labelled internal standards, sample preparation, sample analysis, and data acquisition, processing and interpretation. After attaining proficiency in the method, Ms. Caleffi was involved in its validation, generating data to characterize the intra-laboratory reproducibility. Ms. Caleffi's internship was funded by the EU 6th Framework Integrated Project "ProSafeBeef", in which both Microbioticos and the Agrochemicals Unit are research partners (**Section 3.10.1.)**. Upon return to her home institute, Ms. Caleffi will implement and validate the method for use in risk assessment surveys and regulatory screening for anthelmintic residues in beef. The data generated will be compiled and evaluated by other members of the ProSafeBeef consortium working under the "Chemical Residues" work package. It is intended that Microbioticos will attain ISO17025 accreditation for the method and will also act as a regional hub of training for Latin America, primarily for technology transfer under TCP RLA/5/055.

Ms. Carolina Sheng Whei Miaw, from the Department of Food Engineering, University Centre of Belo Horizonte, Brazil, commenced a 5-month internship in the Agrochemicals Unit on 20 July 2009. Ms. Miaw was trained in the operation, maintenance and troubleshooting of gas chromatographic - mass spect-rometric instrumentation its application to the analysis of residues and contaminants in food and environmental samples. She was involved in the adaptation and validation of a multi-contaminant method for meat and meat products using a QuEChERS (quick, easy, cheap, effective, rugged and safe) sample extraction/cleanup procedure. Ms. Miaw also participated in the FAO/IAEA train-the-trainers workshop "Screening/ post-screening techniques for veterinary drug residues" (Section **4.1.**).

4.3. Training on radiotracer techniques in residues analysis, Panama, 15-26 June 2009

Mr. Rathor (centre) with the MIDA trainees.

Mr. Nasir Rathor undertook a two week expert mission to the Ministerio de Desarrollo Agropecuario Direction National de Sanidad Vegetal (MIDA) in Panama from 15-26 June 2009. The objective was to provide training to the MIDA staff in the use of radiotracer techniques for the determination of contaminants and residues in fruits and vegetables using carbon-14 labeled compounds. The training was funded under TC project PAN/5/019.

Mr. Rathor gave presentations and demonstrations on the theory and practical aspects of:

- Preparation of ^{14}C -stock and working solutions,
- Calculation of the activity of the solutions and how to express it in different units (CPM / DPM, mCi and Bq),
- Calculation of the amount of cold compound in the ^{14}C labeled standard,
- Basic liquid scintillation theory,
- Counting efficiency and effect of different quenching on the recovery of the ^{14}C method,
- Correction of colour quenching
- Use of Berthold LB 124 bench Geiger Mueller detector,
- Calculation of the uncertainty of the analytical procedure,
- Maintenance and troubleshooting of a liquid scintillation counter (LSC)
- Maintenance of the gas chromatograph-mass spectrometer (GC-MS),
- Operation of GC-MS using scan and selected ion monitoring (SIM) modes SIM,
- Retention time locking (RTL),
- QuEChERs methods using ^{14}C chlorpyrifos.

Laboratory experiments were performed to demonstrate the use of radiotracer techniques for the processing of pineapple samples. The uncertainty of sample processing was studied using two different sample choppers and ^{14}C-chlorpyrifos as radiotracer. The application and usefulness of radiotracer techniques for method validation studies was also demonstrated. Pineapple samples were fortified with a mixture of 4 pesticides and ^{14}C-chlorpyrifos. The QuEChERS method was used for sample preparation and the recovery of the target pesticides was calculated after the extraction stage, after the cleanup stage and after evaporation by

counting the activity in a LSC. The extracts were also analysed by GC-MS. Practical training was also provided on the operation and maintenance of the GC-MS instrument.

Pineapple sample preparation for residues analysis by MIDA staff

Mr. Rathor noted that the MIDA laboratory has developed very noticeably since his previous training mission in February 2007, both in the quality system and in building the capacity of the staff, who are young, enthusiastic, willing to learn and very dedicated to their work. He made several recommendations to laboratory management to assist in the further development of the laboratory and the services that it provides through the IAEA technical cooperation project PAN/5/019.

Mr. Rathor training MIDA staff in radiotracer technique.

