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Nuclear Techniques in Food and Agriculture

Agrochemicals Unit

Activities Report

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FAO/IAEA Agriculture & Biotechnology Laboratory
IAEA Laboratories Seibersdorf
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Vienna, Austria

The aims of the Agrochemicals Unit are to provide assistance and support to developing countries in their efforts to ensure the safety and quality of food and agricultural commodities, thereby safeguarding the health of consumers and facilitating international trade. The focus of the Unit's work is on improving member state laboratory practices and methodologies. The main areas of activity in pursuit of these objectives are applied research and development, technology transfer, training, and support for the development of international standards and guidelines.

The Unit's work currently centres 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 will become a major focus of the Unit in the future, and preliminary contacts were made and collaborations initiated during 2008 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 multiresidue methods for organochlorine and polychlorinated biphenyl contaminants in fish and organophosphates in fruit, developed and optimized using carbon-14 labelled tracers; an isotope-dilution method for sulphonamide antimicrobial drug residues in animal products; elaboration of an effective sample processing procedure for fumonisin mycotoxins; and multiresidue methods for contaminants in water by isotope-dilution gas chromatography-mass spectrometry. The Unit also provided support in response to an environmental and food chain contamination incident in Mongolia, through participation in a field investigation and provision of analytical services.

The laboratory interacted with a range of collaborators in its research and development activities, including with the EU projects BioCop, MoniQA, CONffIDENCE and ProSafeBeef. A project was initiated with the World Health Organization (WHO) and the World Organization for Animal Health (OIE) to compile a global survey of laboratory quality systems. The Unit also collaborated with the Entomology Unit through the development of a method to determine concentrations of tetracycline and its main metabolite in medfly diet used in a feeding experiment.

Several training activities were implemented at Seibersdorf in 2007-08, including two "train the trainers" workshops focusing on quality assurance/quality control measures and analytical methodology, and a TC group fellowship training course on the application of GC-MS to contaminant analysis. Fourteen TC fellows and 2 scientific visitors were also trained during 2007-08. In all, 53 individuals were trained in the Agrochemicals Unit for a total of approximately 60 man-months. Training and awareness building activities involving Unit staff outside Seibersdorf included various training courses in Panama and in the IAEA Collaborating Centre in Costa Rica and conferences/summits in China and Canada. 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.

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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 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 often present risks to human health and the environment and may create barriers to international trade in agricultural commodities. Governments increasingly rely on testing throughout the food production chain to ensure the safety and wholesomeness of foods. This encompasses a renewed emphasis on the control of contaminants at their source through feedback mechanisms such as post harvest/slaughter monitoring 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 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 and improving decision making and for the improvement of food safety and environmental protection. Laboratory analytical capabilities and quality assurance and quality control procedures must be strengthened to enhance the role of the laboratory in import/export certification, to provide indicators for the successful use of good production practices and to control food processing practices.

In order to achieve the Subprogramme and Unit objectives, close linkages must be fostered with other subprogramme areas, especially those dealing with insect pest management and crop and animal production. These efforts will help governments in their efforts to ensure the safety and quality of foods throughout the food production chain based on the use of appropriate production technologies, including those developed through international standardization bodies.

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3. RESEARCH AND DEVELOPMENT ACTIVITIES

3.1. Validation of an Efficient Method for the Determination of Organochlorine Pesticides in Fish by Gas Chromatography, with Optimisation Using ^{14}C-lindane and ^{14}C-DDT

Contamination of aquatic resources, including freshwater, estuarine and marine fish and shellfish has been documented in the scientific literature for many regions of the world. Environmental concentrations of some pollutants have decreased over the past 25 years as a result of better water quality management practices. However, environmental concentrations of other contaminants such as heavy metals, pesticides and other toxic organic compounds have increased due to intensifying urbanization, industrial development and use of new agricultural chemicals. Aquatic organisms may bioaccumulate environmental contaminants to more than 1 000 000 times the concentration detected in the water column. Fish and shellfish monitoring serves as an important indicator of sediment contamination and water quality problems and also enables responsible agencies and competent authorities to detect levels of contamination in edible fish and shellfish that may be harmful to human consumers, thus facilitating effective risk management practices. The capability to implement monitoring activities is also important to many countries that export fish and shellfish, both wild-catch and from aquaculture, to enable them to meet the quality requirements to maintain this trade.

The use of target species and target analytes in fish and shellfish contaminant monitoring programs is essential to compare data among sites over a wide geographic area and to protect human health. Criteria for selection of the target analytes in these programmes vary widely depending on specific program objectives. The study summarized here was initiated in response to requests from several countries for assistance in testing for residues of chlorinated pesticides. Although use of some of the organochlorine pesticides was terminated more than 20 years ago in many countries of the world, it is still necessary to test for these compounds, since they were used in large quantities for over a decade and can persist at high concentrations in the environment and consequently in food.

The selection of analytical methods for routine analysis must be based on factors such as available resources, experience, programme objectives and data quality requirements. There are several analytical techniques available for the determination of organochlorine pesticides in fish tissue; however, these employ complex, time consuming and expensive extraction, clean-up and analytical procedures and are difficult or impossible to apply in many developing countries. The aim of this study was to further adapt a simple, rapid and inexpensive method previously validated in the Agrochemicals Unit for the analysis of pesticide residues in fruit and vegetables[1] and in wheat flour[2] for the determination of organochlorine pesticides in edible fish tissue.

[1] Aysal, P., Ambrus, Á, Lehotay, S.J. and Cannavan, A. (2007). Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. Journal of Environmental Science and Health B, 42, 481-490.
[2] Aysal, P., Mushi, G. and Cannavan, A. (2006). Adaptation of the IAEA-ethyl acetate multiresidue method to determine pesticide residues in wheat flour. Book of abstracts of the International Conference on Pesticide Use in Developing Countries: Environmental Fate, Effects and Public Health Implications, 16-20 October 2006, Arusha, Tanzania, 51.

The IAEA–ethyl acetate multi residue method for the determination of pesticide residues in fruits and vegetables is an adaptation of the QuEChERS method[3]. Ethyl acetate is used for the extraction to reduce costs and permit analysis by gas chromatographic (GC) techniques using conventional electron capture and nitrogen-phosphorous detectors (ECD, NPD) as well as mass spectrometric detection, in order to increase the applicability of the method to laboratories where mass spectrometry is not available. The initial ethyl acetate extract is cleaned up by dispersive solid phase extraction with primary-secondary amine sorbent and anhydrous magnesium sulphate to remove many polar matrix components common in food matrices, such as organic acids and certain polar pigments. In 2006, the method was further developed for the analysis of pesticides in wheat flour, which is a dry, absorbent matrix and can cause difficulties with the extraction of analytes. Further modification of this method to enable extraction from oily fish tissue would make the method a powerful one, facilitating the analysis of a wide variety of matrices using a single method, with slight variations, and minimising both the laboratory equipment and analyst training required.

During development/adaptation, the individual steps of the method were optimized using radiolabelled versions of a relatively non-polar and a polar organochlorine pesticide, ^{14}C-lindane and ^{14}C-DDT, respectively. The method was validated using spiked samples of Nile perch, which is one of the recommended predator target species for inland fresh water and great lake waters.

3.1.1. Experimental

Frozen Nile perch fillets were purchased from a local store and analysed to ensure that they were negative for the target analytes. These were used in fortification experiments and to prepare matrix blanks for matrix-matched calibration standards. Upon receipt in the laboratory, partially frozen Nile perch fillets were comminuted using a Stephan chopper, divided into 100 g portions and stored in a freezer pending analysis. Composite samples were re-homogenized with a hand blender prior to weighing out analytical portions.

The twelve representative pesticide reference standards used in the study are listed in **Figure 1**. Radiolabelled pesticides, ^{14}C-lindane and ^{14}C-DDT, of specified concentration and activity, were used in separate experiments to estimate the efficiency and repeatability of the extraction and clean-up steps of the method. Blank samples of Nile perch were spiked with the labelled compounds. After each extraction or clean-up procedure, aliquots of the extracts were removed, added to scintillation cocktail and the activity measured on a liquid scintillation counter and compared with the known added activity.

Total oil content and pH value of the fish samples were determined as 0.28 % ± 0.05 and 6.99, respectively. Since the original sample pH was neutral, the sodium bicarbonate, which was used as neutralizing agent in original methods for fruits and vegetables and cereals, was omitted for the analysis of fish samples.

Portions (15 g) of previously comminuted sample were spiked as necessary for method validation (10, 100 and 1 000 µg/kg) and mixed with 15 g anhydrous sodium sulphate. Ethyl

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

acetate (30 mL) was added at 30°C and each sample was homogenized immediately using a probe blender for 2 minutes. After centrifugation, 10 mL aliquots of the ethyl acetate extract were vortex mixed for 45 seconds with 0.25 g primary-secondary amine sorbent and 1.5 g anhydrous magnesium sulphate ($MgSO_4$). After centrifugation, the supernatant extracts were analysed directly by GC-ECD.

3.1.2. Results

3.1.2.1. ^{14}C-lindane and ^{14}C-DDT recoveries

Table 1 shows the recoveries of ^{14}C-lindane and ^{14}C-DDT during the extraction and dispersive-SPE clean-up steps. Overall recoveries were 82 and 79% for ^{14}C-lindane and ^{14}C-DDT, respectively. The overall precision, expressed as relative standard deviation (RSD), was between 2 and 9%. There was no significant loss of either representative analyte during dispersive-SPE of the fish extracts. The overall method recovery for both analytes averaged 80%.

Table 1. ^{14}C-lindane and ^{14}C-DDT recoveries (Q) and precision (RSD) for extraction and clean-up steps.

C14 labelled pesticide	Extraction[a]		Cleanup[b]		Overall[c]	
	Q (%)	RSD (%)	Q (%)	RSD (%)	Q (%)	RSD (%)
^{14}C-Lindane	90	2.3	91	2.1	82	2.2
^{14}C-DDT	86	8.7	92	3.6	79	8.7

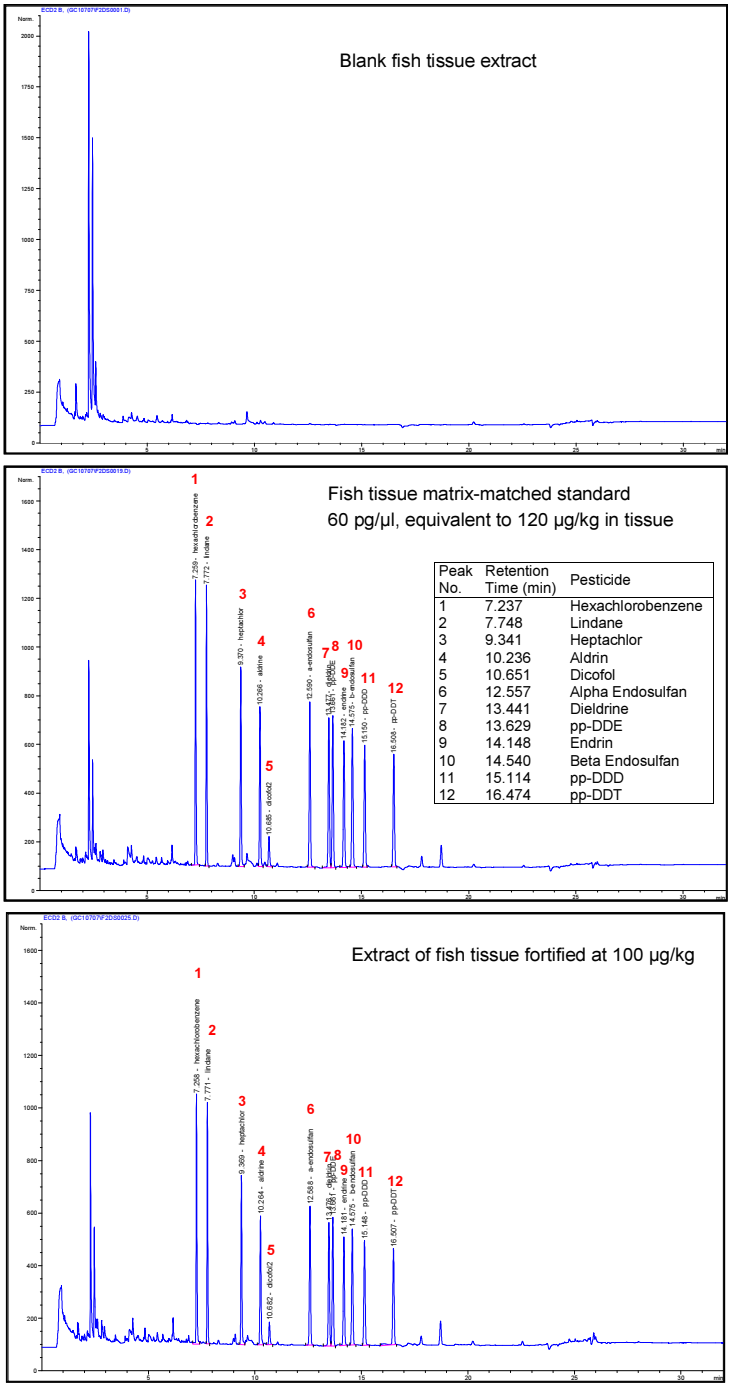
[a] triplicate aliquots and measurements of 8 samples in two different days (5+3) for lindane and 7 samples for DDT; recovery for extraction step only
[b] duplicate aliquots and measurements of samples; recovery for cleanup step only
[c] combination of extraction and cleanup measurements

Since the results of the radiotracer study indicated that the adapted method gave satisfactory results for both a non-polar and a polar representative organochlorine pesticide, further method characterization and validation experiments were designed and performed for the twelve selected compounds by fortification with unlabelled compounds.

Preliminary optimization of the extraction step using ^{14}C labeled pesticides.

3.1.2.2. Recovery of pesticides from fortified fish tissue

Representative GC-ECD chromatograms of a negative fish tissue extract, a calibration standard containing the twelve organochlorine pesticides at a concentration equivalent to 120 µg/kg in fish tissue, and an extract of tissue spiked at 100 µg/kg are presented in **Figure 1.** Peak shapes and resolution for all analytes were acceptable and the matrix extract exhibited no interfering peaks. All analytes could be quantified at concentrations ≤ 10 µg/kg.



Peak No.	Retention Time (min)	Pesticide
1	7.237	Hexachlorobenzene
2	7.748	Lindane
3	9.341	Heptachlor
4	10.236	Aldrin
5	10.651	Dicofol
6	12.557	Alpha Endosulfan
7	13.441	Dieldrine
8	13.629	pp-DDE
9	14.148	Endrin
10	14.540	Beta Endosulfan
11	15.114	pp-DDD
12	16.474	pp-DDT

Figure 1. Representative GC-ECD chromatograms of pesticides in fish tissue.

Figure 2 shows the recovery of each of the twelve pesticides at each of the three fortification levels in fish tissue. The values depicted are averages of results from two different occasions. The accuracy (measured as recovery) and precision (intra-laboratory repeatability, RSD) of the method, averaged for all twelve analytes, are summarized in **Table 2.** All pesticide recoveries fell within the acceptable limits recommended by Codex Alimentarius. For all 12 pesticides in fish at 3 levels, the overall recovery of the method was 90% with a RSD of 8% (n = 479).

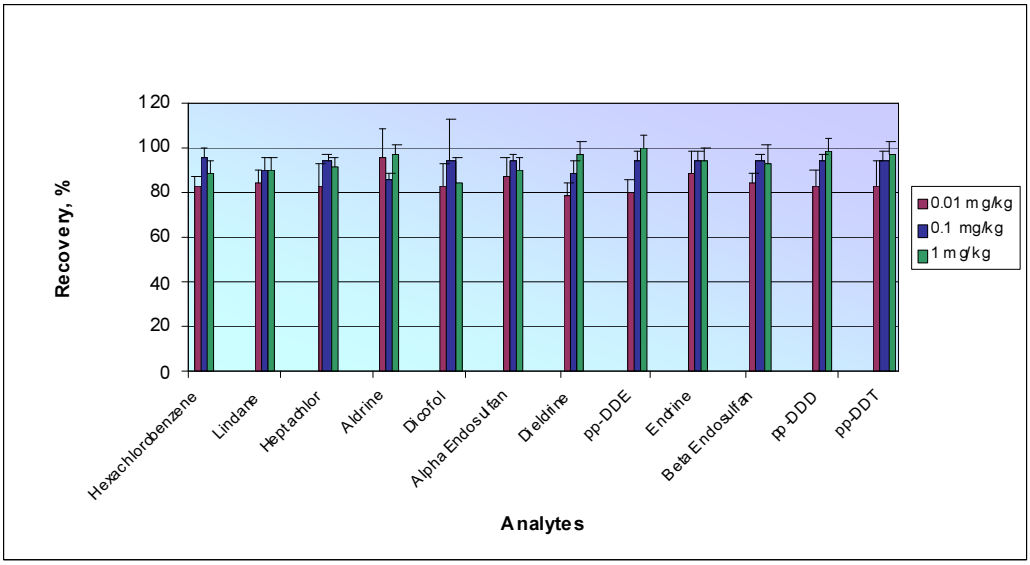


Figure 2. Recovery of the pesticides at three fortification levels (average of 2 occasions).

