Coordinated Research Project (CRP):D52039

Development and Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products”

REPORT OF THE 1ST RESEARCH COORDINATION MEETING

Vienna, 1-5 June 2015
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Introduction

The 1st Research Coordination Meeting (RCM) for the Coordinated Research Project (CRP) “Development and Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products” was held at the IAEA Headquarters, Vienna, 1st to 5th June 2015 (See Annex A for the agenda). The meeting was chaired by Mr. Wim Reybroeck (Institute for Agricultural and Fisheries Research (ILVO), Belgium) and the rapporteur was Ms. Hiranthi Jayasuriya (U.S. Food and Drug Administration, Center for Veterinary Medicine). The full list of participants is included as Annex B.

The purpose of this meeting was to discuss the plans and proposed research of the participating researchers; to promote interactions between the researchers and to prepare recommendations and guidelines to facilitate project tasks. Meeting recalled that the main purpose of the RCM was to assist and encourage the researchers to fulfil their goals while the scope and expectations were to acquire and disseminate new knowledge, stimulate growth of nuclear sciences and technologies in developing countries and ensure that research results are freely available.

Background

In many developing countries, rapid demographic changes and rising incomes have increased the demand for high value food commodities. Increasing international trade in these products has therefore led to dramatic growth in the livestock and aquaculture sectors. Changes in production practices and exacerbating factors such as climate change have resulted in a rise in disease outbreaks and an increased use of agrochemicals, including veterinary medicines, with the concurrent environmental contamination and development of microbial and parasitic resistance to these compounds. At the same time, awareness of food safety is rising and many importing countries have implemented food control regulations to guarantee the quality and safety of imported foods for their consumers.

Many developing countries have also taken steps to put in place control systems that encourage the responsible use of veterinary medicines (and other agrochemicals) to combat drug resistance and comply with international standards. However, they find it difficult to obtain the required know-how and skills, thus hindering their ability to access international markets for food products of animal origin. One significant constraint is the capacity of laboratory services to generate surveillance data using reliable analytical tools.

Objectives of the RCM were to:
1. Bring CRP participants together and stimulate interaction;
2. Discuss the plans and proposed research of individual participating researcher;
3. Facilitate a better understanding of the relationship each researcher has to the overall objectives of the CRP;
4. Promote interaction between the researchers and;
5. Prepare recommendations and guidelines to facilitate project tasks and for the researchers to agree a common approach and way forward;
6. Discuss mechanisms to provide support to research proposals through the generation of incurred residues.
Scope of the meeting and expectations included:
1. Presentation of workplans by CRP members;
2. Ensuring a two way feedback;
3. Finalizing clear work-plans per program of work and proposals;
4. Identifying synergies and networking opportunities;
5. Preparing a meeting report (including rapporteur's notes);
6. Improving understanding of roles in the project.

RCM Presentations

Mr. James J Sasanya, IAEA

A presentation was delivered on the purpose of CRPs, background of this current CRP, its associated objectives and results (including outcomes and outputs), the strategies for effective implementation and potential challenges. The scope and expectations of the the first RCM were also presented.

Mr. Leen van Ginkel, RIKILT, The Netherlands

Title: RIKILT’s Role in Research and Enhancing of Global Laboratory Networking for Food safety.

A short overview was given on the position of RIKILT within Wageningen University and Research, Wageningen, The Netherlands. RIKILT’s main tasks are in the area of food safety and food quality, with a focus on authenticity. Additionally, research is undertaken on new developments in the areas of nanotechnology and new protein sources. Research focuses on new approaches for hazard identification and analytical methods.

RIKILT’s activities are organized around the National and EU-reference tasks, next to technical aspects such as the development and validation of new analytical methods, this includes many quality and training activities. These activities are grouped as “Quality Services” where RIKILT provides proficiency testing programmes and, when necessary custom tailored training, etc.

Current research on aquaculture focuses on improving analytical methods for antibiotics, growth promoting compounds and other pharmaceuticals in both the products and the feed. One of the current activities is incorporating the analysis for aminoglycosides, a very polar class of compounds, in a multi-residue method for antibiotics.
Mr. Wim Reybroeck, Belgium
*Title: Optimization and primary validation of screening tests for the detection of residues of antibiotics and chemotherapeutics in fish and aquaculture products and for the detection of mycotoxins in fish feed.*

For the monitoring of residues of anti-infectious agents in food of animal origin, the use of screening tests is still the most cost-effective approach. In an integrated system different types of screening tests play a role. Sample preparation procedures could be adapted to make screening of fish and aquaculture products possible by means of microbial inhibitor tests (disk plate assays and agar diffusion tests (e.g. Explorer 2.0 + e-Reader)). Different lateral flow devices are commercially available for the simultaneous detection of different antibiotic families (β-lactams, tetracyclines, sulfonamides) in milk in a few minutes. Appropriate sample preparation protocols are developed to integrate these types of dipstick tests into screening of fish and aquaculture for antimicrobial residues. Work if also planned to optimize protocols of Charm II tests involving radiolabeled (C14 and H3) reagents and to limit the number of false positive results when testing residues in fish samples. For the detection of mycotoxins in fish feed, a promising technique is the Randox Mycotoxins Array using biochip format technology. ILVO hopes to support the project by, among others, performing initial validation studies and hence limiting the validation work for transfer laboratories.

Ms. Hiranthi Jayasuriya, USA
*Title: Residue Chemistry and Food Safety Research at the Center for Veterinary Medicine, US Food and Drug Administration*

Ms. Hiranthi Jayasuriya described the work done at the Division of Residue Chemistry, U.S. Food and Drug Administration, to develop and validate methods for drug residues and other chemical contaminants in animal derived foods and animal feeds. She outlined the programs to develop modern analytical methods to replace obsolete assays, the development of methods for the detection of antibiotic and mycotoxin residues in distiller’s grains and As speciation study with the organic arsenical drug Roxasone.

She highlighted the utility of high resolution mass spectrometry (HRMS) in structure elucidation of novel methyl testosterone glucuronides which are proposed as better markers for monitoring methyltestosterone use in Fish. The ability of performing retrospective analysis of exact mass data generated by HRMS instruments makes it an attractive option especially in untargeted analysis.

Mr. Joe Boison, Canada
*Title: Sensitive and Reproducible Analytical Techniques for Monitoring Veterinary Drug Residues and Related Contaminants in Foods*

Objectives of this work includes providing scientific and technical advice and guidance on the development and validation of suitable and efficient analytical methods to support the national and/or regional efforts to ensure that aquaculturalists are using the few drugs and chemicals approved/available for aquaculture production safely; providing guidance to ensure that aquaculture products are free from drug and chemical residues that may pose potential safety hazards to the consumer and also thereby negatively impact international trade. The laboratory has over the past 18-20 years been developing suitable analytical methods using stable isotope internal or surrogate standards wherever possible under ISO/IEC 17025:2005
laboratory accreditation conditions to support the Canadian National Chemical Residue Monitoring Program (CNCRMP) for veterinary drug residues. All the methods developed and used to support the CNCRMP for veterinary drugs in foods of animal origin are validated to international standards to demonstrate that they are fit-for-use in a regulatory control program as required by Codex Alimentarius Commission guidelines. The expectation is to use the extensive skills and experience developed in this laboratory to assist laboratories in developing countries or regions to elevate their standard of analytical support systems to the equivalence of those in developed countries in order to remove barriers to international trade posed by the lack of analytical expertise in some countries. This will entail frequent consultations with leaders of the projects in the laboratories in the participating countries and perhaps one to four face-to-face meetings at defined appropriate locations to discuss progress and strategies to get the work accomplished.

Mr. A. Turan Erdogdu, Turkey

Title: Training and Supporting of Radio-Analytical and Complementary Techniques to Control of Veterinary Drugs and Related Chemicals in Aquaculture.

An overview was provided of Izmir/Bornova Veterinary Control Institute as the National Reference Laboratory for Antibiotic Residue Analyses. In addition to research on innovative analytical techniques, the institute provides training to laboratory personnel from various IAEA member states. Areas of work include antibiotic residue analysis, heavy metals, pesticide analysis, dyes, radio receptor assays, HPLC technique, GC-MS and LC-MS/MS, following ISO 17025/2005 principles. Opportunities for collaboration with contract and agreement holders were discussed/outlined.

Contract Holders Presentations

Each contract holder presented their recent and proposed work for the first year under the project, with an emphasis on how their research will contribute to achieving the CRP objectives.

Mr. Rodrigo Granja, Brazil:

Title: Development and Validation of a Radioimmunoassay Kit For the Screening of Chloramphenicol in Fish and Shrimp Tissues And Related Feeds.

A brief overview of Microbioticos' Laboratories and the relevance to the IAEA CRP program over the last 20 years was presented. The current project follows the lab’s proposal during its 3rd CRP, to develop a radioimmunoassay kit for the screening of chloramphenicol in seafood and related feeds. The process of antibody production, method validation, application to routine sample analysis in national residue monitoring among others were presented. The current study will focus on Tilapia, Cat Fish and Shrimp and attention will be paid to improving antibody sensitivity. The research findings will be disseminated through IAEA TCPs.
Mr. Robinson DJ. Solomon, India
Title: Detection and Monitoring of Veterinary Drugs and Chemical Residues in Feed Materials and Aquaculture Products Using Radioanalytical and Complementary Techniques

The scope of matrices and analytes to be investigated were presented and feedback provided. Samples included feed materials in aquaculture, water, shrimp, fish, Artemia among others. The compounds to be tested were chloramphenicol, tetracyclines, and beta-lactams., Others were toxic metals such as Arsenic, Cadmium, Lead, Mercury and agro-chemicals as Endosulfan and it's toxic metabolites. The instrumentations discussed included HPLC, LC-MS, AAS and related work has been initiated including purchase of analytical reference standards as well as optimisation and streamlining of novel extraction methods.

