Asia Pesticide Residue Mitigation through the Promotion of Biopesticides and for Enhancement of Trade Opportunities

Synthesis of the Lab Training Workshop, 10-14 August 2020

The Lab Training on Good Laboratory Practices was conducted virtually from 10-14 August 2020 through BlueJeans virtual platform. It was organized through the Asia Pesticide Residue Mitigation Project (APRMP) by APAARI and IR4 project (Rutgers University). The project is supported by the Standards and Trade Development Facility (STDF)/World Trade Organization (WTO), the United States Department of Agriculture (USDA), and the Food and Agriculture Organization of the United Nations (FAO) and German Development Agency (GIZ) as knowledge partners. Sevently participants attended the training from the partner countries. The training aimed to improve participants's understanding of lab training practices, such as Good Laboratory Practices (GLP), following Standard Operating Procedures (SOP), maintaining proper lab records and data, handling and storing samples, use of equipment exclusively to analyse pesticide residues, and reporting the data in an approved format.

The workshop took place in a very interactive form. Participants were encouraged to ask their questions and clarifications in the lab methods/ equipment operations related to the project. They were subsequently addressed by Dr. Wayne Jiang, Associate Professor, IR4 project Michigan State University, USA, and Dr. Michael Braverman, IR4 Project, Rutgers University, USA, where were the resource persons for the training.

The virtual lab training began with the introduction from Dr. Wayne, whose activities in the project include laboratory training and conducting pesticide residue studies. He has previously worked on STDF projects in Africa, conducting efficacy studies and residue trials. The key players and funding agencies STDF, IR4, and USDA were acknowledged.

The first day of the training workshop presented an overview of the training, including Good Laboratory Practices (GLP), protocols (work plans), a set of Standard Operating Procedures (SOP), and individual working methods in the laboratory. The most important terminologies to be understood that are specific to the lab training included lab research director, quality assurance, study director, Food and Drug Administration (FDA). It also covered the discussion on lab assignments, lab needs, and communication between trainers and attendees. It further covered details on funding agencies, information on the testing facility, and an outline on terms related to GLP.

The key definitions discussed on the first day are as follows. The **testing facility** is an important terminology to be understood in the lab training. The standard definition for the testing facility as provided by FDA is "a testing facility is a person, who actually conducts a non-clinical laboratory study, i.e., actually using the test article in a test system". The Environmental Protection Agency (EPA) defines the testing facility as a person who actually conducts a study, i.e., actually uses the test substance in a test system. "Testing facility" encompasses only those operational units that are being or have been used to conduct studies. The definition for the same provided by the Organisation for Economic Co-operation and Development (OECD) is "the persons, premises, and operational units that are necessary for conducting the non-clinical health and environmental safety study". A **quality assurance unit** is a person or organizational element,

except the study director, designated by testing facility management to perform the duties relating to quality assurance of non-clinical laboratory studies. **Standard Operating Procedures (SOP)** are the documented procedures that describe how to perform tests or activities normally not specified in detail in study plans or test guidelines. The **Study Director (SD)** is the individual responsible for the overall conduct of a nonclinical laboratory study. Other definitions included study initiation date (the date the protocol is signed), experimental start date (the first date the test substance is applied to the system, experimental starting date (the date on which the first study-specific data are collected), study completion date (the date the final report is signed by SD), and experimental completion date (last date on which data are collected from the study).

Good Laboratory Practices (GLP) are regulations that are intended to ensure the quality and integrity of the data in a laboratory study. It aims in establishing procedures for planning, performance, monitoring, recording, and reporting laboratory studies. GLP is needed if the research focuses on developing agricultural pesticides, toxic chemicals, food controls/additives, and tests of substance with regard to explosive hazards. It should be noted that GLP is not required in case of basic scientific research, to develop new analytical methods and tests used to derive the specifications of a marketed food product.

Public agencies, such as FDA, EPA, and OECD, are responsible for reviewing the test results and determine if they demonstrate the product's safety and efficacy for commercialization. Only when the agencies are satisfied that safety and efficacy have been established adequately is the marketing of the product permitted. During the discussion, it was highlighted that the data will not be submitted to any regulatory agency other than using it internally. The equipment (LC, GC-MS, GC-MS/MS, GC-ECD, GC-FID) availability in different partnering countries, the chemical most suited to be used with that equipment were discussed.