Table 2. Overview of performance characteristics (accuracy and precision) of the method at different levels. Analysis uncertainty is expressed as the relative standard deviation (RSD, n = 84 for each level).

	Fortification Level µg/kg	Accuracy		Precision	
		Average recovery (%)	Codex acceptable range	Reproducibility RSD (%)	Codex acceptable range
Day 1	**10**	84	70-120	12	20
	100	89	70-120	6	20
	1000	95	70-110	9	15
Day 2	**10**	83	70-120	10	20
	100	96	70-120	6	20
	1000	91	70-110	6	15
Overall		**90**		**8**	

3.1.2.3. Limit of Detection (LOD)

The LOD of the method for each analyte was estimated using matrix-matched weighted-regression calibration curves. Limits of detection for the analytes for GC-ECD analysis were within the ranges of LODs of contemporary published methods for organochlorines in fish, including those using advanced analytical techniques such as tandem mass spectrometry. The standard deviations of relative *y* (response) residuals (Srr) of the weighted regression calibration was ≤0.1 for all analytes in the study, thus meeting accepted quality control criteria.

3.1.3. Conclusions

The IAEA-modified QuEChERS method using ethyl acetate at 30°C as extractant and GC-ECD for analysis was successfully validated for 12 representative organochlorine compounds in fish at 3 levels.

This method is a very useful alternative to other published methods for the analysis of organochlorine residues in fish because of its simplicity and cost-efficiency. It can be applied using laboratory apparatus and gas chromatographic instrumentation available in most pesticide laboratories, including those in developing countries. Ethyl acetate is a less expensive solvent than acetonitrile, which is used in the original QuEChERS method, and this cost advantage will become even more marked because of the predicted world shortage of acetonitrile in 2009 and beyond and the consequent increase in the price and difficulty in sourcing this solvent.

It is envisaged that future work in the Agrochemicals Unit will expand the range of analytes covered to include other compounds such as organophosphorous pesticides and PCBs (see **Section 3.2.**) in different target species (matrices), making the method a powerful tool for fish and shellfish contaminant monitoring programmes.

This work was carried out under Project 2.1.3.2, Activity 2.

3.2. Simultaneous Determination of Organochlorine Pesticides and Polychlorinated Biphenyls in Fish Tissue

Persistent organic pollutants (POPs) are a heterogeneous group of substances including polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/F), organochlorine pesticides (OCP) and other organic pollutants such as polycyclic aromatic hydrocarbons (PAH).

These synthetic organic compounds introduced a revolution in the industrial and agricultural sector in the second half of the 20th century. For example, a great number of these compounds were introduced as pesticides to raise crop productivity. It has been estimated that crop loss would double if pesticides were not used. The PCBs, because of their thermal stability, fire and oxidation resistance, and solubility in organic compounds; have been used in various industrial applications including as insulating fluids in electrical transformers and capacitors, as plasticizers, as lubricants, as fluids in vacuum pumps and compressors, and as heat transfer and hydraulic fluids.

These compounds, whether used in agriculture, manufacturing or for other industrial purposes, are frequently found as pollutants in the aquatic environment where they may be accumulated and concentrated by water organisms and eventually result in contamination of the food chain, bioaccumulating to relatively high concentrations in top-level predators such as fish and marine mammals. The ingestion of foods contaminated with persistent lipophilic pesticides and PCBs can result in their accumulation in humans and a consequent risk to human health, especially to vulnerable sectors of the population. For example, these compounds can cross placental barriers and may cause serious neonatal damage even at very low trace concentrations. Organochlorine pollutants have been implicated in a broad range of adverse human health and environmental effects including reproductive failures and birth defects, immune system dysfunction, endocrine disruption, and cancers. Generally, PCBs have a low order of acute toxicity but because of their high fat solubility and poor metabolism, they present a substantial threat to man. Because of their indefinite half life the problems with bioaccumulation are great. A major target organ of PCB contamination is the liver.

In order to protect human health and the environment, it is necessary to have analytical methods to detect and quantify these pollutants. **Section 3.1.** of this report described a multi-residue method for the determination of organochlorine pesticide residues in fish. It is common to have methods developed for the determination of a range of compounds within a class such as this. An approach with wider and more cost-effective potential application is to target multiple classes of contaminants in a single multi-residue method. The method previously described was, therefore, expanded to include a number of PCBs in fish in addition to a range of organochlorine pesticides.

Currently there are no standard methods for the determination of individual PCB congeners; the standard method applied is for the commercial product, arochlor, which comprises an undetermined mixture of PCB compounds. However, PCBs in environmental samples and human tissues are not adequately characterized by this method, and the Environmental Protection Agency (EPA) has suggested that individual PCB congener analysis is preferable, since this will provide a more accurate determination of total PCB concentrations.

3.2.1. Experimental

Polychlorinated biphenyls comprise a group of 209 possible isomers of unsubstituted biphenyls. The nine compounds selected for this study are those recommended by EPA, as listed in **Table 3**. Twelve organochlorine pesticides were included, as described in **Section 3.1.** of this report, giving a total of 21 analytes.

The method was validated in edible tissues of Nile perch, and sample preparation and analysis were as described in **Section 3.1.** Samples were analysed by gas chromatography with electron-capture detection. Gas chromatographic conditions were optimized to provide chromatographic resolution of the selected analytes.

Table 3. Polychlorinated biphenyls included in the method.

1. PCB	2. IUPAC Name
3. PCB-18	4. 2,2´,5-Trichlorobiphenyl
5. PCB-28	6. 2,4,4´-Trichlorobiphenyl
7. PCB-52	8. 2,2´,5,5´-Tetrachlorobiphenyl
9. PCB-101	10. 2,2´,4,5,5´-Pentachlorobiphenyl
11. PCB-118	12. 2,3´,4,4´,5-Pentachlorobiphenyl
13. PCB-153	14. 2,2´,4,4´,5,5´-Hexachlorobiphenyl
15. PCB-180	16. 2,2´,3,4,4´,5,5´-Heptachlorobiphenyl
17. PCB-194	18. 2,2´,3,3´,4,4´,5,5´-Octachlorobiphenyl
19. PCB-209	20. 2,2´,3,3´,4,4´,5,5´,6,6´-Decachlorobiphenyl

3.2.2. Results

Figure 3 shows the recoveries for all spiked pesticides/PCBs in fish as an average of two different occasions. All compounds' recoveries are within the range 65-119 % at all spiking levels in fish. For all 21 analytes in fish at all 3 levels, the overall recovery of the method was 88% with RSD of 8% (n = 840).

The limits of detection for the method ranged from approximately 0.5 µg/kg (heptachlor) to 6.4 µg/kg (dieldrin).

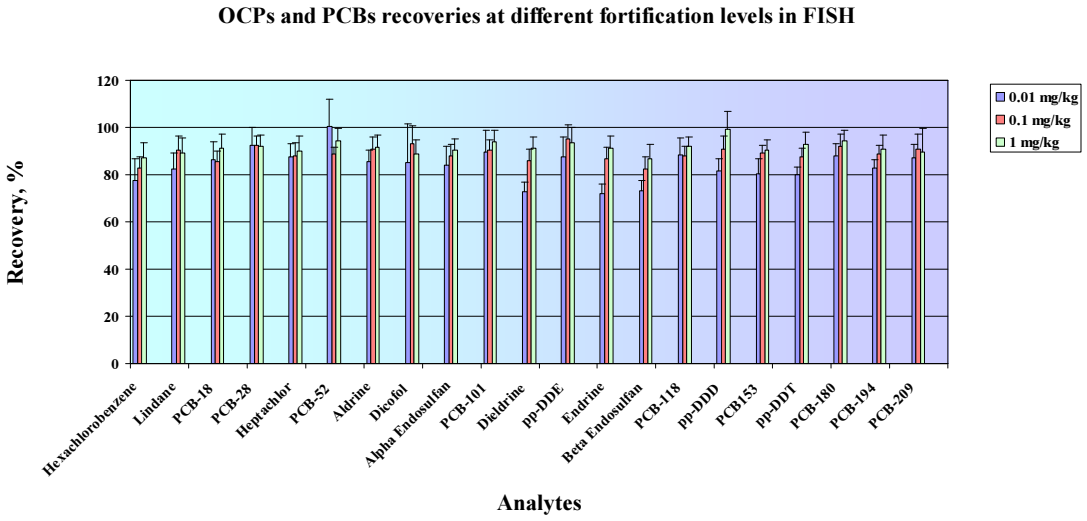


Figure 3. Method recovery for PCBs and organochlorine pesticides.

3.2.3. Conclusions

The method using heated ethyl acetate for extraction, dispersive solid phase extraction for clean-up and gas chromatography with electron-capture detection was successfully validated for 21 representative target organochlorine pesticides and PCBs in fish tissues at 3 fortification levels. The method is simple and cost-efficient and the use of gas chromatography with electron-capture detection makes it applicable in many developing countries, since this instrumentation is a common in pesticide residue laboratories. The multi-target, multi-residue approach provides a powerful tool for monitoring contaminants in food, and this approach will be further developed in future work in the Agrochemicals Unit. The applicability of the method to fish tissue facilitates its use in both a food safety framework and as an indicator of environmental contamination.

This work was carried out under Project 2.1.3.2, Activity 2.

3.3. Validation of a Robust Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry (LC-MSMS) Confirmatory Method for 13 Sulphonamides

The sulphonamides are an important class of antibacterial drugs widely used in veterinary practice and animal production as therapeutic agents and growth promoters. Codex Alimentarius and other national and regional bodies have set maximum residue limits (MRL) for sulphonamides in various animal tissues to protect the health of the consumer from potentially harmful effects of residues of these drugs in animal-derived foods. It is also necessary for countries wishing to participate in international trade in these commodities to adhere to the MRLs. To monitor compliance with these MRLs, highly specific and selective methods must be available to confirm the presence of any sulphonamide(s) at a level greater than the MRL in a sample.

A simple and rapid quantitative method for the simultaneous determination of seven sulphonamides was described in a previous Agrochemicals Unit activities report and in the literature[4]. The method employed high performance liquid chromatography (HPLC) with post-column derivatisation and ultra-violet detection. This method is suitable for screening and quantitation for sulphonamides in both developed and developing countries. However, in order to comply with international analyte identification requirements, a more specific method must be available for confirmation of the screening/quantitative result. The HPLC method previously developed was, therefore, further developed to produce a confirmatory LC-MSMS method. The scope of the method was also broadened to include residues of 13 compounds in the sulphonamide class, in porcine kidney and chicken muscle tissues.

3.3.1. Experimental

The method employs a simple extraction protocol using an inexpensive extraction solvent (ethyl acetate), concentration by evaporation, a hexane wash and reconstitution in LC mobile phase. Tissue samples were finely chopped in a blender and aliquots were weighed into centrifuge tubes The samples were extracted by homogenization with ethyl acetate in the presence of anhydrous sodium sulphate (for porcine kidney) and hydrochloric acid. The extracts were centrifuged and aliquots of supernatant were evaporated to dryness under a stream of nitrogen at 55°C. The residues was redissolved in mobile phase (water/acetonitrile, 85:15 v/v, containing 5 mM formic acid) and washed with hexane. The aqueous extract was analysed by LC-MSMS.

Identification and quantitation were achieved using a triple-quadrupole mass analyser with positive mode electrospray ionization. Quantitation was based on matrix-matched calibration and the inclusion of a stable isotope internal standard (d4-sulphadimidine) to resolve matrix ion-suppression effects and run-to-run variation in instrument response, and to improve the precision of the results.

The method was fully validated on 3 days at three concentration levels, ½ MRL (50 µg/kg), MRL (100 µg/kg) and 1½ MRL (150 µg/kg) for the 13 sulphonamides listed in table 1 in porcine kidney, with a single day validation in chicken muscle.

[4] Dabalus Islam, M., Schweikert Turcu, M., and Cannavan, A. (2008). Comparison of methods for the estimation of measurement uncertainty for an analytical method for sulphonamides. *Food Additives and Contaminants*, **25**, 1439-1450.

The ruggedness of the method was evaluated by introducing minor alterations to seven variables in the extraction procedure and testing method recovery and repeatability against the nominal method. The variables selected were: molarity of the hydrochloric acid at the extraction stage, weight of sodium sulphate added, homogenization time, centrifugation time/speed, evaporation temperature, evaporation just to dryness before removing from the turbo-vap as compared with allowing it to remain in the turbo-vap for 10 minutes after evaporation, and the volume of hexane for the final wash step. Four replicates of blank matrix spiked at 100 μg kg^{-1} were tested for each variable.

3.3.2. Results

All compounds eluted from a reversed-phase column within 8 minutes.

Multiple reaction monitoring chromatograms of two daughter ions derived from the parent ions of each of the 13 sulphonamides (plus the deuterated sulphamethazine internal standard) are presented in **Figure 4**. The method meets the identification criteria specified by the European Union in Commission Decision 2002/657/EC, and included in the current draft revision of the Codex guidelines, for confirmatory methods for compounds licensed for use in food-producing animals.

The precision and accuracy of the method are summarized in **Table 4**. Recoveries, calculated for all compounds using the d4-sulphamethazine internal standard, ranged between 69% and 108.9% for 11 of the analytes, with lower values for sulphaguanidine (23% in chicken muscle and 36% in pig kidney) and sulphaquinoxaline (59% in chicken muscle and 60% in pig kidney). Method performance would be improved for these latter analytes by the inclusion of stable isotope labelled internal standards for each compound. Precision was good for all analytes, with reproducibility ranging between 1.5 and 10.5% in chicken muscle and 2.3-7.9% in pig kidney.

The decision limits (CCα, also shown in **Table 4**) for each compound were calculated according to the calibration curve procedure described in ISO 11843 and recommended in Europe in Commission Decision 2002/657/EC. For the control of compliance, the measurement uncertainty is taken into account by applying the decision limit. Commission Decision 2002/657/EC specifies that the result of an analysis shall be considered non-compliant if the decision limit (CCα) of the method is exceeded.

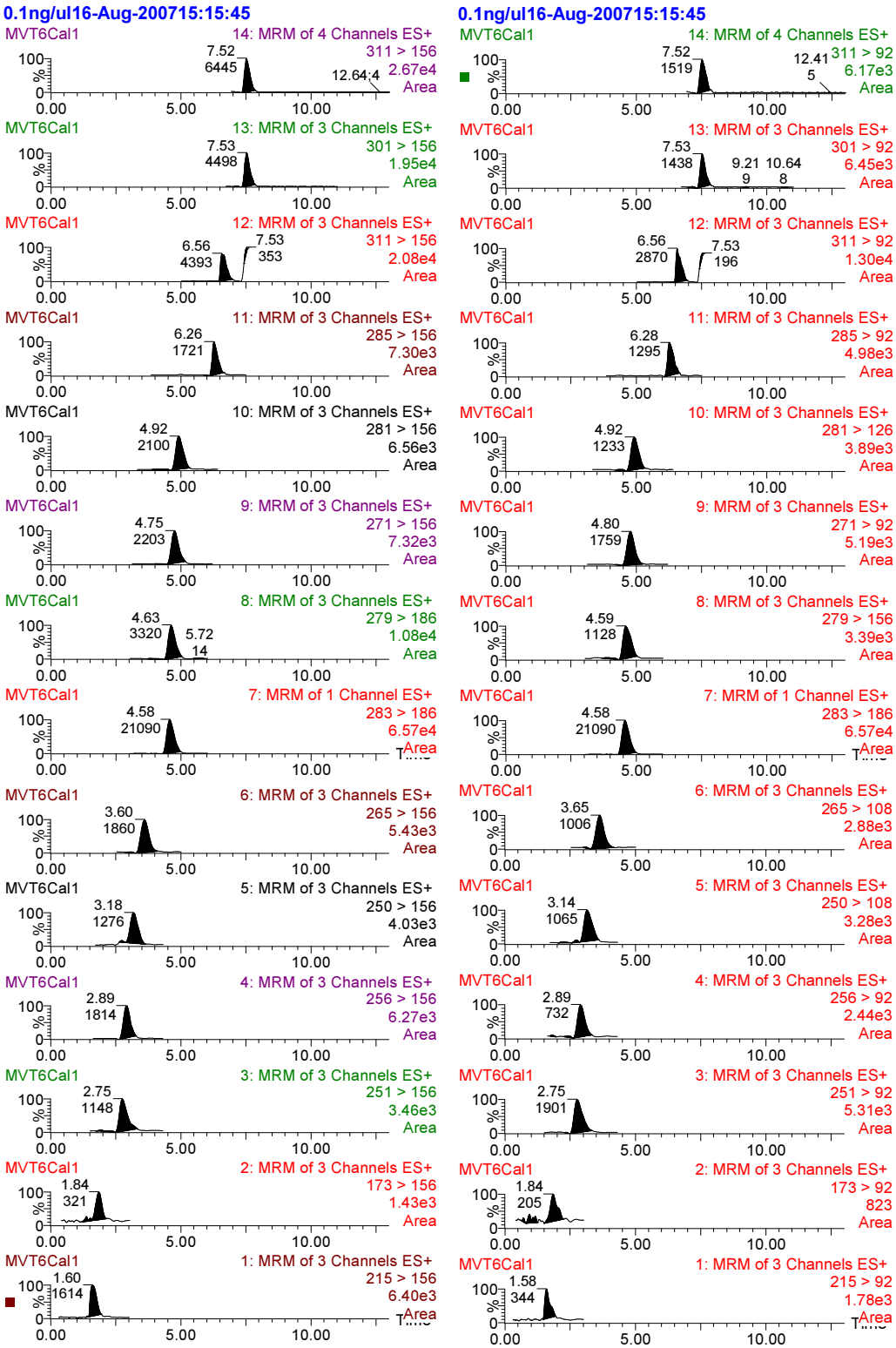


Figure 4. Chromatograms of 2 daughter ions derived from each of 13 sulphonamides.