Mr. Liberty Sibanda, South Africa
Title: Using Radionuclides to Improve Liquid Chromatography and Mass Spectrometry (LC-MS/MS), Gas Chromatography Mass Spectrometry (GC-MS/MS) and Liquid Scintillation Counter Analytical Methods for Monitoring of Aquaculture Production Sites and Produce

The project aims to develop a screening method using Liquid Scintillation Counting method for antibiotics. A multi-antibiotics LC-MS/MS method will also be developed for fish muscle (finfish, mulluscs, abalone etc) aided by stable-isotope internal standards for quantification. An ICP-MS method for analysis of toxic metals (Cd, Pb, As and Hg) will also be extended from finfish muscle to other types of aquaculture products. This ICP-MS technique will be confirmed using Neutron Activation method for positive metal identification. The main types of matrices to be investigated will include feed, muscle and water. Targeted number of publications include (1) Multi-residue LC-MS/MS in different aquaculture products and (2) use of Neutron Activation in heavy metal confirmation in aquaculture and aquaculture products. The possibility of HPLC ionic separation before ICP MS was also suggested.

Mr. Juan Carasco, Chile
Title: Multi-residue Method for the Detection of Veterinary Drug Residues and Other Organic Contaminants in Aquaculture Products and Feed by LC/MS/MS and GC/MS/MS Spectrometry Techniques

It is becoming increasingly important to have a quick turnaround time for analytical results when testing residues of veterinary drugs and other contaminants in food and feed due to the high cost of delayed harvest time of fish or other aquaculture product. On the other hand it is also necessary for a country to have information on all ingredients and foods used in marine farming to ensure food safety.

Laboratory tests that are being used in Chile are methods for single analytes, take time and are more expensive due to man power and cost of consumables. In the past this was due to very few laboratories having the LC-MS/MS equipment, but now most of the laboratories are equipped with these instruments. Thus, the objective of this project is to develop, validate and implement multi-analyte mass spectrometry techniques (according to the new regulations and international requirements) for residues of veterinary drugs in aquaculture products and animal feed in order to ensure safe food from production/sea to the table.
Mr. Zhou Zhu, China  
*Title: Development of Multi-residue Methods of Veterinary Drugs and Growth Promoters by LC-MS/MS to Strengthen the Analytical Capability in Aquaculture Products in South China*

Antibiotics and hormonal growth promoters are commonly and illegally used in aquaculture to control aquaculture-related diseases and improve yields. Low levels of ‘cocktail compounds’ can be used to escape surveillance, but may result in multiple residues of these banned substances such as nitrofuran, methyltestosterone, medroxyprogesterone, trenbolone and diethylstilbestrol etc. Concerns about these residues are related to food safety/possible toxic effects on public health, and the need to meet requirements for international trade. Therefore, it is necessary to improve detection capabilities of analytical methods in monitoring residues in aquaculture products. The research contract holder aims to develop multi-residue confirmatory methods for collecting reliable data on veterinary drug and hormonal growth promoter residues in aquaculture products in South China through this CRP. A brief introduction of the CSI (Chemical Analysis and Physical Testing Division, Shenzhen Center for Disease Control and Prevention, Shenzhen, China) project scientific background, scope, detailed workplans, and expected outcomes were presented.

Mr. Aziz Mukota, Uganda  
*Title: Development, Validation and Optimization of New Cost-Effective Analytical Techniques for Residues of Common Pharmacologically Active Veterinary Substances and Related Contaminants in Aquaculture, and Studying Associated Risk Factors in Uganda*

Aquaculture farming is among the fastest growing agri business in the world including Uganda. The Government of Uganda has identified aquaculture as one of the key drivers of food security, job creation and economic growth after Uganda’s fishing industry suffered a major setback in the 1990’s when its exports were banned by the European market due poor hygiene, bad fishing habits and lack of an established measurement and testing infrastructure to ensure accurate and comparable measurements for various contaminants including pesticide, antibiotic residues, toxic metal contaminants in fish.

The overall aim of this collaborative research will be to Develop, Validate and Optimize new cost-effective analytical techniques based on isotope dilution assay and radio receptor technique to be used for testing residues of common pharmacologically active veterinary substances and related contaminants like pesticides, mycotoxins, and heavy metals in aquaculture products. In addition the research will investigate risk factors associated with poor aquaculture practices along the food chain and their mitigation strategies in Uganda.

Optimized methods will be used to; characterize and profile selected aquaculture products, and cause review of National Standards basing on obtained data which in the long run will lead to increased exports of aquaculture products from Uganda leading to economic development and protection of health of consumers.
Mr. Valdemar L. Tornisielo, Brazil

Title: Investigating Agrochemical Contamination of Water Sediments and Fish in Fish Farms and Studying the Depletion of Antimicrobials in Fish Tissue in Lab Conditions Using Radiolabeled Antibiotics as well as Related Antimicrobial Resistance

According to the FAO, Brazil is the second largest aquaculture producer in the Latin America and the Caribbean. The sector (fresh water or mariculture) is the country’s largest potential for increasing fish supplies to ensure food security. Peru has also increased fish production mainly trout, and both countries have to use large quantity of pharmaceutical drugs and related chemicals to control or prevent diseases and boost productivity where diseases are prevalent. The drugs and other environmental contaminants are unfortunately a risk to public and environmental health and should be controlled using reliable, sensitive and robust techniques. In addition, little is known about depletion patterns of many drugs in various fishes (and production sites) in the country. Lack of such information undermines the understanding and implementation of drug withdrawal periods that are critical in setting of safe levels/standards for these drugs. The present project will research innovative LC-MS/MS technique for reliable and cost effective testing of veterinary drugs and related chemicals (including non-target analysis) and investigate depletion (using the radio-labelled antibiotics) patterns of selected veterinary drugs in fish in controlled/laboratory environment. Use/misuse of veterinary drugs is also associated with development of antimicrobial resistance and more information is available for terrestrial animal production that aquaculture. This project also hopes to investigate antimicrobial resistance patterns associated with aquaculture production in Brazil and Peru.

Mr. Daniel Wunderlin, Argentina

Title: Development of Methods Based on Isotopic Dilution in Connection with LC-MS to Evaluate the Presence of Veterinary Drugs and Related Chemicals in Aquaculture Fish

The proposed project is based on the development of state-of-the-art methods to evaluate the presence, bioaccumulation and persistence of veterinary drugs residues as well as other pharmaceuticals products present in the water and fish growing in aquaculture systems. Methods will involve use of isotopically labeled compounds to improve the certainty, accuracy and precision of analytical measurements in fish, this method is known as isotopic dilution (ID), facilitating proper characterization of matrix effects, which is one of the biggest challenges associated with standard methods of analysis for food products. This isotopic dilution will be linked to liquid chromatography-tandem-mass spectrometry for both screening and quantification of drug and pharmaceutical residues in fish captured from aquaculture systems in Argentina. The combined use of ID with LC-MS/MS would also improve the evaluation and reduction of ionic suppression.

Simultaneously, evaluation of stable isotopes in water and fish (mainly δ15N) will be undertaken, identifying markers of anthropic pollution in the water that could be transferred to fish. This is pertinent given public concerns on the potential impact of consuming fish growing in polluted water.
Ms. Ping Shen, Singapore

Title: Novel Analytical Approach in ASEAN Region to Test Aquaculture Chemicals by Harnessing the Power of High Resolution Mass Spectrometry (HRMS), Triple Quadrupole Mass Spectrometry (MS/MS) and Stable Isotopes.

There are major challenges facing the analysis of residues of aquaculture associated chemicals (veterinary drugs, natural toxins and other chemical contaminants) with regard to testing scope and coverage, laboratory productivity, and the quality of the testing specificity, sensitivity and quantitative accuracy. The Agri-food and Veterinary Authority (AVA) has proposed to explore novel analytical approaches to harness the strength of Triple Quadrupole Mass Spectrometry (MS/MS) on its sensitivity and reproducibility, and High Resolution Mass Spectrometry (HRMS) on its specificity, coverage and screening speed to address these challenges. Isotope labelled internal standards (deuterated or C-13 labeled standards) will be used to develop analytical methods with the desired accuracy in residue quantitation. The developed analytical methods can help AVA as well as the national food safety control laboratories of other ASEAN member states to confidently detect the aquaculture chemicals, further strengthen food safety regulation framework, support risk management, promote good agricultural practices and address trade and public health concerns, especially in the aquaculture sector.