Method validation study at MIDA using ^{14}C-chlorpyriphos

4.4. National Training on "Sampling of fresh fruits and vegetables for compliance with Codex MRLs", Al Ain, UAE, 21-25 June 2009

In April 2009 the IAEA received a request, through the regional FAO Representative, from the United Arab Emirates (UAE) to support a national workshop on the principles and practice of sampling of commodities for pesticide residues analysis. The Agrochemicals Unit was requested to prepare a programme, provide training materials and identify suitable consultants for the national workshop. Dr. Perihan Aysal (Turkish Atomic Energy Authority, Saraykoy Nuclear Research and Training Center) and Dr. Berthold Kettner (Institut für Lebensmittel Arzneimittel- und Umwelt-Analytik GmbH, Germany) were identified as suitable consultants and funded by IAEA TC to undertake an expert mission to the laboratories of the Ministry of Environment and Water-Sector of Technical Affairs-Directorate of Laboratories (MOEW) to lecture at the national workshop in Al Ain, UAE, from 21-25th June, 2009.

Dr. Aysal (3rd from left) and Dr. Kettner (2nd from right) with UAE authorities and workshop participants.

Agrochemicals staff prepared a programme and provided standard training materials from previous train-the-trainers workshops. Additional material was prepared by Unit staff in conjunction with the consultants.

The workshop was attended by approximately 52 participants composed of directors, food inspectors and scientists from different laboratories of MOEW, including Al Ain MOEW Central Lab, Dubai Central Laboratory Department - Food and Environment Section, Fujairah Environment Research Center, Abu Dhabi Food Control Authority and Sharjah Central Food Laboratory. The topics covered included:

- food safety, the role of Codex Alimentarius and requirements of trading food for developing countries
- principles of sampling for the determination of trace organic contaminants
- maximum residue limits (MRL)
- methodologies of sampling fresh fruits and vegetables for compliance with Codex MRLs
- representative and non-representative samples
- statistical methods and error minimization
- sources of uncertainty of the analytical procedure
- sample storage and transportation to the laboratory
- sample preparation and sample processing
- pesticide stability

- sampling tools
- sampling from different fields (airports, seaports, shopping malls, retail markets, supermarkets)
- transportation of the samples to the laboratory.

Sampling from a truck at the Dubai Fruit and Vegetables Market.

The workshop also included practical sessions on sampling. On the 4th day the participants travelled to the Dubai Fruit and Vegetables Market were pre-sampling procedures and representative sampling protocols were demonstrated while trucks were unloading various vegetables and fruits. The instructors demonstrated how representative samples should be taken from the trucks. The participants also visited the Dubai Flower Centre, where imported flowers, fruits and vegetables are stored in a cold warehouse. Instructions were given on how to properly sample packages coming from different countries. The participants also visited a supermarket in Dubai City Centre, where strategies for the sampling of retail packages were explained. On the 5th day of the workshop, the participants visited several orchards and fields where mango, date palm, aubergine, tomato, banana, flowers and water melon are grown and field sampling (pre-harvest) was demonstrated.

The final session of the workshop was held at the Al Ain MOEW Central Laboratory where sample registration and sample preparation procedures were demonstrated using pears and dry ice for the preparation of the analytical sample.

Questions and answer sessions were held each day to give participants the possibility to clarify any issues that may have been unclear.

The knowledge transferred through this workshop will help to harmonize techniques for sampling and sample processing and to minimize errors in pesticide residue analysis, thereby helping to produce reliable and comparable results.

4.5. Regional Training Course on the Estimation of Pesticide Loads in a Microcatchment, Costa Rica, 23 November – 4 December, 2009

Under TCP RLA/5/050 a "black box" monitoring strategy was deployed by Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, and Uruguay to monitor indicators of good agricultural practice (GAP) and compliance with maximum residue limits (MRLs). The approach involved integrated biological and chemical monitoring of water quality at a landscape scale using harmonized protocols and geo-referenced sampling. RLA/5/053 takes this approach a step further by obtaining information about relevant pesticide processes within the sub-catchment and pesticide load to the environment. An additional nine countries are participating: the Dominican Republic; El Salvador; Haiti; Jamaica; Nicaragua; Paraguay; Peru; Spain; and Venezuela. At the first level training course Haiti, Nicaragua and Paraguay were not represented, either because the course pre-requisites could not be fulfilled or for logistical reasons.

The aim of the course was to train technical staff involved in the implementation of RLA/5/053 or related national projects. Topics included: integrated analytical approaches to monitor good agricultural practice (GAP) at a landscape scale, including PIRI, LIMS and GIS; field sampling, assessment of bioindicators and path tracking; and analytical methodologies for pesticide residues in water and sediments. The course was hosted by the IAEA Collaborating Centre on eLearning and Accelerated Capacity Building under the Universidad de Costa Rica, Centro de Investigación en Contaminación Ambiental. The event provided an opportunity to assess progress made during 2009 and develop a website for participants to learn and interact with one another. Ms. Britt Maestroni was a course facilitator.