Table 4. Summary of method validation parameters for sulphonamides in two matrices.

	Precision		Accuracy		Decision limit (CCα)
	Chicken muscle	Porcine kidney	Chicken muscle	Porcine kidney	
	% RSD		% Recovery		MRL=100μg/kg
Sulphaguanidine	8.9	5.0	22..9	35.4	107.2
Sulphanilamide	7.1	7.6	76.1	83.9	112.1
Sulphadiazine	5.5	7.9	89.9	107.8	112.3
Sulphathiazole	3.8	6.8	76.4	99.5	113.0
Sulphapyridine	7.7	7.1	102.1	105.9	114.4
Sulphamerazine	5.6	5.7	100.4	83.4	110.9
Sulphamethazine	1.5	2.7	99.1	96.1	103.9
Sulphamethizole	4.4	2.8	69.2	75.5	105.3
S'methoxypyridazine	2.9	2.3	93.7	87.0	103.3
S'chloropyridazine	4.9	2.9	88..6	90.1	105.3
Sulphadoxine	4.2	2.8	98.6	105.1	104.3
Sulphaquinoxaline	8.0	4.0	59.1	59.8	106.3
Sulphadimethoxine	8.7	3.2	71.4	77.1	104.4

The introduction of minor variations in the seven individual factors described above caused no significant variation in repeatability or recovery, demonstrating the ruggedness of the method.

Ionisation suppression in the mass spectrometer was observed for some of the compounds, as shown in **Figure 5**. Calibration standards were prepared in an extract of negative matrix to compensate for this effect.

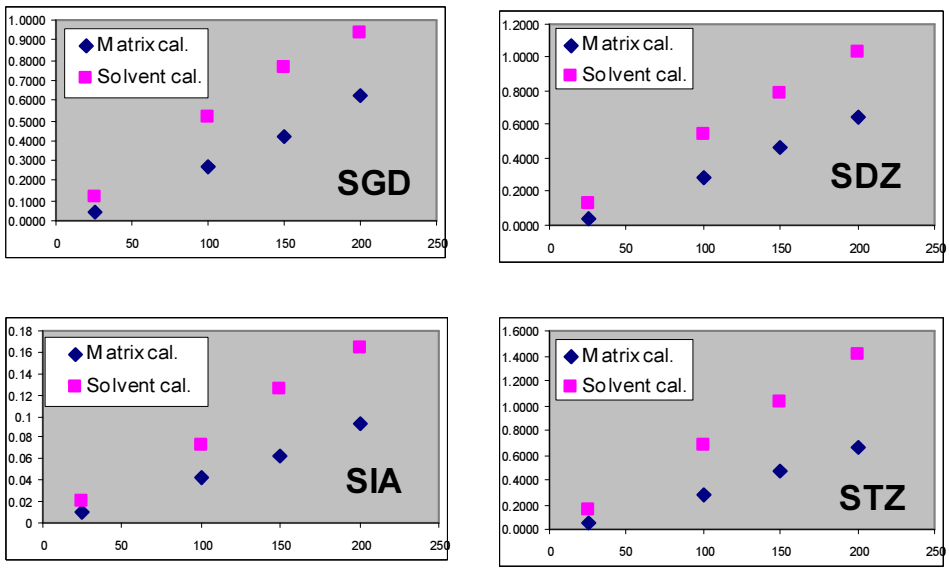


Figure 5. Ionisation suppression caused by the matrix for four of the 13 analytes.

3.3.3. Conclusions

The precision of the method was good for all analytes and accurate quantitation was achieved by using matrix-matched calibrators. The recovery of two of the analytes, sulphaguanidine and sulphaquinoxaline, was relatively low, but nonetheless, those analytes could still be quantified at the target concentration (50 µg/kg). The method meets the identification criteria requirements applied in Europe and recommended by Codex Alimentarius.

The method presented is rapid and relatively inexpensive in comparison to other published methods for confirmation of sulphonamides and is suitable for use in both developed and developing country regulatory laboratories that are equipped with LC-MSMS.

Scientists from twenty developing countries were trained in the method at Seibersdorf in 2007. The method was also presented at the 3rd International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 7-9 November 2007[5].

This work was carried out under project 2.1.3.2, activity 2.

[5] Islam, M., Cannavan, A. and Schweikert Turcu, M. (2007). Validation of a robust liquid chromatography – tandem mass spectrometry (LC-MSMS) confirmatory method for 13 sulphonamides. Book of abstracts of the 3rd International Symposium on Recent Advances in Food Analysis, 7-9 November 2007, Prague, Czech Republic, 170, 235.

3.4. A Simple, Economical and Efficient Sample Processing Procedure for Fumonisin B1 Analysis in Maize

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

The objective of this work was to compare two processing procedures, dry processing and wet (slurry) processing, for the analysis of maize samples for fumonisin B1 (FB1) in order to identify a simple, economical and efficient procedure applicable in developing country laboratories lacking equipment such as large-volume grinders and homogenizers.

3.4.1. Experimental Design

Due to the fact that grinding and preparing a slurry for a whole bulk sample of 20 kg is not a practical laboratory operation, we tested the applicability and efficiency of a multi stage procedure consisting of:

- thorough mixing of 20 kg naturally contaminated maize grains in a concrete mixer;
- subdivision into 2 x 10 kg portions, using a sample divider;
- further mixing and sub-division into 2 x 5 kg portions;
- grinding of a 5 kg portion and addition of water to form a slurry.

The multistage procedure is presented in **Figure 6**.

Maize kernels (20 kg) were thoroughly mixed in a concrete mixer, subdivided into 1 and 5 kg subsamples for the dry and wet procedures, respectively. Each subsample was finely ground and 25 g analytical portions withdrawn for FB1 analysis. In the wet procedure the ground 5 kg sample was homogenized with water (1:3, w/v) until a fine slurry was obtained and an analytical portion of about 75 ml (25 g sample equivalent) was analysed. In further experiments, 1 litre portions of slurry were further homogenized to investigate the effect of double processing.

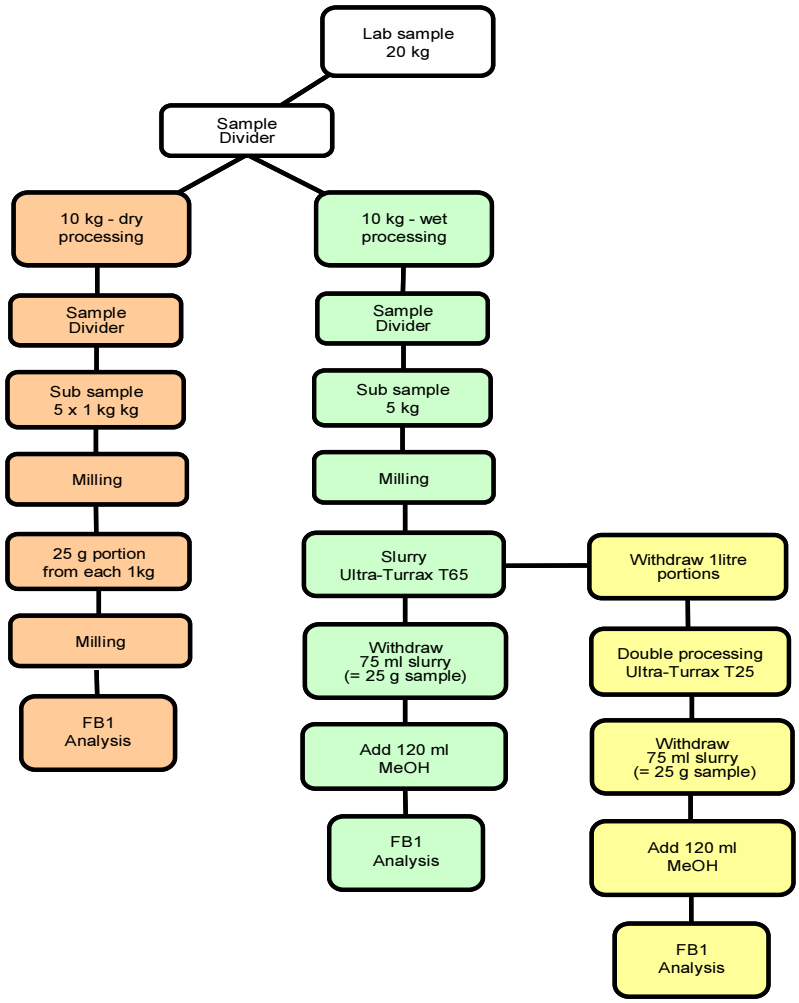


Figure 6. Multistage procedure and experimental design.

3.4.2. The Analytical Method

The analysis of fumonisin B_1 in the naturally contaminated maize sample was carried out by the analytical procedure described by Visconti[6] based on extraction with methanol/water 3:1 (v/v), cleanup on strong anion exchange (SAX) cartridges and reversed phase HPLC analysis with fluorescence detection of the FB1 after derivatization with ortho-phthalaldehyde (OPA). **Table 5** contains a list of equipment used and the approximate investment required to equip a laboratory with the items needed for the wet/slurry processing technique, as compared to large scale dry grinding equipment costing tens of thousands of Euros.

Table 5. List of equipment used.

STEPS OF THE ANALYSIS	EQUIPMENT	Approximate investment for sample preparation equipment (Euro)
Sample preparation and processing	Sample mixer (concrete mixer 80 l capacity); Cemotec 1090 Sample Mill (or similar). Ultra Turrax T65 (or similar)	100 500-4000 2000-4000
Withdrawal of subsamples and analytical portions	In-house sample divider; 1 and 3 digit balance Home made ladle	10 500 5
Sample extraction	Rotary shaker	
Clean-up of sample	SPE 12 port vacuum manifold, SAX cartridge	
Concentration-evaporation of samples	Sample concentrator	
HPLC analysis	Reversed-phase LC Nova pak C18, 3.9x150 mm column, C18 guard column, Scanning Fluorescence detector, 335 nm (Ex), 440 nm (Em)	

3.4.3. Statistical Treatment of Data

Each step of the analytical procedure contributes to the total uncertainty of the result (expressed as relative standard deviation or CV) according to the following equation[7]:

$$CV_{R=}\sqrt{CV_S{}^2 + CV_{SP}{}^2 + CV_A{}^2}$$

[6] Visconti, A. and Doko, B. (1994). Survey of fumonisin production by Fusarium isolated from cereals in Europe, Journal of the Association of Official Analytical Chemists (JAOAC) International, 77, 546-550.
[7] Ambrus, Á. (2004).Reliability of measurement of pesticide residues in food. Accreditation and Quality Assurance, 9, 288-304.

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

CV_L is the uncertainty of the laboratory phase: it is calculated as the ratio of the standard deviation to the mean:

$$CV_L = \frac{SD}{Mean}$$

CV_L conceptually represents the combined uncertainty of sample processing and analysis. Therefore the uncertainty of sample processing can be derived from the following formula:

$$CV_{SP} = \sqrt{CV_L^2 - CV_A^2}$$

CV_A was obtained based on duplicate analysis and estimated using the following formula:

$$CV_A = \sqrt{\frac{\sum d^2}{2n}}$$

where d is the difference between the results of duplicate analyses.

3.4.4. Results

The Fumonisin B1 results were statistically elaborated and used to estimate the uncertainty of the laboratory phase (CV_L), the reproducibility of analysis (CV_A) and the uncertainty of sample processing (CV_{SP}). The summary results obtained for the dry and the wet procedure are presented in table 6.

Analysis of variance of the results indicated no significant difference in the average fumonisin B1 content estimated for the dry and wet procedures. However, for the slurry procedure the sample processing uncertainty, expressed as relative standard deviation, was 5% - 13%, which is significantly lower than the uncertainty of about 36% estimated for the dry procedure[8].

The double processing slurry procedure not only increased the amount of work but also necessitated investement into a small slurry homogenizer (ultra-turrax type). As shown in **Table 6** there was no improvement in the extraction efficiency for fumonisin B1 or the homogeneity of the processed sample.

[8] Maestroni, B., Bettaglio,M.,Ambrus, Á., Rathor, M.N. (2005). Estimation of the uncertainty of sample processing for the analysis of fumonisin FB1 in maize. Congress Proceedings, Vol.1, BCPC International Congress Crop Science and Technology 2005, 31 Oct-2 Nov 2005, Glasgow, UK, pp.411-416.

Table 6. Summary results obtained for the dry and the wet procedure including the FB1 content, the laboratory uncertainty (CV_L), the uncertainty of analysis (CV_A) and the uncertainty of sample processing (CV_{SP})

	Dry Procedure	Trial 1- single slurry	Trial 1- double slurry	Trial 2- single slurry	Trial 2- double slurry
FB1 (µg/g)	2.66	2.30	2025	3.06	3.14
n	49	10	14	7	12
CV_L	0.37	0.07	0.10	0.11	0.43
CV_A	0.10	0.05	0.05	0.05	0.05
CV_{SP}	0.36	0.05	0.10	0.10	0.13

3.4.5. Conclusions

The results demonstrated that the slurry technique provides an advantage over the dry processing procedure in terms of reduced contribution of the processing step to the uncertainty of the analytical result. There is no advantage in applying a second homogenization step. The proposed single processing slurry procedure is presented as a viable and more efficient alternative to the dry processing procedure and is recommended for regular use in analytical laboratories lacking large-volume processing/grinding equipment, and where such equipment can be replaced with appropriate small scale and home made equipment. The equipment required for the slurry technique applied to smaller analytical portions, as described above, is much less expensive than large-volume grinding and processing equipment.

This work was presented as a poster at the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Istanbul, Turkey, 21-25 May 2007[9].

This work was carried out under project 2.1.3.2, activity 2.

[9] Maestroni, B.M., Rathor, M.N. and Cannavan, A. (2007). A simple, economical and efficient sample processing procedure for fumonisin B1 analysis in maize. Book of abstracts of the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May 2007, Istanbul, Turkey, 1474.

3.5. Determination of Organophosphorus Pesticides in Pineapple: Application of ^{14}C-Labelled Chlorpyriphos to Compare the Processing Efficiency, Homogeneity and Optimal Sample Size under Ambient and Cryogenic Conditions

A quick, rapid and cheap method for the determination of selected organophosphorus compounds in pineapple by gas chromatography with a nitrogen-phosphorous detector (GC-NPD) was developed by the Agrochemicals Unit on the request of project counterparts, primarily for PAN/5/017. The method was demonstrated on the occasion of the scientific visit of project counterpart staff to the Unit. The sample processing uncertainty under ambient and cryogenic conditions was established as part of the method development using ^{14}C-labelled chlorpyrifos.

3.5.1. Method

A mixture composed of ethoprophos, terbufos, diazinon, disulfoton, chlorpyrifos-ethyl, ethion, triadimephon, profenophos, triazophos, fenithrothion was used to fortify pineapple fruit at 0.1 mg/kg. A 750 dpm/ml solution of ^{14}C-labelled chlorpyrifos was also applied to the fruit to quickly and precisely establish the homogenization efficiency.

Two different sample processing conditions were compared in the experiments: at ambient temperature and under cryogenic conditions.

The pineapple crowns were removed and the fruits were cut to provide representative portions. Using a syringe, the surface of the fruit was treated with the mixture of the pesticides and ^{14}C-labelled chlorpyrifos. Homogenized pineapple was used as a blank analytical sample and used to prepare positive quality control samples, spiked at 0.1 mg/kg and included in every batch of samples.

After surface treatment, the samples were left for 20 minutes to allow the pesticides to interact with the matrix and the solvent to evaporate. In the case of cryogenic processing the matrix was surface treated one day before the experiment, stored in a freezer overnight and finally processed in the presence of dry ice. The samples were homogenized in a Stephan Chopper for three minutes at ambient temperature, or for five minutes under cryogenic conditions with dry ice (450 g to 1.2 kg of pineapple sample). The homogenization time was established during pre-trials, and was the minimum time necessary to obtain a peel size diameter smaller than 2-3 mm.