Ms. Judith Tsafack Takadong, Cameroon

Title: Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products in Cameroon

Cameroon spends about USD$ 200 million each year in imports to address the deficit in fish production which is estimated to be 230,000 tones. To reverse this situation, the government has planned to build intensive production centres to produce about 100,000 tons of fish per year, including development of aquaculture. Veterinary pharmaceuticals and related substances are widely used in aquaculture and their misuse could result in unsafe residues/contaminants in aquaculture products as well as in the production sites. Furthermore, naturally-existing food contaminants such as mycotoxins, could unintentionally end up in aquaculture products. To support risk management and communication, good agricultural practices and address trade and public health concerns, it is crucial to monitor these residues/contaminants. Through the present project, the Centre for Food and Nutrition Research of IMPM aims to use its recent experience working with radio-receptor assay technique for chemical hazard analysis to develop innovative analytical techniques, to strengthen the national control system and contribute to proper use of veterinary pharmaceuticals and related chemicals in aquaculture. Initially, a Cross-sectional study will be undertaken to identify what and how the veterinary drugs and related chemicals are used in aquaculture in Cameroon. This will be followed by a pilot study to test and validate the sampling procedure, screening method (Radio-receptor assay technique), and the elaboration of Standard Operating Procedures.
Mr. Rey Manuel Cambisaca Saquicela, Ecuador

Title: Determination of Antibiotics in Ecuadorian Aquaculture Products by LC-MSMS; Implementing Methods and Monitoring

As the demand for aquaculture products as a protein source increases, so is the need for public awareness on the safety of such products for public health and meeting international market requirements. This is because of the increasing need to use various veterinary pharmaceuticals and related pharmacologically active substances to control the numerous diseases that affect aquaculture production worldwide including Ecuador. A number of substances are either banned (not permitted for use) due to health implications or when used, their residues should not exceed a certain limit for safety of the consumer. Ensuring that such guidelines are met requires a functional and reliable residue monitoring program supported by a laboratory that meets international standards. Such labs should have the skill and knowhow to not only apply internationally reputable methods but also develop and validate their own methods that are fit for purpose and can be applied elsewhere. A basic foundation for these capabilities exists at the national institute of fisheries. However, it requires strengthening through research on analytical techniques that strengthen the monitoring of veterinary pharmaceuticals and related contaminants in aquaculture products, inputs and environment (water). A program of work through which this will be realised was presented and discussed.

Conclusions of the First RCM

1. Aquaculture is a fast growing industry and there are a number of diseases that hamper productivity. The use of drugs is therefore very common. However, in many cases there are no control programs. This CRP provides an excellent opportunity to strengthen such programs through a network of research institutions willing to compare, evaluate and harmonize workplans. A lot of synergies were identified among the participants and this provides a platform for collaboration.

2. Individual project objectives reflected similarities across regions (and thus priorities).

3. Optimum use of both screening and confirmatory techniques will be investigated in this CRP.

4. Centralized purchase of expensive reagents/standards (especially radio-labelled standards) that can then be apportioned among interested parties is necessary.

5. There was willingness by the CVM/USFDA to support the CRP through provision of incurred materials. However, the challenge of shipment to respective countries was raised.

6. There is some need for specialized training or benchmarking at partner institutions to facilitate certain research project activities.

7. There was need to modify individual workplans to ensure soundness and feasibility.

Recommendations

1. Work with incurred tissue during method validation. FDA/CVM is willing to provide incurred tissue if a pathway is worked out to receive them.

2. Include radio-labelled (stable isotopes) as internal standards during R&D to compensate for matrix effects.

3. Include cheap and easy assays as screening methods to cut down the time and the cost of investigations and method application, and follow up the suspect sample with a confirmatory and quantitative assay.
4. Communicate and collaborate with other contract/agreement holders to better realise project goals
5. Consider CODEX documents as reference during R&D, including sampling guidelines for fish
6. Ensure project scope is realistic and deliverable
7. Ensure there is a clearly defined number of compounds to study and in line with the contract. Nevertheless, flexibility to drop or add more compounds where necessary is encouraged.
8. There is need to establish guidelines or standards for the quality of water used in aquaculture production and this CRP can contribute to the debate
9. There is need to promote untargeted analysis (in addition to targeted analyses).
Agreed Action Plan and Logical Framework

Project Title: Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products in Cameroon

<table>
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<tr>
<th>Lead Partner</th>
<th>Centre for Food and Nutrition Research, IMPM, Yaounde-Cameroon</th>
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<td>Start month</td>
<td>July 2015</td>
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<td>End month</td>
<td>May 2016</td>
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<td>Interaction with other partners</td>
<td>Institute for Agricultural and Fisheries Research (Belgium), Bornova Veterinary Control Institute (Turkey), Uganda National Bureau of Standards</td>
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<tr>
<td>Technical support required</td>
<td>Development and primary validation dossier, and optimum use of the liquid scintillation counter (including reduction of rate of false positives) in residue analysis.</td>
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</table>

OBJECTIVES

1. To perform a survey to identify the different veterinary drugs and related chemicals used in aquaculture in Cameroon and their mode of use.

2. To develop and validate radio receptor assay (RRA) screening methods for residues of veterinary drugs in aquaculture products.

DESCRIPTION OF WORK:

Task 1: To optimize and validate the Charm II screening Assay for Beta-lactams, Tetracyclines, Sulfonamides, Macrolides (including Lincosamides) in fish

Task 2: To optimize and validate an immunological assay for the detection of Chloramphenicol in fish

Task 3: Apply the above methods by monitoring local fish products (may begin second year); may require sourcing of additional funds
DELIVERABLES

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Validated procedure for the analysis of Beta-lactams, Tetracyclines, Sulfonamides, Macrolides (including Lincosamides) in fish</td>
<td>April 2016</td>
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<tr>
<td>2</td>
<td>Validated procedure for the analysis of chloramphenicol in fish</td>
<td>May 2016</td>
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EXPECTED RESULTS

Validated Standard Operating Procedure (s)/protocol (s) and laboratory manual (s) for the analysis of Beta-lactams, Chloramphenicol, Macrolides (including Lincosamides), Sulfonamides and Tetracyclines in fish

Project Title: Multi-residue Method for the Detection of Veterinary Drug Residues and Other Organic Contaminants in Aquaculture Products and Feed by LC-MS/MS and GC-MS/MS Spectrometry Techniques.

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<th>Lead Partner</th>
<th>Labser Laboratory, Rancagua, Chile</th>
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<tr>
<td>Interaction with other partners</td>
<td>FINET (Food Intelligence Net); INPC; RIKILT</td>
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<tr>
<td>Technical support required</td>
<td>Specialized training in validation of methods for veterinary drug residues.</td>
</tr>
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</table>
OBJECTIVES

1. Develop Multi-residue Methods for the Detection of Veterinary Drug Residues in Salmon fish tissue in the first year.


3. Implement and validate multi-residue analysis method (at least for antimicrobials required by SERNAPECA) in the first year.

DESCRIPTION OF WORK:

Task 1: Select for priority the analytes for the multi residues method and may include those monitored by Sernapesca e.g. oxolinic acid, flumequine, ciprofloxacin, enrofloxacin, erythromycin, florfenicol, amoxicillin, sulfonamides, tetracycline, oxytetracycline, chloramphenicol.

Task 2: Establish of the limit of quantification needed according the purpose of the contaminant determination.

Task 3: Review of relevant literature/and other available method (e.g. from the network) as well as international standards and guidelines/regulation for residues of veterinary drugs to select the most appropriate approach.

Task 4: Validation and implementation of LC-MS/MS method and quality control procedures.

Task 5: Application to field samples.

DELIVERABLES

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<th>Description</th>
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<td>1</td>
<td>List of the analytes to be included in the multi residues method.</td>
<td>June 2015</td>
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<td>2</td>
<td>Select method fulfilling the requirements.</td>
<td>August 2015</td>
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<td>3</td>
<td>Implementation of the multi residues method selected. Description of the analytical method in the SOP.</td>
<td>February 2016</td>
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<td>4</td>
<td>Validation of the multi residues method for the first year</td>
<td>May 2016</td>
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<td></td>
<td>Surveillance study (plan)</td>
<td>Post May 2016</td>
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<tr>
<td>6</td>
<td>Dissemination of research work</td>
<td>Post May 2016</td>
</tr>
<tr>
<td>7</td>
<td>Continue with other methods such as for fish feed</td>
<td>Post May 2016</td>
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**EXPECTED RESULTS**

- Multi-residue method for Veterinary Drugs in Salmon tissue developed
- Multi-residue method for Veterinary Drugs in Salmon tissue validated
- Method for multi-residue analysis of veterinary drugs implemented in the monitoring of Salmon

Project Title: Development of multi-residue methods of veterinary drugs and growth promoters by LC-MS/MS to strengthen the analytical capability in aquaculture products in South China

<table>
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<th>Lead Partner</th>
<th>Chemical Analysis and Physical Testing Division, Shenzhen Center for Disease Control and Prevention, Shenzhen, China</th>
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<tr>
<td>End month</td>
<td>July 2016</td>
</tr>
<tr>
<td>Interaction with other partners</td>
<td>Canadian Food Inspection Agency, Saskatoon, Canada; Agri-Food &amp; Veterinary Authority of Singapore, Singapore</td>
</tr>
<tr>
<td>Technical support required</td>
<td>Specialized training and advice in LC-MS/MS method development</td>
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## OBJECTIVE

1. To develop an isotope dilution mass spectrometry assay by LC-MS/MS for the determination of nitrofuran metabolites (AOZ, AMOZ, SEM, and AHD) in different aquaculture products.

2. To write a detailed Standard Operation Procedure (SOP) and to validate the quantitative LC-MS/MS based confirmatory methods according to the international guidelines.

3. To investigate the occurrence of the nitrofuran metabolite residues and hormonal growth promoters in different aquaculture products in South China.