Laboratories in Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, and Uruguay have identified microcatchments and commenced monitoring. They have the necessary staff and resources to implement the RLA/5/053 workplan. Staff from those laboratories provided most of the training for the course, except for the components on pesticide sorption/degradation, GIS and analytical methodology. This is compelling evidence that these countries have not only applied the harmonized approaches developed under the coordinated research project on Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale (D3.20.35), but are able to train others in their use. Argentina and Colombia shared videos and provided instructions and tips for other laboratories on the use and application of Doppler flow meters. El Salvador, Jamaica, Peru and Venezuela have the potential to implement significant, if not all, parts of the programme, whilst the other participants will require additional mechanisms and time to implement the workplan.

Training and exercises on pesticide fate and GIS indicated that expertise varied considerably, ranging from current international standard to little or no capacity. Individual meetings with the participants, summary reports by the new participants, and the responses to a 2009 baseline questionnaire helped clarify the key issues and barriers to implementation of the project activities.

The course concluded with the formulation of a number of recommendations and proposed actions to ensure successful implementation of the project. The full report and other relevant information can be accessed at http://elearning.iaea.org/ATutor/go.php/106/index.php.

5. APPENDICES

Appendix 1

5.1. Staff Publications 2009

Cannavan, A (2009). The current state of analytical methodology for food safety and traceability in developing countries. Book of abstracts of the 4th International Symposium on Recent Advances in Food Analysis, 4-6 November 2009, Prague, Czech Republic, 85.

Byron, DH, **A Cannavan** and RKP. Patel (2009). Report on Activities of the International Atomic Energy Agency (IAEA) Relevant to Codex Work (CX/RVDF 09/18/3-Add 1.). Eighteenth Session of the Joint FAO/WHO Codex Committee On Residues of Veterinary Drugs in Foods, Natal, Brazil, 11 – 15 May 2009

Cannavan, A, PJ Brodesser, and DH Byron (2009). Food Traceability and Authenticity in Developing Countries through Isotope Ratio Techniques. Book of Abstracts of the 10th CSL/JIFSAN Joint Symposium, Food Safety and Nutrition: Methods and Systems for Tracking, Tracing and Verifying Foods, Greenbelt, Maryland, USA, 13-15 May 2009, 59-60.

Cannavan, A (2009). Research and Capacity Building to Meet Food Safety Regulations – a Global Perspective. Book of abstracts of the conference Food Research in Support of Science-Based Regulations: Challenges for Producers and Consumers, Prague, Czech Republic, 21-22 April 2009, 49.

Sasanya, J, M Islam, A Kist, M Danaher, M Whelan, R Granja, **A Cannavan** (2009). A Multi-residue Isotope Dilution LC-MSMS Method to Support Risk Assessment for Anthelmintic Drug Residues in Beef. Proceedings of the International Conference Advancing Beef Safety through Research and Innovation, Dublin, Ireland, 25-26 March 2009, 96.

Danaher, M, M Whelan, B Kinsella, H Cantwell, M McCormack, P Byrne, K Cooper, G Kennedy, **A Cannavan**, A Montes Nino, R Granja, G Trigueros, E van Asselt, A Furey, S Lehotay (2009). Detection of Anthelmintic Residues in Food Using Rapid Polarity Switching UPLC MS/MS Combined with QuEChERS Technology. Proceedings of the International Conference Advancing Beef Safety through Research and Innovation, Dublin, Ireland, 25-26 March 2009

Posters

Carazo, E, IG Ferris,K Gross-Helmert, **B Maestroni** (2009). The Role of eLearning in Supporting Analytical Laboratories. 3rd IUPAC International Workshop on Crop Protection Chemistry in Latin America, Rio de Janeiro, Brazil , 9-12 November 2009.

Ribeiro, DHB; LC Luchini, FG Serafim, MC Sarvini, LB Monza, V Kirs, RM Loewy, I Pino, AM Parada, X Videla, A Nario, JAG Dallos, A Mojica, R Castro, Y Pastor, I Chica, E Carazo, C Chinchilla, J Matarrita, B Mandl, SF Andreoletti, EC Castelli, EN Bastos, **B Maestroni**, I Ferris (2009). Assessing good agricultural practices in production of fruits and vegetables: a coordinated study in seven Latin American countries. 3rd International Workshop on Crop Protection Chemistry in Latin America, IUPAC, Rio de Janeiro, Brazil , 9-12 November 2009.