Before proceeding to the withdrawal of analytical portions the cryogenically prepared analytical sample was left on the bench for a few minutes to allow the CO_2 to sublime.

The extraction and the clean up was performed according to the IAEA modified QuEChERS method[10]. In each experiment six portions of 100 g and six portions of 10 g of the homogenized pineapple were prepared. For every 10 g of matrix, 10 g anhydrous sodium sulphate (Na_2SO_4) and 1.67 g sodium bicarbonate ($NaHCO_3$) were added, followed by 20 ml ethyl acetate. The samples were shaken briefly to mix the salts, solvent and matrix, then each

[10] Maestroni, B.M., Rathor, M.N. and Cannavan, A. (2007). A simple, economical and efficient sample processing procedure for fumonisin B1 analysis in maize. Book of abstracts of the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May 2007, Istanbul, Turkey, 1474.

sample was extracted for 1 minute using an Ultra-Turrax homogenizer. The samples were centrifuged (1 300 g, 3 min). An aliquot of supernatant (10 ml) was cleaned-up by dispersive solid phase extraction using primary-secondary amine (PSA) and vortexed for 1 minute. For each 10 ml extract, 0.25 ± 0.01 g PSA sorbent and 1.5 ± 0.01 g anhydrous magnesium sulphate ($MgSO_4$) were used.

The extracts were centrifuged (750 g, 1 min), the supernatants evaporated to dryness and the final extract reconstituted to a final volume of 1 ml with iso-octane. The extracts were transferred to GC vials and analysed by GC-NPD using instrument parameters elaborated for the method.

Aliquots of the extracts were removed and the activity counted by liquid scintillation counter at various stages of the procedure to provide an estimate of the contribution to the overall uncertainty at each stage of the method.

3.5.2. Results

A calibration curve was prepared using five different concentrations of matrix-matched calibrators. Each curve met the quality control criteria of the standard deviation of the relative residuals (Srr < 0.1) and the correlation coefficient (R^2 >0.99).

Figure 7 shows the recoveries for the different pesticides under ambient and cryogenic conditions for the 30 g recovery samples. The beneficial effects of cryogenic processing conditions on the stability of organophosphorus pesticides can be clearly seen. In general the recoveries obtained under cryogenic processing were greater than 60%, whereas those for processing at ambient temperature for a 30 g analytical portion were less than 60%. This was also observed for 10g and 100 g analytical portions.

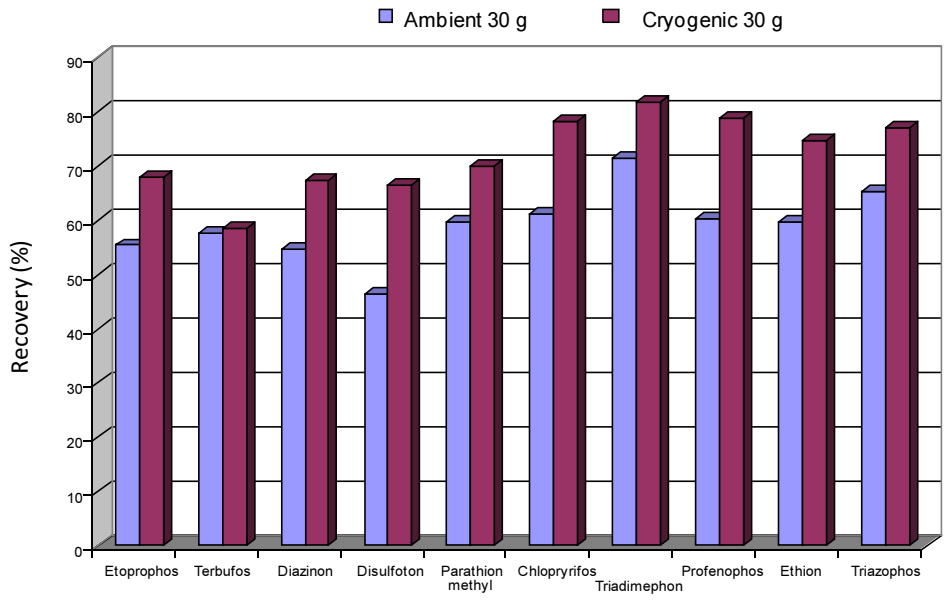


Figure 7. Comparison of sample processing conditions (ambient and cryogenic) for 30 g analytical portion.

To study the processing efficiency under ambient and cryogenic conditions, the activity of ^{14}C-chlorpyrifos was measured in the crude extract just after extraction with ethyl acetate. In this case the variability of the activity from the radiolabelled chlorpyrifos in small (10 g) and large (100 g) analytical portions gave an indication of the processing efficiency. The results of the average ^{14}C-chlorpyrifos recovery for the small and large analytical portions are shown in **Table 7**. The variability (CV%) is lower when cryogenic processing is applied for both the small and the large analytical portions, indicating that the sample is more effectively homogenized and that the contaminated particles are better distributed throughout the analytical sample when the processing is done under cryogenic conditions. Previous studies have also shown that sample processing under cryogenic conditions gives rise to a more homogeneous sample and the recoveries obtained are more representative of the entire sample.

Table 7. Comparison of the recovery and the variability values (CV%) of 14-C-Chlorpyrifos recoveries in small (10 g) and large (100 g) analytical portions.

	10g ambient	100 g ambient	10 g cryogenic	100 g cryogenic
Average (%)	98	89	91	92
Std Deviation	8	3	6	3
CV (%)	7.9	3.4	6.2	3.1
n	17	18	14	16

An analysis of variance (ANOVA) test was used to help determine whether or not the sample was well mixed. In the case of processing the sample at ambient conditions, the recovery values for the small and the large portion were significantly different at the 95% confidence level, indicating that the processing did not generate a homogeneous sample. However, in the case of sample processing under cryogenic conditions, there was no significant difference in the recovery values for the small and the large portions, indicating that the processing generated a well mixed sample.

3.5.3. Conclusions

Pineapple is a difficult matrix to process effectively for pesticide residue analysis at ambient temperature. Unfortunately this is the most common procedure in developing country laboratories, where access to dry ice and frozen conditions is difficult. At ambient temperature, not only is it difficult to obtain a well mixed sample, but also the stability of pesticides is compromised, as was shown by the lower recovery values under ambient conditions for the pesticides in the test mixture used. It is therefore recommended to process the sample under cryogenic conditions.

Notwithstanding the disadvantages of processing pineapple for analysis under ambient conditions, the analytical method developed in the Agrochemicals Unit was shown to perform satisfactorily and has been adopted by the counterpart laboratory in Panama.

This work was carried out under Project 2.1.3.2. activity 2.

3.6. Analysis of Chlortetracycline in Medfly Feed

The Agrochemicals Unit collaborated with the Entomology Unit by providing analytical services for a study to investigate levels of the antimicrobial agent, chlortetracycline, in a medicated diet for medfly larval rearing over a 10 day time period.

The analytical method was adapted from the literature and a preliminary validation performed to characterize the recovery and precision. Briefly, a homogenized sample was extracted with acetone:water:1M HCl (12:6:2) at pH <1.2, the extract was centrifuged and a portion of the supernatant was diluted with 0.02M oxalic acid:acetonitrile (8:2) for analysis by reversed-phase HPLC with fluorescence detection. Quantification was by comparison with a matrix matched calibration curve. Typical recovery for the method was between 80-90%, with typical relative standard deviations of about 4%.

A decrease in the concentration of chlortetracycline was observed over the period of the experiment, with a concurrent increase in a second chromatographic peak. Further investigation identified the second peak a 4-epichlortetracycline, an isomer of chlortetracycline with no antimicrobial activity. A typical chromatogram of a sample from day 10, showing the peaks for chlortetracycline and its 4-epimer, is presented in **Figure 8**.

The results of the study performed by the Entomology Unit will be published elsewhere.

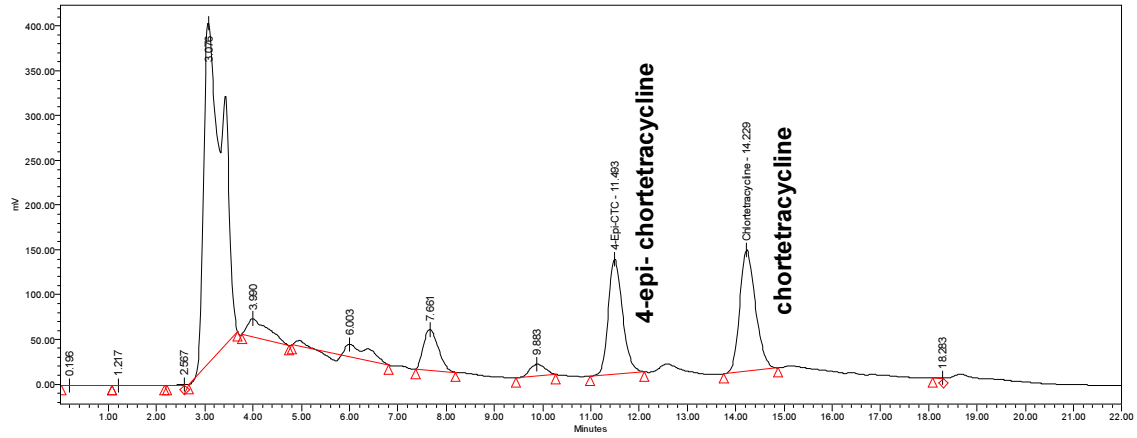


Figure 8. Chromatogram of an extract of medicated medfly larval diet showing peaks for chlortetracycline and 4-epichlortetracycline.

3.7. Analysis of Selected Pesticide Residues in Water by Isotope-Dilution GC-MS

Two methods for the analysis of selected pesticide residues in water samples were adapted in the Agrochemicals Unit during 2008. The methods used either liquid-liquid extraction (method 1, the n-hexane method), which was suitable for non-polar compounds, or solid phase extraction (method 2, the SPE method), for more polar compounds. Analysis was by gas chromatography-mass spectrometry (GC-MS).

The methods were developed within the framework of CRP D5.20.35, to provide analytical tools to monitor the effectiveness of good agricultural practices. A regional TCP, RLA/5/050, also adopted the analytical methodology within its network of laboratories in order to effectively monitor the effects of pesticide use in several environmental compartments.

The methods were adapted for the following analytes: alachlor, atrazine, azinphos-methyl, azoxystrobin, carbaryl, carbofuran, chlorothalonil, chlorpyrifos, cypermethrin, diazinon, endosulfan-alpha, endosulfan-beta, epoxiconazole, lindane, kresoxim-methyl, metolachlor, parathion-methyl, pendimethalin, pirimicarb, profenofos, prometryn, propiconazole and pyrimethanil.

Retention-time locking was used to standardize the parameters for the analysis. Chlorpyrifos methyl was chosen to lock the GC-MS acquisition method at a retention time of 17.41 minutes. Any drift in the retention time of the chlorpyrifos methyl in subsequent analytical runs was automatically compensated for by the gas chromatograph, thus enhancing the methods repeatability and transferability. Stable isotopes were used as internal standards (ISTD) to correct for the large variability in GC-MS response, to permit calculation of analyte concentrations by the isotope dilution principle and to provide information on the performance of the various stages of the analytical method. ^{13}C-DDT and ^{13}C-atrazine were used as ISTD 1, spiked into samples at the start of the extraction procedure for the n-hexane and SPE method, respectively. ^{13}C-sulfotep was used as ISTD 2, and added to the extract prior to evaporation; ^{13}C-triphenylphosphate was used as ISTD 3, added to the extract prior to adjustment of the final volume to 1 ml.

Calibrators were prepared in solvent at five different levels and spiked with the same amount of ISTD as the samples.

3.7.1. Extraction and Clean up Method 1: n-hexane Method

One litre of water was fortified at the desired level with a mixture of pesticide standards and with ISTD 1 (500 ng). Sodium chloride (30 g) and n-hexane (30 ml) were added to the water sample, which was stirred on a magnetic stirrer for 10 minutes to partition the analytes between the water and the n-hexane phase depending on their physico-chemical characteristics. The mixture was allowed to stand for five minutes to separate the n-hexane and aqueous phases. A microseparator apparatus (**Figure 9**) was inserted into the neck of the flask containing the sample, and additional water was added very slowly into the microseparator apparatus to displace and collect the full amount of n-hexane, which contained the extracted pesticides. The n-hexane phase was fortified with of ISTD 2 (500 ng) and evaporated on a rotary evaporator to less than

Figure 9. A microseparator apparatus.

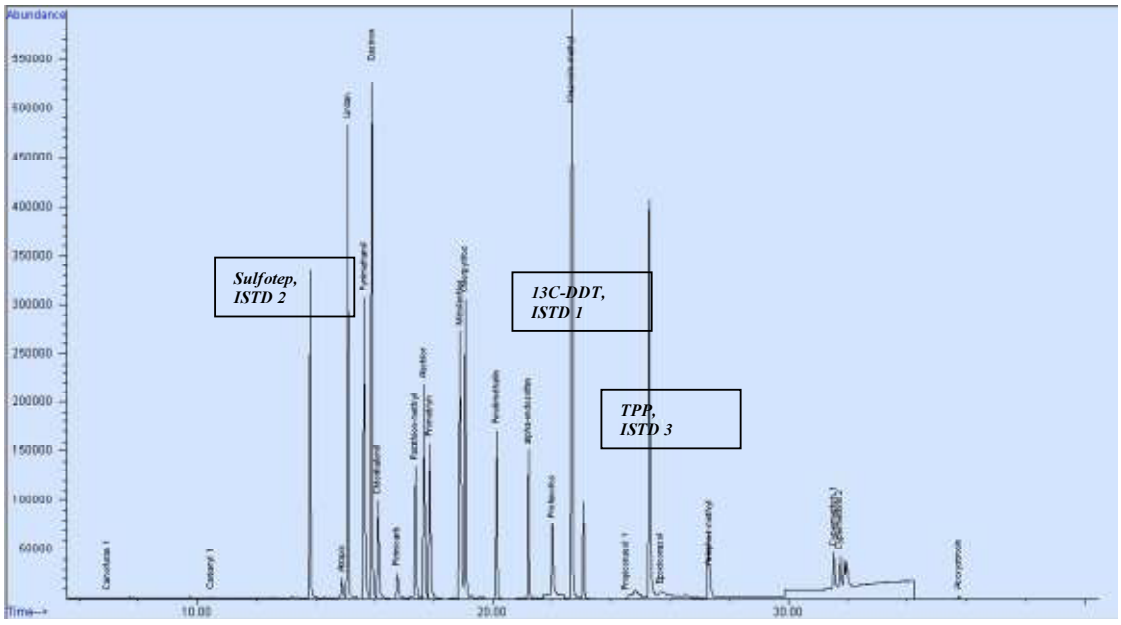


Figure 10. TIC for a water sample fortified at 0.5 ng/ml, extracted by method 1.

1 ml. ISTD 3 (500 ng) was then added, the volume was adjusted to 1 ml and the extract was transferred to GC vials for GC-MS analysis.

Figure 10 shows a typical total ion chromatogram (TIC) for a sample fortified at 0.5 ng/ml (ppb), extracted with n-hexane using the microseparator apparatus and quantified by selected ion monitoring (SIM).

3.7.2. Extraction and Clean up Method 2: SPE Method

One litre of water was fortified at the desired fortification level with a mixture of pesticide standards and with ISTD 1 (500 ng). Phenomenex Strata-X 33 SPE cartridges were conditioned three times with a mixture of methanol/acetonitrile (1:1, 1 ml) and three times with water (2 ml). The fortified water was slowly added to the preconditioned SPE cartridges, and the flow rate adjusted to 6 ml/min. Afterwards, each SPE cartridge was dried under a nitrogen stream. The pesticides were eluted from the SPE cartridges with methanol/acetonitrile (1:1, 6 ml)). ISTD 2 (500 ng) was added and the extract was evaporated to dryness using a turbo-vap apparatus. ISTD 3 was added prior to redissolving the extract in n-hexane (to a final volume of 1 ml). The final extract was transferred to GC vials for GC-MS analysis.