4. To develop a multi-residue confirmatory method by LC-MS/MS with deuterated internal standards for the determination of hormonal growth promoters (methyltestosterone, medroxyprogesterone, trenbolone and diethylstilbestrol etc.) in different aquaculture products (beginning in year 2).

## DESCRIPTION OF WORK:

| Task 1: | Optimizing HPLC method in the analysis of nitrofuran metabolites. This work will be performed on solvent-based standard mixtures. |
| Task 2: | Optimization of mass spectrometric conditions including Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) will be assessed and compared with respect to sensitivity, selectivity and suitability for quantification of the analytes, based on solvent standards. |
| Task 3: | Optimization of sample clean-up procedure. |
| Task 4: | Method validation according to 2002/657/EC decision. |
| Task 5: | Sample (fish, shrimp and crabs) collection from Zhanjiang, Yangjiang, Shenzhen and Shanwei. |
| Task 6: | Review of literature on hormonal growth promoters in fish and aquaculture products (in preparation for the next year). |
DELIVERABLES

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<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard Operating Procedure for the determination of nitrofuran metabolite</td>
<td>December 2015</td>
</tr>
<tr>
<td></td>
<td>residues in fish and aquaculture products.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Validation data for the developed method.</td>
<td>March 2016</td>
</tr>
<tr>
<td>3</td>
<td>Results about the occurrence level of nitrofuran metabolite residues in fish</td>
<td>July 2016</td>
</tr>
<tr>
<td></td>
<td>and aquaculture products in Guangdong Province, China.</td>
<td></td>
</tr>
</tbody>
</table>

EXPECTED RESULTS

Validated isotope dilution LC-MS/MS method for the determination of nitrofuran metabolite residues (AOZ, AMOZ, SEM, and AHD) in fish and aquaculture products.

Overview of the occurrence level of nitrofuran metabolite residues (AOZ, AMOZ, SEM, and AHD) in fish and aquaculture products in Guangdong Province, China.

Project Title: Development of Methods Based on Isotopic Dilution in Connection with LC-MS to Evaluate the Presence of Veterinary Drugs and Related Chemicals in Aquaculture Fish

<table>
<thead>
<tr>
<th>Lead Partner</th>
<th>UNC-National University of Córdoba, Argentina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start month</td>
<td>June 2015</td>
</tr>
<tr>
<td>End month</td>
<td>May 2016</td>
</tr>
<tr>
<td>Interaction with other partners</td>
<td>A regional group could be helpful (Ecuador + Chile + Brazil + Argentina).</td>
</tr>
</tbody>
</table>
OBJECTIVES

1. To develop state of the art methods, based on isotopic dilution and LC-MS, to evaluate the presence, bioaccumulation and persistence of veterinary drug residues as well as other pharmaceuticals present in the water and fish growing in aquaculture systems.

2. To evaluate stable isotopes in water and fish (mainly δ<sup>15</sup>N), looking for markers of anthropic pollution in the water that could be transferred to fish, raising questions on the impact of consuming fish growing in polluted water on the public health.

DESCRIPTION OF WORK:

(First year)

Task 1: To acquire pure veterinary drug standards of interest (both non-labelled and labelled compounds). Starting with antimicrobials, mainly sulfonamides but also including other groups.

Task 2: To develop a screening method based on HRMS (QTOF or similar).

Task 3: To develop a confirmatory method for quantification of veterinary drug residues and related chemicals based on isotopic dilution, LC-ESI, MS/MS by triple quadrupole.

Task 4: To test developed methods using spiked/naturally incurred samples followed by market fish.

Task 5: Start sampling of fish from aquaculture farms in Argentina (3 fish species, at least one farm per species). Sampling will consider seasonal changes (rainy/dry seasons).

Task 6: Preliminary study of aquaculture fish (whole fish, distribution of drugs and metabolites in different tissues/organs).

Task 7: Analysis of <sup>15</sup>N in water, small invertebrates as primary consumers and fish arising from aquaculture systems with different levels of pollution/pollutants.

Task 8: Technical report to IAEA.
DELIVERABLES

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Screening method for sulfonamides by LC-HRMS</td>
<td>Jan-Feb 2016</td>
</tr>
<tr>
<td>2</td>
<td>Quantitative/confirmatory method for sulfonamides by LC-MS/MS using isotopic dilution</td>
<td>March-April 2016</td>
</tr>
<tr>
<td>3</td>
<td>Technical report, including SOPs</td>
<td>May 2016</td>
</tr>
<tr>
<td>4</td>
<td>Peer reviewed publication on methods/application</td>
<td>May 2016</td>
</tr>
</tbody>
</table>

EXPECTED RESULTS

A reliable, easy to use, state-of-the-art method for screening multiple antimicrobials and metabolic products in aquaculture water, feed and fish.

A reliable, sensitive, easy to use, state of the art method for confirmation of multiple antimicrobials and, probably, harmful impurities, in aquaculture water, feed and fish.

Developed methods applied to the study of aquaculture fish and aquaculture environment in Argentina (probably extension to other countries).

Chemical/isotopic markers of anthropic pollution evaluated, namely $^{15}$N as a marker of sewage/manure pollution in aquatic systems dedicated to aquaculture.

Project Title: Development and validation of a radioimmunoassay kit for the screening of chloramphenicol in fish and shrimp tissues and related feeds.

<table>
<thead>
<tr>
<th>Lead Partner</th>
<th>Laboratorio Microbioticos, Campinas, SP, Brazil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start month</td>
<td>June 2015</td>
</tr>
<tr>
<td>End month</td>
<td>May 2016</td>
</tr>
<tr>
<td><strong>Interaction with other partners</strong></td>
<td>Expected with Queen’s University Belfast and Laboratoire d’Hormonologie - Marloie, Belgium</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Technical support required</strong></td>
<td>Chloramphenicol antibody production and characterization; training in LC-MS</td>
</tr>
</tbody>
</table>

**OBJECTIVES**

1. To develop, standardize and validate a radioimmunoassay kit for the determination of Chloramphenicol residues in fish tissues;

2. To develop, standardize and validate an analytical method, using the product outcome from topic A);

3. To conduct an equivalence study of the validated method (topic B) with other technology methods (such as HPLC and Mass Spectrometry);

4. To apply the validated method to samples collected and further to the Official Monitoring Program in Brazil;

5. To participate on inter-laboratory rounds conducted by Microbioticos and International Bodies.
DESCRIPTION OF WORK:

Task 1: Preparation (First Quarter):
- Procurement of equipment and supplies;
- Elaborate sampling project;
- Development of antibody and tritium labelled compound (marker).

Task 2: Begin Introduction of Immunoassay Methods (Second and Third Quarter):
- Clean-up method development/optimization (considering aspects of drug metabolism);
- Validation of Optimized Method
- Analysis of routine samples

Task 3: Compilations and Reports (Fourth Quarter)
- Compilation of analysis data;
- Prepare report and present findings to IAEA;
- Make request and submit applications of CRP to the IAEA;
- Consolidate the outcomes from year 1 (one);
- Application of methods to Brazilian Regulatory Monitoring Program.

Task 4: Additional Work (Year 2 and 3):
- Consolidate the RIA method – Robustness and Applicability;
- Start equivalence studies with other techniques (LC-MS/MS);
- Participate on Inter-laboratory rounds;
- One or more publications anticipated e.g;
  - Immunoassay development and method validation;
  - Application on real samples, results and methods equivalence.

DELIVERABLES

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
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<tbody>
<tr>
<td>1</td>
<td>Antibodies from Brazil / European Institute</td>
<td>October 2015 / Sept 2015</td>
</tr>
<tr>
<td></td>
<td>Developed Chloramphenicol RIA Method</td>
<td>Dec 2015</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>3</td>
<td>Validated Chloramphenicol RIA Method</td>
<td>March 2016</td>
</tr>
<tr>
<td>4</td>
<td>Analysis of Routine Samples</td>
<td>May 2016</td>
</tr>
<tr>
<td>5</td>
<td>One or more publications</td>
<td>From May 2016</td>
</tr>
</tbody>
</table>

**EXPECTED RESULTS**

- Approved SOP/protocol(s) for Chloramphenicol antibody production in Brazil
- Method for the screening of Chloramphenicol residues in seafood and related feeds
- Validated method for the screening of Chloramphenicol residues in seafood and related feeds
- One or more publications in peer review journals

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**Project Title:** Novel Analytical Approach in ASEAN region to test Aquaculture Chemicals by harnessing the power of High Resolution Mass Spectrometry (HRMS), Triple Quadrupole Mass Spectrometry (MS/MS) and Stable Nuclides/Isotopes

<table>
<thead>
<tr>
<th><strong>Lead Partner</strong></th>
<th>AVA/Singapore</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Start month</strong></td>
<td>April 2015</td>
</tr>
<tr>
<td><strong>End month</strong></td>
<td>June 2016</td>
</tr>
<tr>
<td><strong>Interaction with other partners</strong></td>
<td>Canadian Food Inspection Agency</td>
</tr>
<tr>
<td><strong>Technical support required</strong></td>
<td>Synthesis of stable isotope labeled internal standards or compounds if commercially not available</td>
</tr>
</tbody>
</table>
OBJECTIVES

1. To be technically ready with fast screening methods and database for surveillance on veterinary drug residues in major seafood commodities in ASEAN region

DESCRIPTION OF WORK:

Task 1: To identify critical drug groups and isotope labelled internal standards to be covered in this project
Task 2: To purchase reference standards and isotope labelled internal standards
Task 3: To develop generic HPLC methods
Task 4: To establish exact mass database with purchased reference standards
Task 5: To develop generic sample preparation methods

DELIVERABLES

<table>
<thead>
<tr>
<th>No</th>
<th>Description</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>An exact mass database for critical veterinary drugs</td>
<td>June 2016</td>
</tr>
<tr>
<td>2</td>
<td>Generic sample preparation methods for veterinary drug in seafood</td>
<td>Feb 2016</td>
</tr>
<tr>
<td>3</td>
<td>Generic HPLC methods for the targeted veterinary drugs</td>
<td>Feb 2016</td>
</tr>
</tbody>
</table>

EXPECTED RESULTS

1. A surveillance report on commonly detected veterinary drugs in major seafood commodities in ASEAN region, which can be used as reference to help the ASEAN member states to further enhance the laboratory testing capability and improve the aquaculture practices in ASEAN region
Project Title: Using Radionuclides to improve LC-MS/MS, GC-MS/MS and Liquid Scintillation Counter Analytical Methods for Monitoring of Aquaculture Production Sites and Produce.