Loewy RM, M Savini, L Monza, V Kirs, D Baggio, F Gonzaga, A Nario, I Pino, A Parada, X Videl, R Castro, Y Pastor, I Chica, E Carazo, C Chinchilla, J Matarrita, **B Maestroni**, I Ferris (2009). Assessing good Agricultural Practice: Comparative Pesticide Impact in Five Latin-American Countries. 2nd Latin American Pesticide Residue Workshop, Universidad Nacional del Litoral – Facultad de Ingeniería Química, Santa Fe, Argentina, 8-11 June 2009.

Appendix 2

5.2. Staff Travel 2007-2008

Staff Member	Destination	Period	Purpose of Travel
Cannavan, Andrew	Barcelona, Spain	5-6 March 2009	To participate as Chair of the Advisory Board in the 1st annual meeting of the EU FP7 project "Contaminants in food and feed: inexpensive detection for control of exposure" (CONffIDENCE).
	Dublin Ireland	25-26 March 2009	To present a poster and chair a session of the International Conference on Advancing Beef Safety through Research and Innovation and participate in the Work Package 1.4 meeting of the EU FP6 project ProSafeBeef.
	Prague, Czech Republic	20-22 April 2009	To give an invited lecture on "Research and capacity building to meet food safety regulations – a global perspective" at the International Conference on Food Research in Support of Science-Based Regulations.
	Maryland, USA	13-15 May 2009	To present a poster and participate in the 10th CSL/JIFSAN Joint Symposium on Food Safety and Nutrition: Methods and Systems for Tracking, Tracing and Verifying Foods.
	Belfast, UK	28-29 September 2009	To present a keynote lecture on "Food traceability and authenticity – global issues"at the Symposium on Food Safety, Traceability and Authenticity: Opportunities and Challenges for the Agri-Food Industries.

Staff Member	Destination	Period	Purpose of Travel
Cannavan, Andrew	Prague, Czech Republic	4-6 November 2009	To present a keynote lecture on "The current state of analytical methodology for food safety and traceability in developing countries" at the 4th International Symposium on Recent Advances in Food Analysis.
	Marloie, Belgium	26-27 November 2009	To participate as acting chair of the Advisory Board in the 4th Annual Meeting of the EU FP6 Project 'BioCop'.
Maestroni, Britt	Montivideo, Uruguay	1-3 April 2009	To visit participating laboratories and discuss workplans as TO for URU/5/025, RLA/5/050 and RLA/5/053
	Brussels, Belgium	18 November 2009	To give an invited presentation, "Strengthening national food control systems – the FAO/IAEA perspective", at European Food Safety Day: Bringing Results Back to Consumers.
	San José, Costa Rica	26 November–8 December 2009	RLA/5/053 First Level Regional Training Course on the Estimation of Pesticide Loads in a Microcatchment, Revision of Sampling Plans, Use of PIRI, GIS, and LIMS Bioindicator/Bioassay and Analytical Methods.
Rathor, Nasir	Panama City, Panama	15-26 June 2009	To train MIDA laboratory staff on radiotracer techniques in residue analysis
Sasanya, James	Dublin, Ireland	18-22 May 2009	To collaborate with ProSafeBeef project partners in Ashtown Food Research Centre in transferring methodology through Seibersdorf to Brazil.

Appendix 3

5.3. External Collaborations and Partnerships

Institution	Topic
Veterinary Public Health Laboratory, Bangkok, Thailand	Method development and research into causes of chemical contaminants in food, technology transfer to Asia/Pacicfic.
Laboratorios Microbioticos s/c/ Ltda, Sao Paulo, Brazil	Method development for food contaminants, technology transfer to Latin America
University of Costa Rica (UCR), Centro de Contaminacion Ambiental (CICA), Costa Rica	IAEA Collaborating Centre for eLearning and Accelerated Capacity Building for Food and Environmental Protection (EACB)
Institut für Lebensmittel Arzneimittel- und Umwelt-Analytik (ILAU), Germany	Collaborations on research activities linked directly to the CRP D5.20.35 on "Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Land and Water; Groundwater Management and Site Remediation, Australia	
Ministry of Health; State General Laboratory; Environmental Chemistry, Ecotoxicology, Pesticides and Radioactivity Department, Cyprus	
Austrian Agency for Health and Food Safety (AGES), Austria	Collaborations on accelerated capacity building for risk analysis and contaminants in food
Gartner & LVA Analytik, Austria	
Austrian Institute of Technology, Seiberdorf, Austria	Collaboration on nuclear techniques for research into interactions between environmental/food contamination. Collaboration on the use of stable isotope measurements for traceability of foods and animals.
Ashtown Food Research Cenre, Ireland	Partner laboratory in EU Project "ProSafeBeef"
Institute of Agri-food and Land Use, Queens University Belfast, UK	Research and method development activities for food contaminants and food traceability
ASSET Centre, Queens University Belfast, UK	Research activities in isotope-ratio methods for food traceability