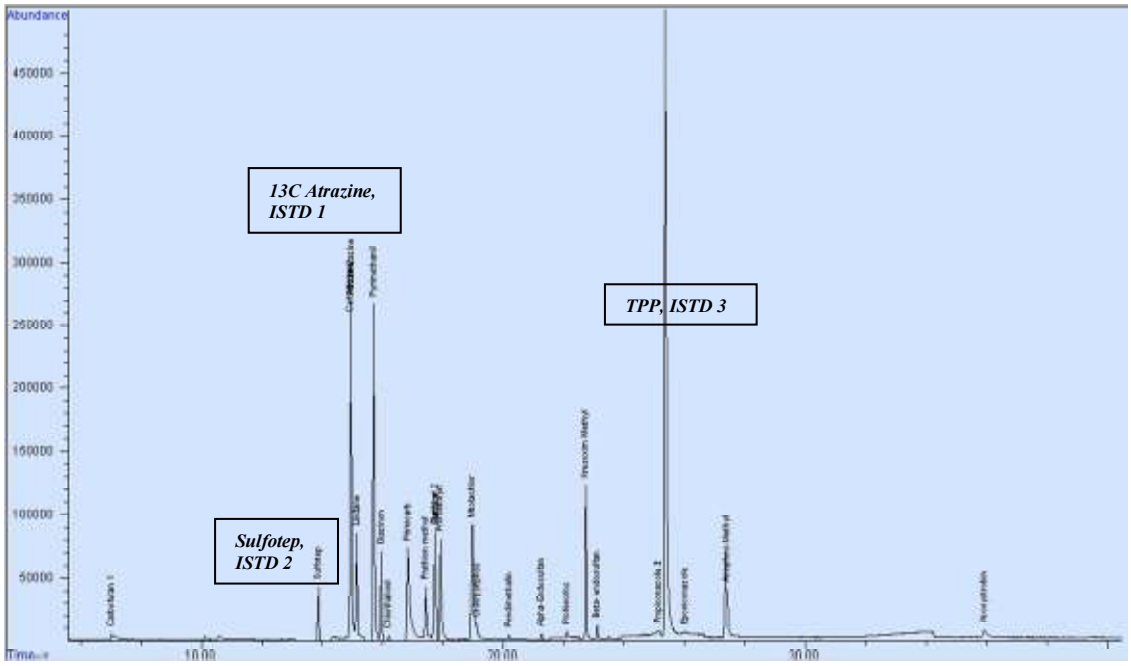


Figure 11. TIC for a water sample fortified at 0.5 ng/ml, cleaned up using method 2.

Figure 11 shows a typical total ion chromatogram for a sample fortified at 0.5 ng/ml, cleaned up using method 2. Quantification of each ion was by SIM.

Typical recoveries for the target analytes ranged from 60-120%, using the results of the more suitable extraction for each of the compounds analysed.

The methods have been successfully transferred to all CRP D5.20.35 contract holders' laboratories as well to all RLA 5/0/50 counterpart laboratories, where they are undergoing on-site validation.

This work was carried out under project 2.1.3.2, activity 4.

3.8. First Research Coordination Meeting of the Coordinated Research Project "Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale" (CRP D5.20.35)

The first Research Coordination Meeting (RCM) of the FAO/IAEA Coordinated Research Project (CRP) D5.20.35 on "Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale" was held at the Centro de Investigación en Contaminación Ambiental (CICA), Universidad de Costa Rica, in San José, Costa Rica from 9-13 July 2007. Ms Britt Maestroni was the scientific secretary for the meeting.

First Research Coordination Meeting in San José, Costa Rica.

The meeting was attended by Research Contract/Agreement Holders from Argentina, Australia, Brazil, Bulgaria, Chile, China, Costa Rica, Cyprus, Ecuador, Germany, Kenya, India, The Philippines, Sweden, as well as observers from Costa Rica, Canada and the IAEA.

The programme of the meeting included presentations on the results of the Consultants' Meeting held at IAEA Headquarters from 6-9 June 2006, including the specific objectives defined for the CRP; a review of the current regional initiatives in the context of integrated analytical approaches to assess the implementation of good agricultural practices (GAP); insights into agricultural non point-source contamination and pesticide monitoring based on first tier risk assessment; the minimum sampling requirements for trend analysis of GAP; available protocols for the analysis of pesticides in water; current water monitoring activities in Cyprus; an introduction to ecological risk assessment, and an example of use of pesticide impact rating index (PIRI) software.

One afternoon was spent at the CICA-UCR laboratory and two protocols for the analysis of pesticides in water (see **Section 3.7.**) were demonstrated to participants. Demonstrations were also given on available FAO/IAEA web resources, such as eLearning courses and LIMS resources.

The discussions among Research Contract/Agreement Holders lead to the preparation of an overall work plan for the next two years of the project.

The establishment of a global network of laboratories will be one of the project's main achievements. Equally important is the information generated under the CRP, which will give a greater understanding of environmental indicators and their relationships to GAP, thus helping to improve pesticide management and facilitate agricultural exports.

The second RCM is planned to take place in Vienna, Austria, from 9-13 February 2009.

This work was carried out under project 2.1.3.2, activity 4.

3.9. 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).

Veterinary drugs are used in the prophylactic and therapeutic treatment of infection in food-producing animals. Anti-parasitic drugs are probably the most widely used veterinary medicines for treating cattle and have been identified by the research team as suitable markers of food safety in beef. A number of these drugs possess toxic properties and residues in edible tissues would be of particular concern to susceptible individuals, especially pregnant women, the elderly and young children.

A review of literature has indicated that a number of important anti-parasitic drugs are frequently not included in residue analysis methods. Many methods used to test for these drugs are single residue methods and appropriate multi-residue methods need to be developed to effectively monitor for as wide a range as possible of these residues in food. The development of a liquid chromatography-tandem mass spectrometry (LC-MSMS) method will provide both a multiresidue screening capacity and analyse confirmatory data in line with the criteria specified in Commission Decision 2002/657/EC and recommended in draft revised Codex guidelines.

The Agrochemicals Unit Head participated in three project meetings during 2007-08, the 'kick-off' meeting in Dublin, Ireland in March 2007, the first WP1.4 meeting, which was hosted at Vienna International Centre on 4 October 2007, and the 18 month WP1.4 meeting in Toledo, Spain, in October 2008.

Development and validation of a multi-residue isotope dilution assay for the determination of 38 anthelmintic drugs or their metabolites by liquid chromatography-tandem mass spectrometry (LC-MSMS) has been completed at Ashtown Food Research Centre (Teagasc, Ireland). A draft standard operating procedure for the method has been transferred to IAEA and a partner laboratory in UK, Queens University Belfast (QUB). LC-MSMS operating parameters have already been optimized at Seibersdorf and sent to Microbioticos to permit them to start setting up the method. The full analytical method is undergoing final refinement and validation at Seibersdorf before transfer to Microbioticos. Transfer of the method is scheduled for early 2009. This will equip Brazil with the analytical tools necessary to perform both a risk analysis and routine testing/monitoring of produce for a wide range of possible anthelmintic residues.

ProSafeBeef workpackage 1.4 meeting, VIC.

Sampling of beef products from retail outlets by OCU began in five European countries in October 2008 and will continue at regular intervals for a two year period. The analytical results from this targeted sampling will be processed by RIKILT to provide an analysis of the risks associated with residues of anthelmintic compounds in beef on the European market. The results will be reported to all stakeholders via OCU consumers' newsletters, web publications and reports to the European Commission.

Sampling will begin in Brazil in 2009, targeted at beef from producers certified for export to the EU. Analysis of the samples using the method transferred via IAEA will facilitate a risk assessment similar to that performed in Europe to be performed in Brazil. The project outcome in Brazil should be the development of the capability for the Brazilian competent authorities to perform appropriate risk assessments and to put in place an effective monitoring and feedback mechanism for the control of anthelmintic drug use in beef production.

Future transfer of the method(s) developed to other IAEA member states, via both Microbioticos and the Agrochemicals Unit at Seibersdorf, will assist in building their risk assessment and management capacities.

A poster on this work was presented at the EuroResidue VI Conference in The Netherlands in May 2008.[11]

This work falls under project 2.1.3.2, activity 2.

[11] Danaher, M, Whelan, M., Cooper, C., Kennedy, G., Bouwmeester, H., Montes Niño, A., Granja, R., Trigueros, G. and Cannavan, A. (2008). Introducing the ProSafeBeef project: Developing a risk-based approach for control of anthelmintic drug residues in beef. Residues of Veterinary Drugs in Food, van Ginkel, L.A. and Bergwerff, A.A., eds., Proceedings of the Euroresidue VI Conference, Egmond aan Zee, The Netherlands, 2008, 1293-1298.

3.10. First International MoniQA Conference, Rome, Italy, 8-10 October 2008

MoniQA is an EU funded Network of Excellence working towards harmonization of analytical methods for monitoring food quality and safety in the food supply chain. It is coordinated by the Vienna-based International Association for Cereal Science and Technology (ICC) and will receive €12.3 million in funding from the European Commission for its activities between 2007 and 2012. MoniQA seeks to establish sustainable integration of leading research institutions, industrial partners and small to medium-sized business working in complementary fields of food analysis to assure food quality and safety. The main objective of the project is to overcome European and worldwide fragmentation in food diagnostic research by integrating key organizations.

The First International MoniQA Conference, entitled "Increasing Trust in Rapid Analysis for Food Quality and Safety", took place in Rome from 8-10 October, 2008. The event brought together a global audience of more than 200 food safety scientists, socio-economists, regulators, industry and trade representatives as well as media correspondents. The conference was divided into sessions on food authenticity, food additives, mycotoxins and phycotoxins, food allergens, chemical contaminants, microbiological contaminants, and horizontal issues. The participants discussed current challenges in avoiding and controlling unwanted substances in the food production chain and shared information on new developments and innovations in rapid and reliable analysis of food contaminants.

The session on "Mycotoxins and Phycotoxins" included presentations on method performance, quality control and measurement uncertainty, sampling issues, and implications for the agri-food industry. Ms. Britt Maestroni, of the Agrochemicals Unit, gave an invited lecture entitled 'Sampling issues for mycotoxins'[12] within the session on mycotoxins and phycotoxins. The presentation focused on the design, implementation and evaluation of effective sampling plans to minimise the misclassification of lots as positive or negative, thus minimizing both buyer's and seller's risks. Data were presented from a study carried out under an IAEA Technical Cooperation Project to determine the variability and distribution of fumonisin B1 in samples taken from lots of maize marketed in Nigeria. The samples were analysed in the Agrochemicals Unit and the results were used to develop a model to predict the buyers' and sellers' risks associated with any given fumonisin sampling plan design developed for maize.

Also of interest to the conference participants and of relevance to the work of the Food and Environmental Protection subprogramme was the session on food authenticity. The use of multi-element stable isotope analysis in combination with multivariate statistics to determine the geographical origin of food was presented as a growing area of research. The Food and Environmental Protection subprogramme has already planned work in the field of traceability and food authenticity to support member states in ensuring food safety, quality and trade access and a CRP is scheduled to commence in 2010 on the application of stable isotope measurements to help control food contamination at source through product traceability, to detect and control food adulteration and to guarantee product authenticity.

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[12] Maestroni, B., Whitaker, T., Cannavan, A., Byron, D.H. (2008). Sampling issues for mycotoxins. Book of abstracts of the 1st MoniQA International Conference, ICC, Rome, Italy, 8-10 October 2008, 35-36.

Further information on MoniQA can be found at http://www.moniqa.org/.

This work was carried out under project 2.1.3.2, activity 2.

3.11. EU 6th Framework Project 'BioCop'

The Agrochemicals Unit Head, as a member of the Project Advisory Board of the EU 6th Framework Integrated Project "New technologies to screen multiple chemical contaminants in food (BioCop)", participated in three project meetings during 2007-08. The field of research of the project is in the development and implementation of new methods to prevent & monitor the occurrence of multiple chemical contaminants in foods through the use of advanced sample preparation techniques & emerging biotechnological screening approaches. The main project objectives are:

- Development of novel screening methods to detect multiple chemical contaminants in foods;
- Training of scientists in the technologies developed;
- Widespread dissemination of project 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.

Most of the work packages within the project have now completed 3 years' research and development work and many of the technologies being explored have been developed to a stage where preliminary validation, or even inter-laboratory validation, is now under way.

An important development has been the inclusion of a new project partner, with the objective of disseminating knowledge generated and transferring technology from the project to countries outside the EU. This should help to harmonize food safety standards and methodology and minimize the impact that new policy-supporting technical developments within the EU will have in countries, especially developing countries, wishing to establish equivalence of food safety standards in order to export food commodities to the EU, and is of potential benefit to a large number of IAEA and FAO Member States. The new project partner, the Veterinary Public Health Laboratory (VPHL) in Bangkok, Thailand, has previously benefited from IAEA TC fellowship training and participation of VPHL staff in FAO/IAEA training courses at Seibersdorf and in South East Asia, is a former contract holder in the IAEA Coordinated Research Project 'The development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries' (D3.20.22), and is a current collaborator with the Agrochemicals Unit in various activities. VPHL staff will be trained in the methods and technology developed through BioCop and will be involved in method validation, demonstration and training of scientists in South East Asia and throughout the world in training events organized and implemented with the assistance of BioCop project partners and with project funds.

BioCop is a large, complex and ambitious project, which is making excellent progress. The results are likely to make a significant impact on the implementation of food contaminant testing and monitoring schemes.

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

This work falls under project 2.1.3.2, activity 2.

3.12. EU 7th Framework Project 'CONffIDENCE'

The kick-off meeting for the EU 7th Framework Programme Project "Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure" (CONffIDENCE) took place in Brussels on 19-20 June 2008. The 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 Agrochemicals Unit Head participated in the kick-off meeting as chair of the project Advisory Board.

The technologies to be developed 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. At the meeting, this point was raised by the Unit Head and accepted by the Project Management Board and it was agreed that countries outside the EU, especially developing countries, should be included as stakeholders in dissemination activities. This will directly benefit many IAEA member states.

The first annual meeting of the project consortium will be held in Barcelona, Spain, in March 2009.

Further information on CONffIDENCE can be accessed at http://www.conffidence.eu.

This work falls under project 2.1.3.2, activity 2.

3.13. Investigation into Environmental/Food Chain Contamination in Khongor Soum, Mongolia, 6-15 March 2008

In late January 2008, the Mongolian Government requested assistance through the UN Resident Coordinator to investigate environmental and food chain contamination associated with the use of toxic chemicals in a small scale gold ore processing operation in Khongor Soum, Darkhan Uul Aimag, approximately 200 km from Ulaanbaatar. A contamination incident in April 2007 had resulted in pollution of the ground, air and drinking water with mercury and cyanide and had caused intoxication of the local population and loss of livestock. A decontamination operation was carried out at the site following the pollution incident, but persistent reports of human health problems, high levels of animal abortions and congenital anomalies in pigs, sheep and cows prompted the more recent request for assistance. Teams from WHO, UNEP and FAO were fielded to investigate the situation. FAO was requested to provide an assessment of the effect of the suspected environmental contamination on locally produced vegetables, milk, water and animal health.

The Agrochemicals Unit Head, as a member of the FAO team, carried out field investigations, assessed laboratory results, evaluated laboratory capacity and liaised with other UN organizations and Mongolian authorities in attempting to elucidate the cause of the reported problems in order to make recommendations for follow-up actions. Food, animal and environmental samples were taken for analysis in the Agrochemicals Unit and at other laboratories. Milk and water samples were screened for pesticide contamination in the Agrochemicals Unit and for mercury in the State Specialized Inspection Agency Central Laboratory in Ulaanbaatar. Blood samples from cattle in the affected area were tested for brucellosis in the State Central Veterinary Laboratory (SCVL) and the Veterinary Research Institute (VRI) in Ulaanbaatar; the capabilities of both of these laboratories have been enhanced through participation in IAEA TCPs in recent years. Environmental samples were also tested for radionuclides in the residues laboratory of the SCVL; which was established under an IAEA TCP. Samples were tested for heavy metals in the Central Geological Laboratory, Ulaanbaatar and the National Veterinary Research and Quarantine Service in Korea.

Taking blood and milk samples in Khongor Soum.

Preliminary results of the investigations were in agreement with the conclusions of the Mongolian authorities, that neither mercury nor cyanide was likely to be the cause of the current human and animal health problems reported at that location. However, there is likely to be pollution from artisanal gold mining, power plant waste and various other industrial operations observed at the site, which may have a bearing on the reported symptoms. Brucellosis was also confirmed in cattle in the region, which may be a factor in the observed incidence of cattle abortions. An assessment of the laboratory capacity and the ability to respond to emergencies or issues such as

that under investigation indicated that there is a need for considerable capacity building in this field.

A two-year FAO Technical Cooperation Project is being formulated to further investigate this and related problems in other regions of the country.

This work falls under project 2.1.3.2, activity 2.

3.14. EuroResidue VI Conference on Residues of Veterinary Drugs in Food, Egmond ann Zee, The Netherlands, 19-21 May 2008

The EuroResidue conferences, currently held every four years, are amongst the world's most important meetings on residues of veterinary drugs in food and the environment. The conference covers aspects such as analytical techniques, pharmacological and toxicological studies, and registration and regulation of veterinary drugs. At the EuroResidue VI conference, approximately 400 regulators and experts from countries throughout the world and from various scientific disciplines met to discuss developments and problems in the field of residue analysis and to exchange ideas.