<table>
<thead>
<tr>
<th><strong>Lead Partner</strong></th>
<th>Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Start month</strong></td>
<td>April 2015</td>
</tr>
<tr>
<td><strong>End month</strong></td>
<td>June 2016</td>
</tr>
<tr>
<td><strong>Interaction with other partners</strong></td>
<td>Department of Agriculture, Forestry and Fisheries (DAFF), Nuclear Energy Corporation of South Africa (NECSA); RIKILT-The Netherlands, UoC-Argentina, Rodrigo-Brazil, MINREST-Cameroon, UNBS-Uganda, CFIA- Canada, Bornova - Turkey</td>
</tr>
<tr>
<td><strong>Technical support required</strong></td>
<td>Procurement of stable isotope internal standards for mycotoxins and antibiotics, and RIA reagents.</td>
</tr>
</tbody>
</table>

**OBJECTIVES**

1. To develop a multi-antimicrobial LC-MS/MS method in different aquaculture products utilizing stable isotope-labelled internal standards.

2. Application of neutron activation analysis in heavy metal positive identification and confirmation in comparison to ICP-MS/MS.

**DESCRIPTION OF WORK:**

**Task 1:** Development of a multi-steroid extraction method in different aquaculture matrices.

**Task 2:** Selection of a multi-extraction method for residues (that may include macrolides, aminoglycosides, quinolones, sulphonamides, and tetracyclines) in different aquaculture matrices.

**Task 3:** Setting and optimization of chromatographic parameters for group separation of the different steroids.

**Task 4:** Setting and optimization of MS/MS parameters (ionization and fragmentation), individual groups initially and then combining them.

**Task 5:** Investigation and elimination of matrix effects in detection and quantification.
   (a) Method validation of the multi-steroid LC-MS/MS method.
   (b) Method validation of the multi-antimicrobial LC-MS/MS method.

**Task 6:**
   (a) Application of multi-steroids extraction method in LC- and GC-MS/MS analysis.
   (b) Optimization of chromatographic parameters.
   (c) Stable isotope internal standard application for quantitation using labelled steroid standards.
   (d) Method validation.

**DELIVERABLES**

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Multi-antibiotic extraction method</td>
<td>October 2015</td>
</tr>
<tr>
<td></td>
<td>(a) Steroids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) Antimicrobials</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(a) Validated multi-steroids LC-MS/MS method</td>
<td>March 2016</td>
</tr>
<tr>
<td></td>
<td>(b) Validated multi-antimicrobials LC-MS/MS method</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Validated multi-steroids GC-MS/MS method</td>
<td>April 2016</td>
</tr>
</tbody>
</table>
EXPECTED RESULTS


Project Title: “Detection of veterinary drugs and chemical residues in feed materials and aquaculture products using Radio-analytical and complementary techniques”

<table>
<thead>
<tr>
<th>Lead Partner</th>
<th>DMM, School Of Biotechnology, Madurai Kamaraj university, Madurai, INDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start month</td>
<td>July 2015</td>
</tr>
<tr>
<td>End month</td>
<td>June 2016</td>
</tr>
<tr>
<td>Interaction with other partners</td>
<td>AGES (Vienna, Austria) through IAEA</td>
</tr>
<tr>
<td>Technical support required</td>
<td>Specialized training regarding LC-MS and GC-MS usage</td>
</tr>
</tbody>
</table>

OBJECTIVE:

1. To determine and identify veterinary drug residues in aquaculture products
2. To identify heavy metal contamination in aquaculture products
3. To identify endosulfan and its toxic metabolites in aquaculture products
DESCRIPTION OF WORK:

<table>
<thead>
<tr>
<th>Task 1 (Year 1): Purchase of antibiotic standards chloramphenicol, tetracyclines, and β-lactam from SIGMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task 2: Improve the sensitivity of our current LC/UV method to make it suitable for use under current analytical requirements for chloramphenicol analysis.</td>
</tr>
<tr>
<td>Task 3: Select other antibiotics chloramphenicol, tetracyclines, and β-lactam that may be added to the scope of the improved method.</td>
</tr>
<tr>
<td>Task 4: Identification of specific marker in aquaculture product for chloramphenicol and development of radio-receptor assays using liquid scintillation counter for the radio labeled chloramphenicol</td>
</tr>
<tr>
<td>Task 5: Optimize and validate the scope-expanded for improved method</td>
</tr>
<tr>
<td>Task 6: Use the validated method for the analysis of at least 60 field samples to evaluate the applicability and suitability of the method for routine analysis.</td>
</tr>
</tbody>
</table>

Year 2
Task 1: Purchase of reference standards for arsenic, cadmium mercury and lead from SIGMA
Task 2: Optimize and validate our current method for heavy metal analysis.
Task 3: Apply the validated heavy metal analysis method for the analysis of at least 60 field samples

Year 3
Task 1: Purchase of reference standards for endosulfan and its metabolites from SIGMA
Task 2: Optimize and validate our current method for endosulfan and its metabolites.
Task 3: Apply the validated endosulfan method for the analysis of a selected number of at least 60 field samples
Task 4: Identification of specific marker in aquaculture product that interact with endosulfan and development of specific radio-receptor assays using liquid scintillation counter for the radio analyte, 14C-endosulfan
Task 5: Comparison and validation of developed radio-receptor method with other complementary analytical methods

DELIVERABLES

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Development of radio analytical and complementary methods/tools for screening, quantification and confirmation of Veterinary Drugs in aquaculture products, feed and water.</td>
<td>August 2015</td>
</tr>
</tbody>
</table>
Development of reliable methods/tools for screening, quantification and confirmation of agriculture chemical and heavy metals in aquaculture products, feed and water August 2016

Methods/tools for screening, quantification and confirmation of agriculture chemical in aquaculture endosulfan residues in feed and water and development of specific radio-receptor assays using liquid scintillation counter for the radio analyte, $^{14}$C-endosulfan August 2017

**EXPECTED RESULTS**

Approved protocols for multi-residue analysis for Vet. Drug, Agricultural chemicals and heavy metals and the spiking experimental protocols in aquaculture products, feed and water etc.,

Identification of impact of environmental influence on aquaculture farming

Implementation of new guidelines based on the level of residues observed as well developed methods.

Project Title: Development, validation and optimization of new cost-effective analytical techniques for residues of common pharmacologically active veterinary substances and related contaminants in aquaculture, and studying associated risk factors in Uganda

<table>
<thead>
<tr>
<th><strong>Lead Partner</strong></th>
<th>Uganda National Bureau of Standards, Kampala, Uganda</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Start month</strong></td>
<td>July, 2015</td>
</tr>
<tr>
<td><strong>End month</strong></td>
<td>June, 2016</td>
</tr>
<tr>
<td><strong>Interaction with other partners</strong></td>
<td>ILVO- Belgium; University of Cordoba Argentina, MINREST-Cameroon, OVI-South Africa, Bornova - Turkey.</td>
</tr>
</tbody>
</table>
### OBJECTIVES

1. Develop new user friendly sample preparation techniques for analysis of veterinary drugs residues for the selected aquaculture product samples

2. Develop, optimize and prepare protocols/standards operating procedures for new isotopic/nuclear analytical techniques/methods for determination of veterinary drugs residues in aquaculture products

3. To validate the developed methods

### DESCRIPTION OF WORK:

| Task 1: | Literature review of available multi-residue HPLC analytical methods for determination of antimicrobials residues (including selection of priority drug residues) |
| Task 2: | Make test runs with different selected protocols |
| Task 3: | Optimize equipment conditions to get best separations and runtimes for HPLC |
| Task 4: | Optimize equipment conditions to get best results for Charm II assays for beta-lactam, sulfonamides, tetracyclines, macrolides (including lincosamides) |
| Task 5: | Develop a standard operating procedure for HPLC analysis of antibiotic residues e.g. tetracyclines in aquaculture products |
| Task 6: | Perform validation of antimicrobial residue method with Charm II and HPLC |
| Task 7: | Participation in proficiency tests for antimicrobial residues |
**DELIVERABLES**

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard Operational Procedures for the detection of antimicrobial residues in fish by HPLC</td>
<td>November 2015</td>
</tr>
<tr>
<td>2</td>
<td>Standard Operational Procedures for the detection of antibiotic residues in fish by Charm II assays</td>
<td>December, 2015</td>
</tr>
<tr>
<td>3</td>
<td>Validation data for HPLC method and Charm II assays for the detection of antimicrobial residues in fish</td>
<td>June, 2016</td>
</tr>
</tbody>
</table>

**EXPECTED RESULTS**

1. Validated protocol for multi-residue analysis of antimicrobials using HPLC
2. Validated protocol for antimicrobial residue analysis using Charm II assays

Project Title: Training and Supporting of Radio-Analytical and Complementary Techniques to Control of Veterinary Drugs and Related Chemicals in Aquaculture

**Lead Partner**

Department Of Toxicology / Bornova Veterinary Control Institute

**Start month**

April 2015

**End month**

June 2016

**Interaction with other partners**

UNBS, Agri Food Vet

**Technical support required**

Training on improved communication of scientific work by the laboratory Staff; Input to facilitate the training on various contaminants e.g. on hormones analysis in food for capacity building in the laboratory.
OBJECTIVES

1. To produce, novel internal standards for tetracyclines and antihelmintics

2. To provide accreditation of all analysis which are performed by LC-MS-MS in the laboratory additional to accredited analysis.