Institution	Topic
International Union of Pure and Applied Chemistry (IUPAC), Chemistry and the Environment Division	Collaboration on compendium of agrochemicals information
Waters Corporation, Milford, MA, USA	Information dissemination, Food safety Summits
Agilent Technologies, PA, USA	Training for member state scientists in analytical techniques
RIKILT Institue for Food Safety, The Netherlands	Research into causes of food contamination with veterinary drug residues
Chinese Academy of Agricultural Sciences (CAAS) Institute for Application of Atomic Energy Department of Agro-Ecological Environment, China	Development of methodology for food traceability and residues analysis
Technical University Munich	Development of radioassay protocols
World Health Orgaization (WHO), Lyon Office for National Epidemic Preparedness and Response	Global survey of laboratory quality Standards
World Organization for Animal Health	
International Federation for Animal Health	Quality control of trypanocidal drugs in sub-Saharan Africa
UNIDO	
UNODC	

Appendix 4

5.4. Trainees, Fellows and Scientific Visitors

Name	Country	Duration	Topic of Training
Trainees			
Emiri, Ms. A.	Albania	2 weeks	Train-the-trainers workshop on Screening/Post-Screening Techniques for Veterinary Drug Residues, 12-23 October 2009
Abdus Samada, Mr. M.	Bangladesh	2 weeks	
Chowdhury, Mr M.A.Z.	Bangladesh	2 weeks	
Tshepo, Mr. R.	Botswana	2 weeks	
Nunez Olguin, Ms. M.V	Chile	2 weeks	
Boateng-Kagyah, Ms. E.B.	Ghana	2 weeks	
Lanovia, Ms. T.	Indonesia	2 weeks	
Md Rodzi, Ms. M.	Malaysia	2 weeks	
Matazagal, Ms. L.S.	Mexico	2 weeks	
Ziyate, Ms. N.	Morocco	2 weeks	
Junius, Ms. S.S.	Namibia	2 weeks	
Carillo de Vera, Ms. M.G.	Paraguay	2 weeks	
Onyino, Mr. R.	Seychelles	2 weeks	
Premarathne, Ms. J.M.K.J.K.	Sri Lanka	2 weeks	
Wangphol, Mr. S.	Thailand	2 weeks	
Baccino, Ms. M.N.	Uruguay	2 weeks	
Nakibuuka, Ms. M.M.	Uganda	2 weeks	
Mujonko, Ms. A.	Zambia	2 weeks	
Makaya, Ms. W., Ms N	Zimbabwe	2 weeks	
Chaverri Suarez, Mr. F	Costa Rica	2 weeks	
Fellows			
Assey, Ms. S.A.	Tanzania	12 days	Residues analysis
El Hawari, Mr. K.	Lebanon	3 months & 12 days	Residues analysis
Firmansyah, Mr. R.	Indonesia	1 month & 10 days	Residues analysis
Nguyen, Ms. Van	Vietnam	2 months	Residues analysis
Scientific Visitors			
Jaber, Mr. F.	Lebanon	10 days	Residues control