On the first day of the conference, the Agrochemicals Unit Head presented a paper entitled 'An investigation into the possible natural occurrence of chloramphenicol in poultry litter'. This work was carried out under the CRP 'Development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries' (D3.20.22) by the Department of Livestock Development (DLD) in Thailand in collaboration with the Agrochemicals Unit and a research agreement holder Queen's University Belfast, UK. The study was designed to investigate claims from poultry producers in Thailand that residues detected in their products of the antibiotic chloramphenicol, which is banned for use in food-producing animals, were caused by natural biosynthesis of the compound in the production environment rather than by abuse of the drug. The detection of chloramphenicol in exported foods has caused many trade disputes, rejection of shipments from importers and loss of revenue to the exporters in recent years. The possibility of contamination from natural sources has been claimed by producers in Thailand, as well as in other regions of the world, but with no supporting evidence. The findings of the study performed in Thailand did not support this claim for the particular production systems in place there. Discussions following the presentation led to proposals collaboration with major European laboratories for follow-up investigations into analytical strategies to distinguish contamination from naturally produced chloramphenicol and the chemically synthesized version.

The Agrochemicals Unit Head also participated in a workshop on 'Associated and third countries and new (EU) Member States', convened by Dr. J. McEvoy of the EC Food and Veterinary Office. Discussion centred on the difficulties encountered in establishing equiv-alence of food safety standards to facilitate export of food commod-ities to EU, and on implementing EU legis-lation for new EU Member States. Presen-tations were given by Dr. P. Kanari (State General Laboratory, Cyprus) on the residues control system in Cyprus, which had benefited from the establishment of a residues screening laboratory under an IAEA TCP, and by Dr. S. Kanarat (DLD, Thailand), a research contract holder in CRP D3.20.22, on the control of residues in the poultry export industry in Thailand.

It was noteworthy that several participants at the conference were former participants in training courses or workshops run at Seibersdorf, at VIC, or on a regional basis, both from countries who are now EU Member States (Poland, Czech Republic, Estonia, Cyprus) and from developing countries elsewhere (including Thailand, Brazil, Argentina, Chile, Mexico). Several participants had also been involved in CRP D3.20.22, and some material was presented in poster sessions that arose from work in that CRP.

The conference provided an opportunity to keep abreast of relevant developments and maintain and initiate collaborations of benefit to IAEA Member States, and to build awareness of the role and impact of the FAO/IAEA programme in food safety research and capacity building.

This work falls under project 2.1.3.2, activity 2.

EuroResidue VI Conference in Egmond ann Zee, The Netherlands.

3.15. Joint FAO/IAEA/OIE/WHO Technical Meeting on Global Survey of Laboratory Quality Systems, Lyon, France

The Agrochemicals Unit Head travelled to the WHO Lyon Office on 30 August 2007 to represent the Joint FAO/IAEA Programme at the Joint FAO/IAEA/OIE/WHO Technical Meeting on Global Survey of Laboratory Quality Systems. The meeting discussed a strategy for developing a global inventory of laboratory quality systems and external quality assessment schemes (EQAs). The aim is 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). A two-part questionnaire was designed for distribution to laboratories, competent authorities and EQA providers and a preliminary survey to test the questionnaires in a limited number of countries was initiated in November.

Follow up teleconferences were held in early 2008 to refine the questionnaires and plan a full survey.

The data from the full survey, to be carried out in 2009, will be validated, collated and analysed by the WHO office in Lyon. The final inventory will be published and disseminated globally.

This work falls under project 2.1.3.2, activity 2.

4. TRAINING ACTIVITIES

4.1. Agrochemicals Unit "Train-the-Trainers" Activities for Food Safety and Consumer Protection

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 residues, endocrine disruptors, mycotoxins and pesticides in food. 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 international trade in food commodities.

The above activities are all interconnected. For example, interns or fellows under specific Technical Cooperation Projects are trained in the Agrochemicals Unit through direct involvement, under the supervision of Unit staff, in the development and validation of analytical methods and procedures which are needed in their countries. The method protocols produced are disseminated through publication in the scientific press and the internet and through their use for training future fellows and in training courses. The trainees gain knowledge and expertise through hands-on experience of method development and analytical strategies including ^{14}C-radiotracer and stable isotope techniques in food contaminant analysis, sample processing and preparation, instrumental analysis using a variety of modern techniques such as thin layer chromatography (TLC) high performance liquid chromatography (HPLC), gas chromatography (GC), isotope dilution methods using hyphenated mass spectrometry (MS) techniques (GC-MS, LC-MSMS), routine maintenance and optimization of such instruments, protocols for method validation, and laboratory quality assurance and quality control principles. Training in these topics is also provided through inter-regional training courses and workshops, generally held annually at Seibersdorf under the auspices of the FAO/IAEA Training and Reference Centre for Food and Pesticide Control. These events are designed on a "train the trainer" basis, so that the knowledge gained at the workshops or courses will be widely disseminated upon the return of the participants to their home institutes. The training courses held at Seibersdorf have also been augmented by a number of regional courses and workshops organized by Unit staff and hosted in various Member States.

Development of these skills and transfer of the technology and methodology is very important in terms of assisting laboratories in Member States to realize their role in their countries' food safety systems and in helping to ensure that those systems meet the requirements for food safety and trade as specified in Codex standards. Increased expertise and awareness of these issues also helps Member States to play a more active role in the development of these guidelines and standards through participation in the appropriate Codex committees.

Another critical aspect of the training activities is the interaction between the participants, leading to the creation of global networks of laboratories and institutes that can share information, experience and expertise. The Agrochemicals Unit plays a lead role in the creation, expansion and maintenance of these networks.

Seminar in Shenzen, China, as a follow-up to the Seibersdorf workshop "Introduction to Screening and Confirmatory Methodology for Veterinary Drug Residues".

Over the past 10 years, more than 75 TC fellows and interns have been trained individually in the Agrochemicals Unit and taken their expertise back to their home institutes. Since 2005, the Agrochemicals Unit has also run 4 training workshops at Seibersdorf, involving approximately 90 participants from more than 50 developing countries. Feedback from our trainees is generally very good, and many follow-up training courses, workshops and seminars have been organized and implemented in their home countries by participants in the courses and workshops at Seibersdorf, using the knowledge gained and training materials provided. Some of the events organized by Seibersdorf workshop participants that indicate the success of the "train the trainers" approach in 2007-08 were: a Regional Workshop for West Africa (Senegal , Burkina Faso, Niger, Gambia and Mali) on analytical methodology for residues in food, in Bamako in August 2007; a training course in the Centro de Control de Insumos y Residuos Tóxicos and lectures on analytical methods for toxic residues in Peruvian export commodities at the VIII Alimentary Industries National Congress in the La Molina National Agrarian University; Lima Peru; and a seminar on methodology for veterinary drug residue analysis in Shenzen Center for Disease Control and Prevention, China, in March 2008.

4.2. FAO/IAEA Train-the Trainers Workshop on "Screening and Confirmatory Methodology for Veterinary Drug Residues"; Seibersdorf, 12-30 November 2007

In recent years, the issue of veterinary drug residues in animal-derived foods has become increasingly important in many 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. The control of veterinary drug residues is achieved through the application of good farming and animal husbandry practices. Analytical laboratories play an important role in the verification of the quality of food commodities, the provision of feedback to the competent authorities on the effectiveness of the production and residue control practices, identification of new or re-occurring residue problems, and in the provision of information 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. In order to produce internationally acceptable results, laboratory staff must be proficient in the appropriate screening and confirmatory methodology, as well as being familiar with the principles of ISO Standard 17025. These residue control programmes are important not only with regard to international trade, but also to guarantee the safety, quality and security of domestic food supplies.

To assist IAEA and FAO Member States in implementing effective control programmes for veterinary drug residues, the Joint FAO/IAEA Programme has run several training courses and workshops for analysts and regulators over the past few years. The latest of these was a training workshop on screening and confirmatory methods for veterinary drug residues, held at the FAO/IAEA Training and Reference Centre for Food and Pesticide Control at Seibersdorf, 12-30 November 2007. The objectives of the workshop were to strengthen the awareness of scientists and laboratory managers of the relevant guidelines and regulations and the theoretical and technical aspects of screening and confirmatory methods for the detection of veterinary drug residues; 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

Train the trainers – Screening and Confirmatory Methodology for Veterinary Drug Residues.

practices. The workshop was designed on a 'train the trainers' basis, and it is expected that further training events will take place in the participants' home institutes using the knowledge gained and the training materials provided by IAEA.

The programme comprised on site lectures and laboratory work in the following subjects:

- Codex standards, guidelines and recommended international codes of practice for the control of the use of veterinary drugs, including complying with maximum residue limits
- Veterinary drug residue testing in the context of food safety
- Principles of sampling
- Laboratory accreditation, equivalency of food control procedures and mutual recognition
- Quality assurance systems and quality assurance/quality control measures in analytical laboratories
- Statistical treatment and interpretation of analytical results
- Sample preparation
- Screening techniques (microbial inhibition tests, immunoassays)
- Confirmation of results
- Chromatographic theory and practical applications of TLC, HPLC, GC-MS, LC-MS
- 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 from 21 developing countries; on a regional basis, 4 from Latin America, 8 from Africa, 9 from East Asia/Pacific and 1 from Eastern Europe. Twelve participants were female and 10 male. Lectures and laboratory practical sessions were led by staff of the Agrochemicals Unit and by invited guest lecturers from internationally renowned laboratories, academia, international govern-mental and non-governmental organizations and both the public and the private sector. All participants engaged fully in lectures, practical sessions and discussions. A half day visit was accommodated by the veterinary drug residue testing laboratories of the Austrian Health and Food Safety Agency (AGES), at which the participants had the opportunity to interact with the analysts working there, ask questions and exchange information on methods and instrumentation used for various classes of drugs.

A screening method practical session.

On the final day of the workshop the participants, working in four groups, gave presentations summarizing the issues important to their countries with

respect to the control of veterinary drug residues, the current and future roles of their laboratories in the 'farm to fork' approach to food safety, and the most important lessons learned from the workshop. The importance of maintaining a network of laboratory contacts and collaborators in different countries was emphasized. The presentations were of a high standard

Feedback from the participants and external lecturers, both at the end of the workshop and through further contact with many of the participants since, has indicated that the workshop was extremely successful. Background and training material was also provided on a workshop CD to a number of applicants from the many countries that were not able to be accommodated at the workshop. Feedback from those individuals has also been good, and there is already a strong demand for future similar events.

This work falls under project 2.1.3.2, activity 2 (2007).

4.3. FAO/IAEA Train-the-Trainers Workshop; "Introduction to Quality Assurance/Quality Control Measures in Pesticide Residue Analytical Laboratories", Seibersdorf, 13 October – 7 November 2008

Although workshops on this topic have been run previously (most recently in 2005 and 2006), the workshop programme is continually developed, updated and refined to reflect the dynamic nature of food safety issues, quality systems and the responses demanded of analytical laboratories.

Train-the-trainers workshop in Seibersdorf.

The objectives of the workshop were to introduce and elaborate the QA/QC principles relevant to pesticide residue analysis according to the ISO 17025 Standard and Good Laboratory Practice (GLP) Guidelines, and to discuss the various roles of quality assured laboratories in the application and monitoring of the effectiveness of good agricultural practices. The programme included lectures, laboratory practical sessions and demonstrations, discussion and feedback sessions and visits to a national regulatory laboratory and a private laboratory engaged in pesticide residue analysis, both of which are accredited to ISO 17025. Topics covered at Seibersdorf included: the principles of laboratory quality control and quality assurance systems and the implementation of ISO 17025 and GLP; radiotracer techniques in residues analysis; basic statistics in residue analysis; sampling procedures; quality control of analytical methods including sample preparation, extraction, clean-up, instrumental analysis for detection, quantification and confirmation of residues; estimation of uncertainty; method validation; new developments and trends in pesticide residue analysis and risk assessment.

The workshop had 21 participants, 10 female and 11 male. Developing countries from various regions of the world were represented, with 7 participants from Asia, 7 from Africa, 4 from Latin America and 3 from Europe. The majority of participants were analysts with some experience in pesticide residue analysis, though a few candidates were included from institutes wishing to develop a capability for pesticide residue analysis for regulatory purposes. It was considered that the information provided in the workshop, and the opportunity to network with other residues laboratories, would be of benefit to those countries in optimizing the use of their available resources to plan and develop their laboratories.

Thin layer chromatography practical session.

Lectures and practical sessions were presented by members of the Agrochemicals Unit, and other IAEA and FAO staff. A number of external lecturers also contributed, including representatives of CVUA Stuttgart (EU Community Reference Laboratory for Pesticides),

The European Food Safety Authority (EFSA), The Austrian Agency for Health and Food Safety (AGES), Wessling Hungary, AGROVET, Waters Corporation, Agilent and Thermo Fisher Scientific.

The discussion and feedback sessions held at various stages of the workshop provided opportunities for the participants to consider and debate aspects of particular relevance to their own laboratories between themselves and with the staff and experts present. These sessions were lively and all workshop participants contributed.

For the final week of the workshop, the participants were encouraged to think in broader terms and to consider the role that their laboratories play in supporting good agricultural practices, enhancing food safety and enabling trade in food commodities. Within this context, the need for laboratories to produce reliable data through the implementation of quality assurance systems and quality control procedures was emphasized. The workshop concluded with a round-table discussion on these issues.

Immediate feedback on the effectiveness of the workshop was gained via feedback sessions during the workshop and a detailed evaluation questionnaire completed by the participants on the final day. The feedback received provided confirmation of the importance of these workshops and other training activities to our Member States. Feedback on the workshop sessions on radiotracer techniques in residues analysis verified the value of nuclear techniques in residues analysis and research. The charts below (**Figure 12**) summarizing the participants' responses with respect to some important aspects of the workshop indicate its effectiveness and suggest successful future outcomes in individual countries.

Continued feedback and interchange between the laboratories and the Agrochemicals Unit will be maintained through email and by telephone.

This work was carried out under project 2.1.3.2, activity 6.

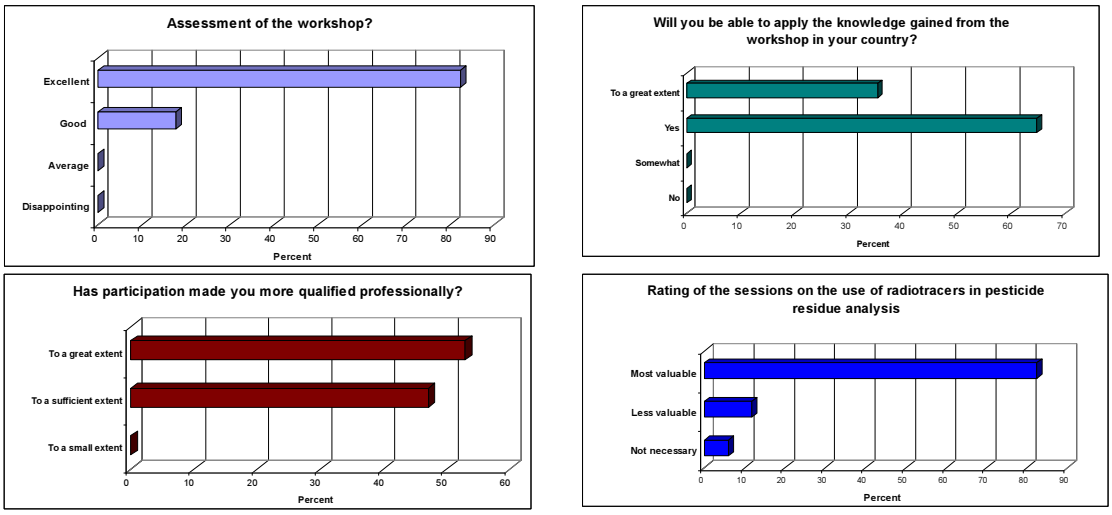

Figure 12. Participants' feedback to various aspects of the workshop.

4.4. Training Course on the Application of Gas Chromatography-Mass Spectrometry for the Control of Food and Environmental Contaminants

An intensive fellowship training course on gas chromatography-mass spectrometry (GC-MS) was held in the Agrochemicals Unit from 10 November to 5 December 2008, funded under TCP RLA/5/050.

Recently in Latin America there have been significant improvements in the availability and use of advanced analytical instrumentation. Equipment such as GC-MS has been purchased and installed in many laboratories with the intent to contribute to better food and environmental safety. To help facilitate the optimal use of the GC-MS instruments for screening and confirmatory analysis of contaminants in food and the environment a four week theoretical and practical GC-MS training course was organized.

Six analysts from Argentina, Brazil, Bolivia, Chile, Colombia, Costa Rica and Lebanon participated for the whole duration of the training, while two additional analysts from Tanzania and Slovenia participated at the first week of the course.

The training was organized in four different modules, each supervised by a different expert.

The first module was provided by Mr Rick Parmely, from Restek Corporation, who gave technical training on the theory of gas chromatography-mass spectrometry and provided extensive information on the maintenance and troubleshooting of the Agilent 5975c MSD system, which was used as a model system for the training. Detailed training was provided on technical aspects of the theory of mass spectroscopy, gas chromatography, the instrument inlets, capillary column selection, the mass-selective detector (MSD), the vacuum system, ion sources, the quadrupole mass filter, GC detectors, data acquisition methods, and troubleshooting.