3. To develop, provide validation and accreditation of new antimicrobial residue analysis additional to antibiotic analyses which are performed in the laboratory.

4. To provide accreditation of heavy metals analysis in foods.

5. To provide accreditation of dye substance analysis in foods.

6. To provide accreditation of hormones analysis in food. To provide training study for laboratory stuffs who responsible for hormone analysis in the laboratory.

7. To use of screening tests much more
DESCRIPTION OF WORK: Preparation

Task 1 To find a suitable institution to language training and supporting this training study financially

Task 2: To prepare/acquire internal standards (isotope labeled) of interest

Task 3: Identify the need for new analytical methods for antibiotics and other pharmaceutical compounds (analytes/matries).

Task 4. Implementing a selected method for the identified compound / matrix combination and preparation of a SOP

Task 5. Validation of the implemented method

Task 6: To apply for accreditation for analysis which are validation completed.

Task 7 Dissemination though publication of the new method

Subsequently similar tasks will be performed for other compound classes or matrices

To apply for accreditation for analysis of heavy metals analysis in foods.

To apply for accreditation for analysis of mycotoxin analysis in feed

Search for capacity building and development for training studies for persons who come from other institutions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Training English language identify need for new analytical method</td>
<td>June - September 2015</td>
</tr>
<tr>
<td>2</td>
<td>To begin method developments for new antibiotic analysis</td>
<td>June 2015</td>
</tr>
</tbody>
</table>
3  Implementation of new analytical method
   To contact institution interested in hormone analysis in food to take a training course for hormones analysis.  January 2016
   June 2015

4  To apply for accreditation for analysis for which validation is completed.  April 2016
   To begin method developments for pesticides analysis by LC-MS-MS

5  To beginning for new screening tests for residue analysis

5  Complete 1,2,3,4 and 5th of clauses

**EXPECTED RESULTS**

Laboratory staffs will be more efficient on international studies and affairs at end of the language training

Antibiotics, heavy metals and dye substance analysis will be accredited and it will help us for more reliable results.

New antibiotic residue analysis studies will be developed and accredited.

Project Title: Investigating agrochemical contamination of water sediments and fish in fish farms and study the depletion of antimicrobials in fish tissue in lab conditions using radiolabeled antibiotics as well related antimicrobial resistance.

<table>
<thead>
<tr>
<th>Lead Partner</th>
<th>Centro de Energia Nuclear na Agricultura CENA, Piracicaba SP, Brazil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start month</td>
<td>July 2015</td>
</tr>
<tr>
<td>End month</td>
<td>April 2016</td>
</tr>
</tbody>
</table>
### Interaction with other partners
- Center of Nuclear Energy in Agriculture (São Paulo-Brazil)
- Biological Institute of São Paulo (São Paulo-Brazil)
- APTA Vutuporanga (São Paulo-Brazil)
- Universidad Nacional del Altiplano (Puno-Peru)
- Proyecto Especial Lago Titicaca (Puno-Peru)
- Microbioticos – Brazil
- UoC - Argentina

### Technical support required
- Sampling protocol for sediments, fish and shrimp
- Specialized training in LC-MS/MS methods at Netherlands (RIKILT)

## OBJECTIVES

1. Visit to the main productions regions of fish and shrimp farms in Brazil and Peru for technical inspections and sampling;
2. Developing and validation of a multiresidue LC-MS/MS method for detection and quantification of antimicrobial residues in fish, shrimp, sediment and water samples;
3. Monitoring for antimicrobial residues in rainbow trout, water and sediment samples coming from the Titicaca Lake in Puno-Peru.

## DESCRIPTION OF WORK:

Task 1: Optimization and adjust of the method extraction of the antibiotics for the matrixes (sediments, water and fish). Development of a chromatographic method for the detection and quantification of antibiotic residues using an LC-MS/MS system.

Task 2: Validation of the analytical method (detection capability, specificity, recovery, robustness, ...).

Task 3: Visit Puno, Peru to sample rainbow trout, sediment and water at the fish farms by Lake Titicaca. Load water samples on SEPAK columns and transport to Brazil.

Task 4: Visit shrimp farms in Rio Grande do Norte, Brazil, to sample water, shrimp and sediment.

Task 5: Analysis of the samples of water, sediment and rainbow trout coming from Puno-Peru.
**DELIVERABLES**

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sampling protocol (s)</td>
<td>September 2015</td>
</tr>
<tr>
<td>2</td>
<td>Validated analysis protocol</td>
<td>February 2016</td>
</tr>
<tr>
<td>3</td>
<td>Sample analysis and generation of relevant data</td>
<td>April 2016</td>
</tr>
<tr>
<td>4</td>
<td>Project report</td>
<td>May 2016</td>
</tr>
</tbody>
</table>

**EXPECTED RESULTS**

Knowledge about the use of antimicrobials in fish and shrimp farming; Validated chromatographic method for the detection and quantification of residues of antimicrobials in sediments, water, rainbow trout and shrimp; Sampling scheme to collect and analyze sediments and shrimp from shrimp farms in Brazil, and sediments, water and rainbow trout from Puno-Peru. Report with a summary of the results.

Project Title: “Determination of Antibiotics in Ecuadorian aquaculture Products by LC-MS/MS; Implementing Methods and Monitoring”

<table>
<thead>
<tr>
<th>Lead Partner</th>
<th>Instituto Nacional de Pesca, Ecuador</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start month</td>
<td>June 2015</td>
</tr>
<tr>
<td>End month</td>
<td>December 2016</td>
</tr>
<tr>
<td>Interaction with other partners</td>
<td>RIKILT Wageningen UR (The Netherlands), LABSER (Chile), SAG (Chile) and FDA (U.S.), and other Research Institutions.</td>
</tr>
<tr>
<td>Technical support required</td>
<td>Development of Multi-residue Methods and Extraction techniques in marine products; Specialized training in LC-MS/MS and GC-MS/MS.</td>
</tr>
</tbody>
</table>
OBJECTIVES

1. Develop and validate new isotopic analytical method for the analysis of residues of pharmacologically active substances by LC-MS/MS
2. Participate in inter-laboratory/inter-institutional method development, validation and/or optimization
3. Participate in technical meeting and training programs
4. Dissemination of research work

DESCRIPTION OF WORK:

To reach the objectives described, the following tasks will be performed during the first contract period.

Task 1: Identify new methods needed for Ecuador's national residue monitoring program and select the most appropriate method by consulting research bibliography and the laboratory network on methods for analysis of multi-residues, e.g. sulfonamides, tetracyclines and quinolones by LC-MS/MS

Task 2: Implementation of the method in the laboratory of the National Fisheries Institute

Task 3: Optimization and validation of the method multi-residue and participation in PT’s when available.

Task 4: Surveillance study using new analytical method on selected aquaculture products

Task 5: Fully description of the analytical method in the SOP and preparation of the scientific publication (PM)

Task 7: Method extension to other analytes or further methods implementation

DELIVERABLES

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Selection of the methods</td>
<td>July 2015</td>
</tr>
<tr>
<td>2</td>
<td>Development and optimization of the selected methods (SOP)</td>
<td>February 2016</td>
</tr>
<tr>
<td>3</td>
<td>Validation of the method</td>
<td>May 2016</td>
</tr>
<tr>
<td>4</td>
<td>Implementation for surveillance (sample plan)</td>
<td>August 2016</td>
</tr>
<tr>
<td>5</td>
<td>Dissemination of research work (publication)</td>
<td>December 2016</td>
</tr>
<tr>
<td>6</td>
<td>Continuation with other methods</td>
<td>Advance to 2nd contract</td>
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EXPECTED RESULTS

<p>| | |</p>
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<tbody>
<tr>
<td>Application of developed, optimized and/or validated methods to</td>
<td>Application of developed, optimized and/or validated methods to</td>
</tr>
<tr>
<td>test residues of pharmacologically active substances.</td>
<td>test residues of pharmacologically active substances.</td>
</tr>
<tr>
<td>Surveillance results of residues of pharmacologically active</td>
<td>Surveillance results of residues of pharmacologically active</td>
</tr>
<tr>
<td>substances nationwide aquaculture products.</td>
<td>substances nationwide aquaculture products.</td>
</tr>
<tr>
<td>Scientific publication of research work</td>
<td>Scientific publication of research work</td>
</tr>
</tbody>
</table>
## LOGICAL FRAMEWORK MATRIX