Appendix 5

5.5. Coordinated Research Projects (CRP) and Technical Cooperation Projects (TCP)

CRP Title	Scientific Secretary
Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices (D5.20.35, 2006-2011)	B Maestroni
Development of radiometric and allied analytical methods to strengthen national residue control programs for antibiotic and anthelmintic veterinary drug residues (D5.20.36, 2009-2014)	A Cannavan, R Patel
TCP Title	**Technical Officer**
Strengthening Capabilities to Control Veterinary Drug Residues in Foodstuffs (ALG/5/025)	R Patel, A Cannavan
Veterinary Drug Residue Monitoring Programme (ANG/5/003)	R Patel, A Cannavan
Veterinary Drug Residue Monitoring Programme (BEN/5/003)	R Patel, A Cannavan
Establishing a Veterinary Drug Residue Laboratory (BGD/5/027)	R Patel, A Cannavan
Certification of Exported Animal Products Using Nuclear and Other Analytical Techniques (CHI/5/046)	A Cannavan, R Patel
Zoonotic (diseases that can be transmitted from animals to humans) Disease Control and Analysis of Veterinary Residues in Foods (ERI/5/005)	R Patel, A Cannavan
Enhancement of Quality Assurance for the Analysis of Veterinary Drug Residues (INS/5/033)	A Cannavan, R Patel
Monitoring of Residues in Livestock Products and Surveillance of Animal Diseases (MON/5/012)	A Cannavan, J Crowther
Determining Drug Residues in Bovine Meat Exports (NIC/5/007)	A Cannavan, R Patel, J Brodesser
Establishing a South American Regional Network of National and Reference Laboratories for Pharmacologically Active Substances and Contaminants in Food of Animal Origin Through Implementation of Approved Nuclear & Conventional Analytical Techniques (ARCAL, RLA/5/055)	R Patel, A Cannavan

Monitoring of Chemical Residues and Food-borne Pathogens (SRL/5/039)	A Cannavan
Monitoring Veterinary Drug Residues in Livestock Products (NIR/5/036) (Footnote a)	R Patel, A Cannavan
Capacity for Monitoring Pesticide Residues for Compliance with Minimum Risk Levels and Good Agricultural Practice According to ISO 17025 (BOL/5/017)	I. Ferris, B Maestroni
Providing Technical Assistance and Training for the Control of Chemical Residues in Food (BZE/5/003)	B Maestroni, I. Ferris,
Assessment of the Impact of Pesticide Use in Lake Tota, Boyacá, Colombia (COL/5/022)	I. Ferris, B Maestroni
Monitoring of Pesticide Residues in Food Products (IVC/5/027)	J Brodesser, B Maestroni
Upgrading of Food Safety System (MAK/5/005)	J Brodesser, B Maestroni
Monitoring Pesticide Residues in the Production of Tropical Fruit (Pineapples and Melons) and Controlling Analytical Quality with the Aid of Nuclear Techniques (PAN/5/017)	B Maestroni, I Ferris
Supporting the Accreditation of a Pesticides Residue Laboratory (PAN/5/019)	B Maestroni, I Ferris
Strengthening Laboratory Capacity to Assess the Implementation of Good Agricultural Practices in the Production of Fruit and Vegetables in Latin America (RLA/5/050)	I Ferris, G Dercon, B Maestroni
Implementing a Diagnosis System to Assess the Impact of Pesticide Contamination in Food and Environmental Compartments at a Catchment Scale in the Latin American and Caribbean (LAC) Region (ARCAL CII) (RLA/5/053)	I Ferris, B Maestroni, G Dercon
Improving Laboratory Capacity for Food Safety (TAD/5/004)	S Fesenko, I Ferris, B Maestroni
Determining Pesticide and Antibiotic Residues in Food for Local and Export Consumption (URU/5/025)	B Maestroni, I Ferris

Appendix 6

5.6. Abbreviations

ABL	FAO/IAEA Agriculture & Biotechnology Laboratory
CCα	Decision Limit
Codex	Joint FAO/WHO Codex Alimentarius Commission
CRP	Coordinated Research Project
ECD	Electron Capture Detector
FAO	Food and Agriculture Organization of the United Nations
GC	Gas Chromatography
GCMS	Gas Chromatography- Mass Spectrometry
GIS	Geographical Information System
GPS	Global Positioning System
HPLC	High Performance Liquid Chromatography
IAEA	International Atomic Energy Agency
ISTD	Internal Standard
LC	Liquid Chromatography
LC-MSMS	Liquid Chromatography-Tandem Mass Spectrometry
LIMS	Laboratory Information Management System
LOD	Limit of Detection
LOQ	Limit of quantitation
MRL	Maximum Residue Limit
NPD	Nitrogen Phosphorous Detector
OCP	Organochlorine Pesticides
OPA	Ortho-phthalaldehyde
PCB	Poly-chlorinated Biphenyl
PSA	Primary-Secondary Amine
RSD	Relative Standard Deviation
SIM	Selected Ion Monitoring
TCP	Technical Cooperation Project
TIC	Total Ion Chromatogram
WS-CRDS	Wavelength scanned cavity-ringdown spectroscopy

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

International Atomic Energy Agency
Wagramerstrasse 5
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