The second module was run by Mr Eddie Fonseca from CICA laboratory, Costa Rica and Mr Berthold Kettner from ILAU laboratory, Germany. Mr Fonseca presented some practical information on the use of the GC-MS for pesticide residue analysis in Costa Rica. Mr Kettner introduced the concepts of mass spectral interpretation and gave practical demonstrations on data analysis.

The third module was covered by Mr Jaume Santamaria, from Agilent, Spain. The Agilent expert gave very detailed information on the use of the instrument as well as demonstrating the use of the Chemstation software to set up data acquisition and data analysis methods, retention time locking (RTL), and the use of deconvolution software.

Ms Celine Lesueur, from LVA laboratory, Austria, supervised the final module, which focused on the QuECheRS extraction/clean-up method and the use of the GC-MS software to quantify and confirm pesticide residues in food and environmental matrices. One day was spent at the LVA laboratory to give the participants an understanding of the requirements of a routine laboratory in terms of quality and confirmatory aspects.

During the course the participants used the Agrochemicals Unit's GC-MS instrument, gaining hands-on experience of maintenance and trouble shooting procedures such as cleaning the ion source, disassembling and reassembling the MS interface, changing liner and septa,

connecting and disconnecting the column, performing splitter calculations for different split ratios to ECD, NPD and MSD, evaluating tune reports, and checking for leakages.

The training course was very positively evaluated by all participants after the course. It was recommended to organize a second training event as soon as possible.

This work was carried out under project 2.1.3.2, activity 10.

4.5. Training in Panama and Seibersdorf under TC Projects PAN/5/015 and PAN/5/017

Mr N. Rathor undertook an expert mission to the laboratory of "Ministerio de Desarrollo Agropecuario" Direction Nacional de Sanidad Vegetal (MIDA) in Panama, from 5-16 February 2007. His tasks were to train the laboratory staff on pesticide residue analysis in fruit commodities, including radiotracer techniques using ^{14}C-radiolabelled pesticides. Work undertaken consisted of theoretical and practical exercises on:

- safety of laboratory operations,
- sampling of fresh fruits and vegetables,
- sample preparation and processing,
- sample extraction,
- clean up
- determination of pesticide residues using GC-NPD and GC-MSD, including maintenance and troubleshooting
- calibration and quantification calculations
- use, handling and routine maintenance of general analytical equipment,
- principles of method validation
- application of radiolabelled pesticides for method optimization
- daily applications of quality assurance/control measures according to ISO17025, including establishment and use of control charts
- preparation of SOPs
- QUeChERS multiresidue method,

Eight technicians were trained in the practical aspects and a number of lectures were presented to institute staff covering the topics listed above.

Mr Rathor also discussed various issues with the Director of MIDA, Ing A. Espino, and the Sub-Director General of Instituto De Investigacion Agropecuaria de Panama' (IDIAP), Mr Benjamin Name, who was interested in possibilities for cooperation with the IAEA. It is intended that a project proposal that would include collaboration with the Agrochemicals and the Soil Science Units at Seibersdorf will be submitted.

Ms Brenda Checa Orrego preparing samples for analysis in the Agro-chemicals Unit.

As a follow-up to the training mission carried out in February by Mr Rathor, two Scientific Visitors from Panama, Ms Brenda Checa Orrego and Ms Llarys Aparicio Aguilar, visited the Agrochemicals Unit for 8 days in April 2007. The objective was to revisit the concepts of quality assurance and quality control measures for pesticide residue analysis in agricultural production in Panama, and to receive training on quick multiresidue procedures for pesticide residues in fruit and vegetables (see **Section 3.5.**).

The laboratory exercises specifically aimed at studying the

stability of selected organophosphorus pesticide residues in fruit under cryogenic and ambient processing conditions. The performance parameters were characterized for a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for the analysis of pesticide residues in pineapple and melon with the aid of ^{14}C-labelled chlorpyriphos, and the method was validated.

Project PAN5/015 ended in December 2007 and was replaced by a new project, PAN/5/017 **"**Monitoring Pesticide Residues in the Production of Tropical Fruit (Pineapples and Melons) and Controlling Analytical Quality with the Aid of Nuclear Techniques". The objective of the new technical cooperation project is to improve food safety in the production of tropical fruits in Panama. Under this project, a national workshop on "El laboratorio de Análisis de Residuos de Plaguicidas como Herramienta para el cumplimiento de las Buenas Prácticas Agrícolas" was held in Panama from 17-19 December 2008. Ms Maestroni provided technical input to the national workshop, including oral presentations and laboratory demonstrations. The national workshop unanimously endorsed the need to advertise and publicize the use of nuclear techniques in agriculture. It was recommended that IAEA, through a new TCP, PAN/5/019, "Supporting accreditation of a pesticides laboratory" should provide the funding to produce two technical videos for decision makers, the public at large and for possible wider media distribution, to show what is being done at MIDA.

This work was carried out under project 2.1.3.2, activity 10.

4.6. Saskatoon International Workshop on Validation and Regulatory Analysis

The Saskatoon International Workshop on Validation and Regulatory Analysis (SaskVal 2007) was held in Saskatoon, Saskatchewan, Canada, 10-13 June 2007. The objectives of the workshop were to discuss the validation of analytical methods used in residue control programmes and the subsequent use/acceptance of the results if challenged by producers or courts of law. Associated regulatory issues, such as residue programme design and sampling, were also considered. The primary focus was on residues (veterinary drugs, pesticides) and contaminants in foods and related issues. Topics covered in plenary sessions included: risk assessment and the role of expert laboratories; programme design and implementation – method validation and approaches to sampling; requirements for screening and confirmatory methods; and methods of analysis for residues and contaminants.

The audience included approximately 130 laboratory analysts and regulators and included participants from developing countries, including two IAEA TCP counterparts (Indonesia and Benin) and several Scientific Investigators from the recently completed CRP "Development of strategies for the effective monitoring of veterinary drug residues in livestock and livestock products in developing countries" (D3.20.22). It was noteworthy that the results of research carried out under this CRP were represented in several presentations, both oral and poster, at the workshop.

As a member of the workshop's Scientific Committee, the Agrochemicals Unit Head was involved in planning the meeting with respect to the topics to be covered, identification of keynote speakers and evaluation of submissions for oral and poster presentations. The Unit Head presented a keynote lecture on 'Developing country regulatory concerns and collaborative work to protect consumers and facilitate international trade', including a summary of the work of the Joint FAO/IAEA Programme in this field. The Unit Head also co-chaired a plenary session on 'Risk assessment: the role of expert laboratories', which prompted lively discussion and necessitated the formation of an ad hoc expert panel for a question and answer session, with many questions coming from developing countries wishing to meet regulatory requirements for the export of food commodities. A poster entitled 'Comparison of methods for the estimation of measurement uncertainty for an analytical method for sulphonamides', was also presented, which summarized work carried out in the Agrochemicals Unit. There was significant interest in the poster and several requests for further information.

Individual discussions with various participants led to several offers of collaboration, for example through provision of lectures and training material for training courses, inclusion on the roster of experts for TC missions, offers to act as host institutes for TC fellows, and enquiries as to possible inclusion as research agreement holders in future CRPs. Discussions with Dr Nilmini Wijewickreme, Director, Food Safety, Cantest, resulted in her visiting the counterpart team of TCP SRL/5/039 whilst on a trip to Sri Lanka, with consequent possibilities for further assistance to the project from Cantest.

This was a very successful event which facilitated discussion between scientists, regulators and other stakeholders with regard to chemical residues and contaminants in food. The topics covered were relevant to both the developed world and the representatives of developing countries who were present, and the information disseminated and the contacts and networks initiated should help in capacity building and in directing resources in the developing

countries to the most relevant topics. Feedback from developing countries also informed policy makers in the developed countries of the problems faced by their trading partners in the developing world in trying to meet technical requirements that may be unrealistic or arbitrary. Since many of the workshop participants are in a position to be involved in technical panels providing advice for the formulation of food safety regulations, this will hopefully have a positive effect on future regulations and legislation.

The workshop concluded with a recommendation to hold a second workshop in four years time, to fit in with the cycle of the two other major veterinary drug residue conferences/symposia, 'EuroResidue' (The Netherlands) and 'the Hormone Symposium' (Belgium), which are also held on a four year cycle. The existing Scientific Committee was provisionally retained for planning the next workshop.

The SaskVal 2007 Book of Abstracts is available in the Agrochemicals Unit, ABL, Seibersdorf.

This work was carried out under project 2.1.3.2, activity 2 (2007).

4.7. Asia/Pacific Food Safety Summit, Qingdao, China, 15-18 April 2007

The Agrochemicals Unit Head participated in a Food Safety Summit for the Asia/Pacific region, which was jointly organized by the FAO/IAEA Joint Programme and Waters Corporation, with full funding from the latter. The programme was designed to build awareness in the region of international and national regulations and guidelines on food safety with regard to chemical residues and contaminants in food and the laboratory procedures and analytical techniques available to meet the requirements in these regulations and guidelines. The event had approximately sixty participants, mainly from China, Korea and Malaysia. The Unit Head introduced the food safety activities of the Joint FAO/IAEA Programme and presented a keynote lecture on 'International guidelines for the control of veterinary drug residues in food', and sat on an expert panel for question and answer sessions. The participants interacted actively with the presenters and had many questions on guidelines and regulations, the 'farm to fork' approach for food safety, and technical aspects of residues analysis.

This was the second collaboration with Waters Corporation for this type of meeting and once again it proved very successful, both in terms of outreach for the Agency and awareness building amongst and regulators in the region.

This work was carried out under project 2.1.3.2, activity 2 (2007).

Asia/Pacific Food Safety Summit in Qingdao, China.

4.8. Training in the IAEA Collaborating Centre for eLearning and Accelerated Capacity Building for Food and Environmental Protection (EACB), Costa Rica.

In July 2007, the Centro de Investigación en Contaminación (CICA) of the University of Costa Rica was inaugurated as an IAEA Collaborating Centre for eLearning and Accelerated Capacity Building for Food and Environmental Protection (EACB). CICA was designated as the lead institution acting in cooperation with the Advanced Radiation Technology Institute (ARTI) of the Korea Atomic Energy Research Institute and the Food Science and Technology programme (FST) of the National University of Singapore.

Latin America's crop protection market is predicted to grow by over 3% per annum until 2010. This is in response to producing higher and better quality staple commodities and biofuels. Although agrochemical use could improve the current food security situation, it could also generate excessive residues in commodity exports thus creating barriers to trade and environmental contamination, with the potential risk of encouraging the use of more toxic pesticides with serious consequences for small farmers and aquatic organisms. Trade issues are addressed by randomly sampling food produce and checking for compliance with maximum residue limits (MRLs) while harmonizing MRLs within specialty crop groups.

Under the TC Project RLA5050, a regional network of laboratories was created to monitor pesticide residues in water, soil and air as indicators of good agriculture practice (GAP), to prevent pesticide contamination at the source. Eight participating laboratories in the region are generating geo-referenced results for pesticide residues in water/soil/sediments for sub-catchments characterized in terms of pesticides, landscape, climate and stream data. These issues are also being addressed through a coordinated research project (CRP) D5.20.35 in which 5 of the 10 research contract holders are also counterparts of the Latin American regional project. Close collaboration has been fostered between the TCP and CRP participants, the Agrochemicals Unit and the EACB Collaborating Centre to optimize technology transfer and promote the natural synergism between the activities.

Three training events and one RCM (see **Section 3.8.**) were organized in 2007-08 under the auspices of the Collaborating Centre. Ms. Britt Maestroni participated, with other staff members of the Food and Environmental Protection Subprogramme, in the organization and implementation of these activities, including technical training and transfer of analytical methodology and radiotracer techniques from the Agrochemicals Unit.

The Joint IAEA/FAO Regional Workshop, "Integrated Analytical Approaches to Assess GAP", was held at CICA from 16-20 July, 2007, funded under TCP RLA/5/050. The workshop was attended by 15 scientists from the Latin America region and 5 lecturers. The objective was to facilitate technology transfer amongst participating analytical laboratories by exchanging information and establishing an expert network to strengthen the role of the laboratories in the implementation of good

Training at the EACB Collaborating Centre.

agricultural practice.

The above workshop was followed by the 2008 FAO/IAEA regional workshop,: "Integrated Analytical Approaches to Monitor, Control and Comply with Maximum Residue Limits for Pesticides", from 9 to 13 July 2008", and the TC RLA/5/050 Regional Training Course, "Refinement of Analytical Methodology, LIMS, Bioassays and QA/QC Measures", 16-20 July 2008, both events hosted by CICA.

Ms. Maestroni provided technical support for both events by giving lectures on analytical quality control measures and multiresidue methods for pesticides, assisted participants in developing the agenda for the RLA5050 coordination meeting to be held in February 2009, and assisted CICA staff in setting up and running laboratory demonstrations.

The underlying strategy behind these inter-related activities in the region is to strengthen laboratory capabilities through accelerated capacity building, thus supporting sustainable regional development. This approach depends on active participation of all stakeholders, and on communication and effective feedback mechanisms between the laboratory and interested parties. Current feedback indicates that the relevant stakeholders are fully committed and the approach promises to be successful.

Key issues arising from the training activities and meetings include:

- The need for sound sampling strategies and minimum data sets to compare results and to develop a portfolio of regional case studies;
- The use of information and communication technologies as a critical and cost-effective means to accelerate technology transfer, georeferencing of samples, and communicating laboratory results to stakeholders to address food safety issues;
- Chemical monitoring of pesticide residues in water, soil and sediments is fundamental to generate valid georeferenced results;
- Biodiversity indices and bioassay can provide a quick and cheap assessment of the impact of farming systems on water quality and complement analytical techniques, i.e.; biological and chemicals monitoring should be integrated as far as possible;
- Analytical quality management is not negotiable for the analytical laboratory. Open source laboratory information management systems (LIMS) such as Inkosi can help constrain costs associated with the procedures, maintenance of instrumentation and training of laboratory staff;
- The involvement of stakeholders and the scientific community at large is critical to prevent pesticide misuse and to ensure laboratories function most effectively and contribute to more sustainable food production;
- The regional network of pesticide laboratories now provides a framework for strengthening the pesticide safety net in Latin America but resources are limited and the growing diversity of needs complicates efficient technology transfer.

The Agrochemicals Unit and the EACB Collaborating Centre are working together to help address these issues.

This work was carried out under project 2.1.3.2, activities 8 and 10.

4.9. Fellows, Scientific Visitors and Interns

Two fellows from the Laboratoire Central Vétérinaire, Bamako, Mali commenced training in the Agrochemicals Unit in March 2007. Ms Safiatou Berthé Dem was trained for 4 months on pesticide and veterinary drug residue analysis and Mr Mamadou Diallo received 4 weeks training on laboratory quality assurance/quality control. Mr Jean Pierre Ouattara, a fellow from the Laboratoire National de Santé Publique in Ouagadougou, Burkina Faso, commenced a 4 month training programme on pesticide residue analysis, including the application of isotopic techniques, in April. Mr Ouattara's work contributed to the development and validation of an efficient multiresidue method for organochlorine pesticides in edible fish tissue, as described in **Section 3.1.** of this report. The method developed will be implemented in Mr Ouattara's home institute.

Two Scientific Visitors from Panama, Ms Brenda Checa Orrego and Ms Llarys Aparicio Aguilar, visited the Unit for 8 days in April 2007 as a follow-up to a training mission carried out in February by Mr Nasir Rathor to the Ministerio de Desarrollo Agropecuario laboratories in Panama under TCP PAN/5/015, Quality Assurance in Pesticide Residue Analysis for Agriculture Production. The Agrochemicals Unit training activities for this project are described in **Section 4.2.** of this report.

A scientist from the Centre for Environmental Studies, PCSIR Laboratories, Karachi, Pakistan, joined the Unit in April 2007 for a 6 month period. Ms. Hina Siddiqi is currently studying for a PhD in analytical chemistry. During her internship, she was trained in pesticide residue analysis in water, soil and agricultural products and contributed to the development and validation of analytical methods for pesticides and polychlorinated biphenyl contaminants in fish, as described in **Sections 3.1. and 3.2.** of this report. The development work carried out will contribute to her PhD thesis. Since the completion of her internship, Ms. Siddiqi has been working to apply the methods developed at the PCSIR Laboratories in Pakistan.

In 2008, the Agrochemicals Unit accommodated 11 TC Fellows. Ms N. Sibanc (Slovenia) and Mr J. Jimenez Gonzalez (Colombia) joined the Unit in October for 1 month to participate in the training workshop 'Introduction to QA/QC measure in pesticide residue analytical laboratories' (see **Section 4.2.**). Ms S. A. Assey (Tanzania)), Ms M. L. Trigo Orsini (Bolivia) and Mr K. El Hawari (Lebanon) also commenced in October and participated in the above workshop as part of longer fellowships. Ms V. E. Kirs (Argentina), Ms A. Parada Carrasco (Chile), Ms D. Dutra and Ms D. H. Baggio Ribeiro (Brazil), Ms C. S. Mosquera Vivas (Colombia), and Mr M. A. Masis Mora (Costa Rica) joined the Unit in November for one month's group fellowship training on the application of gas chromatography-mass spectrometry in pesticide residue analysis (see **Section 4.3.**).