<table>
<thead>
<tr>
<th>Project Design Elements</th>
<th>Verifiable Indicators</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall Objective:</strong></td>
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</tr>
<tr>
<td>To enhance national control programs for residues of veterinary pharmaceuticals and related chemicals in aquaculture products and feeds (including water) by helping food safety laboratories develop and or strengthen radio-analytical and complementary techniques.</td>
<td>Number of functional laboratories and residue monitoring programs</td>
<td>Reports by national or importing country food safety authorities; IAEA reports;</td>
<td>CRP conducted; suitable committed participants identified; findings transferred and applied; transparent reporting; system in place</td>
</tr>
<tr>
<td><strong>Specific Objectives:</strong></td>
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<td></td>
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</tr>
<tr>
<td>1. To develop a reliable, tailored monitoring strategy based on the use of validated tests including radio-receptor or radioimmunoassays for screening, group-specified and confirmation of residues and contaminants in aquaculture products and in water and feed used in aquaculture, to ensure consumer safety and strengthen Member State competitiveness in international food markets;</td>
<td>- Number of active laboratories testing contaminants in aquaculture and feed samples in support of national programs</td>
<td>1. National laboratory, project reports; transferable methods available to share</td>
<td>Suitable participants with realistic work plans identified; funds available and remitted in time; prompt procurements where applicable; ideas are feasible and where a technical contract is in place, immediate support.</td>
</tr>
<tr>
<td>2. To develop or optimize protocols (incorporating innovative sample preparation techniques) and instrumentation for radio-receptor or radioimmunoassays to limit costs, to prevent false positive test results and to improve the detection capability;</td>
<td>2. Kits developed or optimized</td>
<td>2. Project and/or laboratory reports</td>
<td>Suitable participants with realistic work plans identified; funds available and remitted in time; prompt procurements where applicable; ideas are feasible and where a technical contract is in place, immediate support.</td>
</tr>
<tr>
<td>3. To develop SOPs supported by validation dossiers;</td>
<td>3. Analytical methods developed and/or validated and applied</td>
<td>3. Project and/or laboratory reports</td>
<td>Suitable participants with realistic work plans identified; funds available and remitted in time; prompt procurements where applicable; ideas are feasible and where a technical contract is in place, immediate support.</td>
</tr>
<tr>
<td>To develop procedures for producing test materials (e.g. blank and incurred test material) vital for method validation and daily use;</td>
<td>4. Number of procedures developed and transferred for application;</td>
<td>Reports of developed and/or validated and applied methods</td>
<td>Suitable participants with realistic work plans identified; funds available and remitted in time; prompt procurements where applicable; ideas are feasible and where a technical contract is in place, immediate support.</td>
</tr>
<tr>
<td>To research and identify markers as indicators for the production conditions of aquaculture.</td>
<td>5. Methodologies in place and strategies established</td>
<td>5. Reports of developed and/or validated and applied methods</td>
<td>Suitable participants with realistic work plans identified; funds available and remitted in time; prompt procurements where applicable; ideas are feasible and where a technical contract is in place, immediate support.</td>
</tr>
<tr>
<td>Outputs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.a.i.1. Develop proposal; identify suitable researchers; harmonize workplan.</td>
<td>Hold Consultant’s Meeting (CM); announce project and review proposals; plans for hold 1st RCM</td>
<td>Meeting reports; reports of submitted and reviewed proposals; RCS records;</td>
<td>CM held and CRP announced as planned;</td>
</tr>
<tr>
<td>2. Project Management and Coordination Operational;</td>
<td>Concept was initiated, CM held, CRP proposal prepared and project announced</td>
<td>NACA records; meeting preparations</td>
<td>Funds available; CRP initiated as planned</td>
</tr>
<tr>
<td>3. Screening, quantitative and confirmatory techniques for monitoring and control of pharmacologically active compounds, mycotoxins and other relevant chemicals in aquaculture, feeds and water are developed, validated and applied;</td>
<td>Relevant methods (including validation data), produced and in use</td>
<td>Technical, RCM and laboratory reports; publications</td>
<td>Research implemented as planned; suitable CSI identified</td>
</tr>
<tr>
<td></td>
<td>4. Standard Operating Procedures, protocols and laboratory manuals of analytical methods for pharmacologically active compounds, mycotoxins and other relevant chemicals in aquaculture, feeds and water are developed and disseminated;</td>
<td>Protocols, standard operating procedures (SOPs), laboratory manuals produced and in use</td>
<td>Technical, RCM and laboratory reports; publications</td>
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<td></td>
<td>5. Enhanced laboratory competences according to international standards</td>
<td>Ability to meet ISO 17025:2005 requirements</td>
<td>Technical, RCM and laboratory reports; publications;</td>
</tr>
<tr>
<td></td>
<td>6. Information disseminated to Member State laboratories on optimum use of liquid scintillation counters for radio-receptor assays of food residues and contaminants</td>
<td>Relevant information gathered and in use</td>
<td>Technical, RCM and laboratory reports; publications;</td>
</tr>
<tr>
<td></td>
<td>7. Reports on markers/indicators along with optimized analytical methods produced</td>
<td>Quality data available and protocols, SOPs, laboratory manuals produced</td>
<td>Project reports</td>
</tr>
<tr>
<td></td>
<td>8. Protocols and data produced to support implementation of Codex guidelines and contribute to setting guidelines on respective contaminants</td>
<td>Codex recommended analytical methods in IAEA database; data shared with risk assessors (e.g. JECFA, JMPR)</td>
<td>IAEA/Nucleus reports; Codex meeting reports</td>
</tr>
<tr>
<td></td>
<td>9. CRP report including TECDOC or special issue publication produced</td>
<td>Quality data available and report(s) drafted</td>
<td>CRP review reports</td>
</tr>
</tbody>
</table>

### Outcomes:

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</thead>
<tbody>
<tr>
<td>1.</td>
<td>MS food safety laboratory capabilities established and/or strengthened, with analytical methods that meet national and international standards, are developed and operational in the monitoring of veterinary pharmaceuticals and related chemicals in aquaculture products and feeds;</td>
<td>1. Tangible institutional capacity e.g. ability to develop and apply analytical methods; train others;</td>
<td>1. Role of laboratory in residue monitoring appreciated and CRP implemented as planned;</td>
</tr>
</tbody>
</table>
2. Networking among food safety laboratories initiated or enhanced across regions;

2. Practical evidence of inter-laboratory work; exchange visits; sustained sharing of methods, resources and knowledge; co-authorships of publications

2. Laboratory, technical and/or scientific reports;

- Competent research group identified and there is willingness to collaborate;

3. Developed techniques disseminated widely e.g. through the IAEA TC program

Fellowships or Scientific Visits hosted under TC, bilateral or any other program

3. Laboratory and/or training reports

There is keenness to share and disseminate findings; availability of funds

3. Laboratory, technical and/or scientific reports;

- Competent research group identified and there is willingness to collaborate;

4. Improved understanding of markers/indicators of production systems associated with inputs for aquaculture production, and unintended contaminants

4. Number of methods to verify indicators; list of some markers or indicators identified; reference values established for each indicator or condition

4. Project and/or research reports; publications

4. Aquaculture products from polluted environments can be distinguished using isotopic or complementary methods; suitable CSIs identified

4. Project and/or research reports; publications

5. Adherence to international standards for trade in aquaculture promoted among states

5. More Member States applying international trade standards to food safety programs; more laboratories contributing to Codex meeting/session discussions

5. National and CODEX reports

5. CRP findings disseminated

**ACTIVITIES:**
(against each output)

<table>
<thead>
<tr>
<th>Project Design Elements</th>
<th>Verifiable Indicators</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Hold CM and develop proposal.</td>
<td>Meeting held 23-27 June 2014; proposal developed</td>
<td>CM report</td>
<td>Suitable experts identified;</td>
</tr>
<tr>
<td>1.2 Submit proposal to CCRA; Announce project</td>
<td>CCRA feedback obtained, proposals received</td>
<td>CCRA report</td>
<td>Proposal prepared and submitted</td>
</tr>
<tr>
<td>1.3 Invite applications for the participation in CRP; evaluate proposals</td>
<td>Evaluated research contracts and agreements forwarded for consideration</td>
<td>Contracts issued</td>
<td>Contracts issued in time</td>
</tr>
<tr>
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</tr>
<tr>
<td>2 Hold RCMs (1, 2, 3)</td>
<td>Meetings held and work plans prepared; projects begins and is progressing</td>
<td>RCM reports; progress reports</td>
<td>Contracts renewed, programs of work in place and implemented; meetings held as planned; funds available</td>
</tr>
<tr>
<td>3.1 Implement the development and validation of analytical methods for veterinary pharmaceuticals, mycotoxins and related chemicals in aquaculture products, feeds and water</td>
<td>Coordinate and review of project activities according to presented program of work; RCM held</td>
<td>Review and meeting reports</td>
<td>Meeting held; projects implemented according to work plan</td>
</tr>
<tr>
<td>3.2 Implement the application of analytical methods for veterinary pharmaceuticals, mycotoxins and related chemicals in aquaculture products, feeds and water</td>
<td>Coordinate and review project activities according to presented program of work;</td>
<td>Review reports</td>
<td>Projects implemented according to work plan</td>
</tr>
<tr>
<td>4. Implement the development of SOPs, protocols, manuals; Implement the dissemination of SOPs, protocols, manuals and transfer of developed techniques</td>
<td>Analytical methods and experiences documented; Inter-laboratory studies to test transferred methods; hosting of FEs or SVs</td>
<td>Laboratory reports; End of Mission reports; RCM reports</td>
<td>Expertise available and appropriate program of work in place</td>
</tr>
<tr>
<td>5. Enhance laboratory competences according to international standards e.g. through inter-laboratory studies, hosting of fellowships/scientific visits or fielding expert missions</td>
<td>Participation in inter-laboratory studies; Proficiency Test (PT) schemes; expert missions</td>
<td>Laboratory reports; End of Mission reports; RCM reports</td>
<td>Capabilities build and there is willingness to participate in PTs and share expertise</td>
</tr>
<tr>
<td>6. Dissemination of information on the use of liquid scintillation counters for radio-receptor (and related) assays in chemical and natural residue analysis</td>
<td>Number of reported instruments used in residue analysis, participation in inter-laboratory studies; PT schemes; expert missions</td>
<td>Mail communications; progress and lab reports; lab reports; End of Mission reports; RCM reports</td>
<td>CRP members capable of running radio-receptor assays using their own instrumentation</td>
</tr>
</tbody>
</table>
7. Conduct research to evaluate the presence of unexpected contaminants (e.g. pesticides, pharmaceuticals and personal care products) and biotoxins (e.g. mycotoxins and cyanotoxins) in aquaculture products, looking to associate contaminants with their source by isotopic ratio mass spectrometry (IRMS) and complementary techniques