This work was carried out under project 2.1.3.2, activity 10.

4.10. Training Course on Contaminants in Food: Metabolic Fate and Analytical Approaches

Ms Marivil Islam participated in a training course on 'Contaminants in Food: Metabolic Fate and Analytical Approaches', organized and funded jointly by the School of Advanced Residue Analysis in Food (SARAF), the EU 6th Framework Integrated Project 'BIOCOP' and the CASCADE Network of Excellence. The five-day training took place at the Laboratoire d'Etude des Résidus et des Contaminants dans les Aliments (LABERCA) in Nantes, France, 26-30 March 2007. It was an intensive training course comprising both theory and practical sessions in the laboratory, and covering four main topics: contaminants in food: context and regulatory aspects; analytical chemistry and food safety; metabolism of contaminants; and risk assessment**.**

The training enabled Ms Islam to expand her knowledge on how to identify chemical contaminants and their metabolites in food, how to perform metabolic fate studies using bioassays with confirmation of results by gas- or liquid-chromatography/tandem mass spectrometry (GC or LC-MSMS), and how to use the information gathered for risk assessment purposes. It also provided the opportunity to gain experience in some advanced analytical chemistry techniques based on mass spectrometry and bioassays, including biosensors, electrochemical sensors and the use of DNA chips ('transcriptomics'), and to acquire up to date information on food safety regulations

The knowledge gained through this training course will, to a large extent, be transferred to fellows, interns, project counterparts and colleagues within the Agency and will also enhance the effectiveness of research and development activities in the Agrochemicals Unit.

This was a staff development activity.

5. APPENDICES

5.1. Staff Publications 2007-2008

Dabalus Islam, M, M Schweikert Turcu, A Cannavan (2008). Comparison of methods for the estimation of measurement uncertainty for an analytical method for sulphonamides. Food Additives and Contaminants **25**, (12), 1439-1450.

Granja, RHMM, AM Montes Niño, F Rabone, RE Montes Niño, **A Cannavan**. AG Gonzalez Salerno (2008). Validation of radioimmunoassay screening methods for β-agonists in bovine liver according to Commission Decision 2002/657/EC. Food Additives and Contaminants **25** (12), 1475-1481.

Maestroni, B, T Whitaker, **A Cannavan**, DH Byron (2008). Sampling issues for mycotoxins. Book of abstracts of the 1st MoniQA International Conference, ICC, Rome, Italy, 8-10 October 2008, 35-36.

Kanarat, S, N Tangsirisup, N Nijthavorn, C Elliott, **A Cannavan** (2008). An investigation into the possible natural occurrence of chloramphenicol in poultry litter. Residues of Veterinary Drugs in Food (eds LA van Ginkel, AA Bergwerff), Proceedings of the Euroresidue VI Conference, Egmond aan Zee, The Netherlands, 2008, 37-42.

Danaher, M, M Whelan, K Cooper, G Kennedy, H Bouwmeester, A Montes Niño, R Granja, G Trigueros, **A Cannavan** (2008). Introducing the ProSafeBeef project: Developing a risk-based approach for control of anthelmintic drug residues in beef. Residues of Veterinary Drugs in Food, (eds LA van Ginkel, AA Bergwerff), Proceedings of the Euroresidue VI Conference, Egmond aan Zee, The Netherlands, 2008, 1293-1298.

Schad, GJ, A Allanson, SP Mackay, **A Cannavan,** JNA Tettey (2008). Development and validation of an improved HPLC method for the control of potentially counterfeit isometamidium products. Journal of Pharmaceutical and Biomedical Analysis **46**, 45-51.

Islam, M, A Cannavan, M Schweikert Turcu (2007). Validation of a robust liquid chromatography – tandem mass spectromentry (LC-MSMS) confirmatory method for 13 sulphonamides. Book of abstracts of the 3rd International Symposium on Recent Advances in Food Analysis, 7-9 November 2007, Prague, Czech Republic, 235.

Aysal, P, A Cannavan (2007). The IAEA-ethyl acetate multiresidue method to determine pesticide residues in fruits and wheat flour. Book of abstracts of the SETAC Europe 17th Annual Meeting, Multiple stressors for the environment and human health - present and future challenges and perspectives, 20-24 May 2007, Porto, Portugal, 107.

Whitaker, TB, MB Doko, **BM Maestroni**, AB Slate, BF Ogunbanwo (2007). Evaluating the performance of sampling plans to detect fumonisin B1 in maize lots marketed in Nigeria. Journal of AOAC International **90**, 1050-1058.

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

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

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

Cannavan, A. (2007). Developing country regulatory concerns and collaborative work to protect consumers and facilitate international trade. Book of abstracts of the Saskatoon International Workshop on Validation and Regulatory Analysis, 10-13 June 2007, Saskatoon, Canada, 17.

Dabalus-Islam, M, A Cannavan, M Schweikert Turcu (2007). Comparison of methods for the estimation of measurement uncertainty for an analytical method for sulphonamides. Book of abstracts of the Saskatoon International Workshop on Validation and Regulatory Analysis, 10-13 June 2007, Saskatoon, Canada, 61.

Maestroni, BM, MN Rathor, A Cannavan (2007). A simple, economical and efficient sample processing procedure for fumonisin B1 analysis in maize. Book of abstracts of the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May 2007, Istanbul, Turkey, 1474.

5.2. Staff Travels 2007-2008

Staff Member	Destination	Period	Purpose of Travel
Cannavan, Andrew	Dublin, Ireland	28-30 March 2007	To participate in the start-up meeting for the EU FP6 project "Improving the quality and safety of beef and beef products for the consumer in production and processing" (ProSafeBeef).
	Qingdao, China	15-18 April 2007	To present a keynote lecture and participate in a Food Safety Summit for the Asia/Pacific region.
	Istanbul, Turkey	21-25 May 2007	To participate in the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, to present a poster, attend the satellite meeting of the EU FP6 project 'BioCop', and contributing as an invited panelist to a FAO workshop on 'Sampling procedures for mycotoxins determination in food and feed'
	Saskatoon, Canada	10-13 June 2007	To participate in the Saskatoon International Workshop on Validation and Regulatory Analysis (SaskVal 2007) as a member of the Scientific Committee, to present a keynote lecture, to chair a plenary session and to present a poster of research work carried out in ACU.
	Lyon, France	30 August 2007	To represent the Joint FAO/IAEA Programme at the Joint FAO/IAEA/OIE/WHO Technical Meeting on Global Survey of Laboratory Quality Systems.
	Belfast, Northern Ireland, UK	4-5 December 2007	To participate as an Advisory Board member in the 2nd Annual Meeting of the EU FP6 Project 'BioCop'

Staff Member	Destination	Period	Purpose of Travel
	Ulaanbaatar, Mongolia	6-15 March 2008	To investigate environmental and food contamination in Khongor Soum as part of a UN team (WHO, UNEP, FAO)
	Egmond aan Zee, The Netherlands	19-21 May 2008	To give an oral presentation on work carried out under CRP D5.20.22.
	Brussels, Belgium	19-20 June 2008	To participate as Chair of the Advisory Board in the kick-off meeting of the EU FP7 project "Contaminants in food and feed: inexpensive detection for control of exposure" (CONffIDENCE)
	York, UK	2-3 September 2008	To participate as an Advisory Board member in the 3rd Annual Meeting and open day of the EU FP6 project 'BioCop'
	Toledo, Spain	10 October 2008	18 month meeting of work package 1.4. of the EU FP6 project 'ProSafeBeef'
Maestroni, Britt	Rott an Inn, Germany	4-7 June 2007	Collaborate with ILAU in development of a method for pesticides in water
	San José, Costa Rica	16-20 July 2007	RLA/5/050 Regional Workshop on Integrated analytical approaches to assess GAP
	San José, Costa Rica	9-13 July 2008	RLA/5/050 Regional Workshop on Integrated analytical approaches to monitor, control and comply with MRLs for pesticides
	Rome, Italy	8-10 October 2008	To present a paper at the First International MoniQA Conference 'Increasing Trust in Rapid Analysis for Food Safety'
	Panama City, Panama	15-19 December 2008	To present lectures and lab demos in a training workshop and to evaluate PAN/5/017; to plan PAN/5/019
Islam, Marivil	Nantes, France	26-30 March 2007	Training course of contaminants in food: metabolic fate and analytical approaches

Staff Member	Destination	Period	Purpose of Travel
	Prague, Czech Republic	7-9 November 2007	To present a poster at the 3rd International Symposium on Recent Advances in Food Analysis
Rathor, Nasir	Panama City, Panama	5-16 February 2007	To train laboratory staff on radiotracer techniques and residue analysis
Aysal, Perihan	Porto, Portugal	20-24 May 2007	To present a paper at the SETAC Europe 17th Annual Meeting

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/Pacific.
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 Research Center, Seiberdorf, Austria	Collaboration on research into interactions between environmental/food contamination
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
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

Institution	Topic
RIKILT Institute 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, Germany	Development of radio assay protocols
World Health Organization (WHO), Lyon Office for National Epidemic Preparedness and Response	Global survey of laboratory quality Standards
World Organization for Animal Health	
National Veterinary Research and Quarantine Service (NVRQS), Republic of Korea	Analytical methodology for food and environmental contaminants

5.4. Trainees, Fellows and Scientific Visitors

Name	Country	Duration	Topic of Training
Trainees (2007)			
Dervishi, Ms I	Albania	3 weeks	Training workshop on Screening and Confirmatory Methodology for Veterinary Drug Residues, 12-30 November 2007
Hadjamar, Ms D	Algeria	3 weeks	
Hasanuzzaman, Mr MD	Bangladesh	3 weeks	
Fan, Ms L	China	3 weeks	
Guihua, Ms L	China	3 weeks	
Gonzalez, Mr M	Costa Rica	3 weeks	
Ghazi, Mr A	Egypt	3 weeks	
Gedlie, Mr SH	Ethiopia	3 weeks	
Opuni, Mr F-MK	Ghana	3 weeks	
Widiastuti, Ms R	Indonesia	3 weeks	
Ahmed, Ms A	Iraq	3 weeks	
Dominguez, Ms ML	Mexico	3 weeks	
Narantungalag, Ms S	Mongolia	3 weeks	
Mukete, Ms E	Namibia	3 weeks	
Iglesias Benitez, Mr O	Paraguay	3 weeks	
Lucas Aguirre, Mr OA	Peru	3 weeks	
Arciaga, Ms A	Philippines	3 weeks	
Munasinghe, Mr DM	Sri Lanka	3 weeks	
Tangsirasip, Ms N	Thailand	3 weeks	
Ben Hassine, Mr T	Tunisia	3 weeks	
Bvumbi, Mr DS	Zimbabwe	3 weeks	
Fellows (2007)			
Diallo, Mr M	Mali	1 month 13days	
Berthe Dem, Ms S.	Mali	3 months	
Ouattara, Mr JPN	Burkina Faso	4 months	
Scientific Visitors (2007)			
Checa Orrego, Ms BI	Panama	8 days	
Aparicio Aguilar, Ms LW	Panama	8 days	

Name	Country	Duration	Topic of Training
Trainees (2008)			
Sakhi, Mr AA	Afghanistan	4 weeks	Training workshop "Introduction to quality assurance/quality control measures in pesticide residue analytical laboratories"
Jandric, Ms Z	Bosnia and Herzegovina	4 weeks	
Millar, Ms J	Dominica	4 weeks	
Chin Pampillo, Mr JS	Costa Rica	4 weeks	
Fianko, Mr JR	Ghana	4 weeks	
Oyiengo, Ms EW	Kenya	4 weeks	
Kwon, Mr JW	Rep of Korea	4 weeks	
Ahmad, Mr AR	Malaysia	4 weeks	
Gambin, Ms MD	Malta	4 weeks	
Shrestha, Mr S	Nepal	4 weeks	
Sane, Ms B	Senegal	4 weeks	
Chauke, Ms SPN	South Africa	4 weeks	
Pongpinyo, Mr. P	Thailand	4 weeks	
Ben Mansour, Ms A	Tunisia	4 weeks	
Awadh, Mr GAM	Yemen	4 weeks	
Moono, Mr G	Zambia	4 weeks	
Fellows (2008)			
Trigo Orsini, Ms ML	Bolivia	2 months	QA/QC training workshop and training course on GCMS in residues analysis
Jimenez Gonzalez, Mr JR	Columbia	1 months	QA/QC training workshop
El Hawari, Mr K	Lebanon	6 months	QA/QC training workshop and modern techniques in residues analysis
Sibanc, Ms N	Slovenia	1 month	QA/QC Training workshop
Assey, Ms SA	Tanzania	3 months	QA/QC training workshop and modern techniques in residues analysis
Kirs, Ms VE	Argentina	1 month	Group fellowship training course on GCMS in residues analysis
Dutra, Ms D	Brazil	1 month	
Baggio Ribeiro, Mr DH	Brazil	1 month	
Parada Carrasco, Ms A	Chile	1 month	

Name	Country	Duration	Topic of Training
Mosquera Vivas, Ms CS	Colombia	1 month	Group fellowship training course on GCMS in residues analysis
Masis Mora, Mr MA	Costa Rica	1 month	

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)	Maestroni, Britt
TCP Title	**Technical Officer**
Strengthening Capabilities to Control Veterinary Drug Residues in Foodstuffs (ALG/5/025)	Patel, Rajendra Cannavan, Andrew
Veterinary Drug Residue Monitoring Programme (ANG/5/003)	Patel, Rajendra Cannavan, Andrew
Veterinary Drug Residue Monitoring Programme (BEN/5/003)	Patel, Rajendra Cannavan, Andrew
Establishing a Veterinary Drug Residue Laboratory (BGD/5/027)	Patel, Rajendra Cannavan, Andrew
Certification of Exported Animal Products Using Nuclear and Other Analytical Techniques (CHI/5/046)	Cannavan, Andrew Patel, Rajendra
Enhancement of Quality Assurance for the Analysis of Veterinary Drug Residues (INS/5/033)	Cannavan, Andrew Patel, Rajendra
Monitoring of Residues in Livestock Products and Surveillance of Animal Diseases (MON/5/012)	Cannavan, Andrew Crowther, John
Strengthening the National Capacity for the Production of Veterinary Vaccines (MYA/5/015)	Crowther, John Cannavan, Andrew
Determining Drug Residues in Bovine Meat Exports (NIC/5/017)	Cannavan, Andrew Patel, Rajendra Brodesser, Peter Josef
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)	Patel, Rajendra Cannavan, Andrew
Monitoring of Chemical Residues and Food-borne Pathogens (SRL/5/039)	Cannavan, Andrew
Monitoring Veterinary Drug Residues in Livestock Products (NIR/5/036)	Patel, Rajendra Cannavan, Andrew
Regulatory Control and Monitoring of Contaminants and Residues (BKF/5/005)	Brodesser, Peter Josef Maestroni, Britt

TCP Title	Technical Officer
Capacity for Monitoring Pesticide Residues for Compliance with Minimum Risk Levels and Good Agricultural Practice According to ISO 17025 (BOL/5/017)	Ferris, Ian Maestroni, Britt
Assessment of the Impact of Pesticide Use in Lake Tota, Boyacá, Colombia (COL/5/022)	Ferris, Ian Maestroni, Britt
Monitoring of Pesticide Residues in Food Products (IVC/5/027)	Brodesser, Peter Josef Maestroni, Britt
Upgrading of Food Safety System (MAK/5/005)	Brodesser, Peter Josef Maestroni, Britt
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)	Maestroni, Britt Ferris, Ian
Strengthening Laboratory Capacity to Assess the Implementation of Good Agricultural Practices in the Production of Fruit and Vegetables in Latin America (RLA/5/050)	Ferris, Ian Dercon, Gerd Maestroni, Britt
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)	Ferris, Ian Maestroni, Britt Dercon, Gerd
Improving Laboratory Capacity for Food Safety (TAD/5/004)	Fesenko, Sergey Ferris, Ian Maestroni, Britt

5.6. Abbreviations

ABL	FAO/IAEA Agriculture & Biotechnology Laboratory
ACU	Agrochemicals Unit
APU	Animal Production Unit
CCα	Decision Limit
CRP	Coordinated Research Project
ECD	Electron Capture Detector
ENT	Entomology Unit
FAO	Food and Agriculture Organization of the United Nations
FB1	Fumonisin B_1
GC	Gas Chromatography
GC-MS	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
MRL	Maximum Residue Limit
NPD	Nitrogen Phosphorous Detector
OCP	Organochlorine Pesticides
OIE	World Organization for Animal Health
OPA	Ortho-phthalaldehyde
PBU	Plant Breeding Unit
PCB	Poly-chlorinated Biphenyl
PSA	Primary-Secondary Amine
RSD	Relative Standard Deviation
SIM	Selected Ion Monitoring
SSU	Soil Science Unit
TCP	Technical Cooperation Project
TIC	Total Ion Chromatogram
WHO	World Health Organization

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