<table>
<thead>
<tr>
<th>Project Design Elements</th>
<th>Verifiable Indicators</th>
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<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inputs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1. IAEA funding of CM; experts</td>
<td>CM conducted</td>
<td>CM report</td>
<td>Funds available and experts identified</td>
</tr>
<tr>
<td>1.2. Agency input: Project officer; NACA, CCRA input</td>
<td>Proposal submitted to CCRA</td>
<td>CCRA report</td>
<td>Proposal developed</td>
</tr>
<tr>
<td>1.3. Agency input: Contracts issued; MS applications</td>
<td>Quality proposals received and contracts issued</td>
<td>NACA records</td>
<td>Project applications received, reviewed, funds availed</td>
</tr>
<tr>
<td>2.0 Agency (RCM and workshop funds)</td>
<td>Coordination meeting and workshop planned</td>
<td>Agency records; Host Government Agreement received (as applicable)</td>
<td>Meeting scheduled</td>
</tr>
<tr>
<td>3.1. CSI resources; Agency funds (for contracts); Agency technical support</td>
<td>Committed CSI with necessary resources, working closely with Agency</td>
<td>NACA records</td>
<td>Suitable CSIs</td>
</tr>
</tbody>
</table>

8. Review harmonized protocols and upload them on IAEA database; Collate relevant data on contaminants and share findings with Codex Alimentarius risk assessors and managers

| Protocols and data on contaminants produced; information papers prepared for Codex meetings/sessions | IAEA/Nucleus reports; Codex meeting reports | Research conducted as planned and credible data collected |

9. Produce and publish CRP report (TECDOC or special issue)

<p>| Reports complied into TECDOC and/or peer reviewed publications | CRP/review reports | CRP completed as planned and quality data produced |</p>
<table>
<thead>
<tr>
<th>3.2. CSI resources; Contracts; Agency technical support</th>
<th>Committed CSI with necessary resources, working closely with Agency</th>
<th>NACA records; project reports</th>
<th>Suitable CSIs; resources available</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Agency support (may include TC program); CSI resources;</td>
<td>Transferable technology developed</td>
<td>Project records; TCP reports</td>
<td>Suitable CSIs; resources available</td>
</tr>
<tr>
<td>5. CSI and Agency input that may include TC program (support for Scientific visits/exchanges)</td>
<td>CRP participants planning to share experiences</td>
<td>Project records; TCP reports</td>
<td>Suitable CSIs; resources available</td>
</tr>
<tr>
<td>6. CSI resources and Agency input (technical contracts)</td>
<td>Functional technical contract and mechanism for transfer of technology/materials in place</td>
<td>Project records;</td>
<td>Suitable CSIs; resources available</td>
</tr>
<tr>
<td>7. CSI (infrastructural, human and financial) resources; and Agency resources (human and financial) to support development/validation and application of methods for non-targeted analysis</td>
<td>Reliable methods in place and being applied; data collection ongoing</td>
<td>Project and lab reports</td>
<td>Projects on selected compounds performed according to workplan; competent people CSI identified and relevant tools in place</td>
</tr>
<tr>
<td>8. CSI and Agency technical input to compile and disseminate protocols and exposure data; Agency (staff funding for Codex Meetings)</td>
<td>CRP implementation ongoing or conducted as planned; Relevant Codex meetings planned for</td>
<td>CRP and Codex meeting reports</td>
<td>CRP implemented and related outputs disseminated as planned</td>
</tr>
<tr>
<td>9. Agency input (including funds for Final RCM; a workshop) and CSI resources</td>
<td>Programs of work being implemented as planned and sound project reports submitted</td>
<td>Project/review reports; NACA records</td>
<td>CRP implemented as planned</td>
</tr>
</tbody>
</table>
### Annex A: Agenda

Provisional Program for the First Research Coordination Meeting (RCM) for the IAEA Coordinated Research Project (CRP): “Development and Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products” Vienna, Austria, 1 – 5 June 2015; Vienna International Centre, Room MOE03 (Building M, Room 03)

<table>
<thead>
<tr>
<th>June 1st</th>
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<tbody>
<tr>
<td><strong>Start</strong></td>
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<tr>
<td>08:20</td>
<td>09:10</td>
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<td><strong>Presentation</strong></td>
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<tr>
<td><strong>Presentations</strong></td>
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<td>14:00</td>
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<tr>
<td>Time</td>
<td>Session</td>
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<td>17:00</td>
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<tr>
<td><strong>June 2nd</strong></td>
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<tr>
<td>08:45</td>
<td>09:00</td>
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<tr>
<td><strong>Presentations</strong></td>
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<tr>
<td><strong>Presentation</strong></td>
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<tr>
<td>Time</td>
<td>Activity</td>
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<tr>
<td>15:15</td>
<td>All</td>
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<tr>
<td>17:00</td>
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</table>

**June 3rd**

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker/Activity</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:45</td>
<td>James and rapporteur</td>
<td>Summary of day 2</td>
</tr>
<tr>
<td>09:00</td>
<td>Mr. Joe Boison</td>
<td>Sensitive and Reproducible Analytical Techniques for Monitoring Veterinary Drug Residues and Related Contaminants in Foods</td>
</tr>
<tr>
<td>09:30</td>
<td>Mr. Rey Manuel Cambisaca Saquicela</td>
<td>Determination of Antibiotics in Ecuadorian Aquaculture Products by LC-MSMS; Implementing Methods and Monitoring</td>
</tr>
<tr>
<td>10:00</td>
<td>All</td>
<td>Break</td>
</tr>
<tr>
<td>10:30</td>
<td>Mr. Leen Van Ginkel</td>
<td>RIKILT’s Role in Research and Enhancing of Global Laboratory Networking for Food Safety</td>
</tr>
<tr>
<td>11:00</td>
<td>All</td>
<td>Group discussions (effective implementation of the CRP and establishment of collaboration); Prepare workplans (research contracts)</td>
</tr>
<tr>
<td>12:00</td>
<td>All</td>
<td>Lunch and Excursion - Austrian Agency for Health and Food Safety (<a href="http://www.ages.at/en/startseite/">AGES; http://www.ages.at/en/startseite/</a>): including presentations by the hosts</td>
</tr>
<tr>
<td>18:30</td>
<td>All</td>
<td>Joint dinner (Japanese restaurant)</td>
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</table>

**June 4th**

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker/Activity</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:45</td>
<td>James and rapporteur</td>
<td>Summary of day 3</td>
</tr>
<tr>
<td>09:00</td>
<td>All</td>
<td>Preparation of workplans continued</td>
</tr>
<tr>
<td>10:30</td>
<td>All</td>
<td>Break</td>
</tr>
<tr>
<td>11:00</td>
<td>All</td>
<td>Preparation of workplans continued</td>
</tr>
<tr>
<td>12:00</td>
<td>All</td>
<td>Lunch</td>
</tr>
<tr>
<td>14:00</td>
<td>All</td>
<td>Lunch</td>
</tr>
<tr>
<td>14:00</td>
<td>All</td>
<td>Presentation and critique of workplans</td>
</tr>
<tr>
<td>15:15</td>
<td>All</td>
<td>Break</td>
</tr>
<tr>
<td>15:45</td>
<td>All</td>
<td>Modify workplans (as necessary); draft final meeting report</td>
</tr>
</tbody>
</table>

**June 5th**
08:45 | 09:00 | James and rapporteur | Review of day 4

09:00 | 10:30 | All | Drafting of final report continued

10:30 | 11:00 | All | Break

11:00 | 12:30 | All | Finalize report including conclusions, recommendations, presentations, rapporteur’s notes and developed workplans

12:30 | 13:45 | All | Lunch

13:45 | 14:30 | Chair; James | Final remarks; end of meeting

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**Annex B: List of Participants**

<table>
<thead>
<tr>
<th>Title</th>
<th>Last Name</th>
<th>First Name</th>
<th>Country</th>
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