Training on writing a protocol and making changes to the protocol was covered on the second day. SOP that would be followed in lab during the experiments was briefed and outlined on standard method practiced. This was followed by True or False and MCQ sessions to engage the participants. Information on calculation of the citation index and impact factor were hinted. The participants were advised to quote the funding agencies in all the scientific articles coming out of this project.=

The protocols and SOPs related to the GLP were discussed in detail. The protocol is a document which clearly indicates the objectives and methods for the conduct of the study. **Protocol should include** the following key aspects of the experimental study:

- Descriptive title and purpose of the study
- ID of test, control and reference substance
- Name and address of the sponsor and testing facility
- Appropriate dates
- Justification for selection of test system
- The number, body weight, sex, source of supply, species, strain, substrain and age of the test system
- Procedure for ID of the test system
- Description of the experimental design, including control of bias
- A description of the diet, including acceptable levels of contaminants, if applicable
- Route of administration and the reason for its choice

- Each dosage level in appropriate units and the method and frequency of administration
- Type and frequency of tests, analyses and measurements
- Records to be maintained
- Date of protocol approval by the sponsor and the dated signature of the study director
- Proposed statistics

Any changes or revisions made to the protocol, after the approval from the study director should be addressed and the reasons should be documented through proper channels. The amendment includes the intended change to the protocol after the study initiation date. Deviation represents the unintended departure from the protocol after the study initiation date. An example of the protocol used for IR-4 projects was discussed. It included the project number, objectives, study director's name, signature, date, initial, and the details of the funding agents.

SOPs prepared for the project should be approved by the management. SOP is considered as the integration between science and the data integrity that provides the instructions in the lab. The historical and updated new versions of the SOP should be immediately in the working lab. The SOP should be established for test system room preparation, test system care and include details on receipt, sample ID, storage, handling, mixing, and method of sampling of the test, control, and reference substances. The other important details covered in the SOP includes the following:

- Test system observations
- Laboratory or other tests
- Handling of test systems found moribund or dead during study
- Collection and ID of specimens
- Data handling, storage, and retrieval
- Maintenance and calibration of equipment
- Transfer, proper placement, and ID of test systems

Major components in SOP documents used in Dr, Wayne was discussed in detail. It included the author version, title of the project/work, subtitle and numbering, a footer containing SOP number and Page number, management's signature, and date. The study director (SD) plays an important in the successful execution of the project. SD is the single point of study control who has overall responsibility for the conduct, interpretation, analysis, documentation, and reporting of the study. SD must ensure the protocol is approved and followed, data are accurately recorded and verified, take responsibility, and sign the GLP compliance statement. Quality assurance unit (QAU) helps in assuring management that facilities, equipment, personnel, methods, practices, records, and controls comply with GLPs. The importance of publishing articles, impact factor calculation and acknowledging the funding agents were covered briefly.

There was a general query from the participants on conducting the experiments in an accredited laboratory for pesticide residue analysis and participant was doubtful that accredited lab result are more acceptable and reliable than other lab. Dr. Wayne addressed stating maintaining the proper record and following a good GLP practices and conducting experiments by sticking to SOP could highly increase the accuracy in any labs.

The most important aspect of the analysis which includes collection, handling, and storage of samples was covered during Day 3 of the training workshop. A detailed information on the handling on volatile samples/deteriorating samples were covered. The training has MCQ questions on sampling methods. Shipping of

the products and filling out the sample receipt were covered in detail providing information on the package material to be used and refrigeration medium to be used to keep the samples under controlled temperature. Importance of having a real time monitoring system for the low-temperature storage systems were covered. Low temperature grinding of the samples to prevent the loss of volatile material was provided. Different equipment available for the low temperature grinding and cryogenic grinding was provided.

Once the samples are received for the analysis, the sample condition should be checked upon the receipt, identified with shipping form/protocol, and logged in. Unique lab numbers should be cross-referenced to field sample numbers for easy tracking and identification. The residue sample shipping data sheet format used under the IR-4 project was shared and discussed. The form included information on test substance, crop, trial location, field research director, number of samples shipped, sample ID, the treatment used, date harvested, date sampled, lab ID, signature, and date of the approver.

It is important to note the condition of the sample while receiving and shipping. Based on the type of storage environment samples could be either kept frozen, thawed, stored in dry ice, or remains fresh or never frozen. The condition of the package in which the samples are kept should also be mentioned whether the sample bag intact or broken or open and content mixed. Some examples of the storage system used in Dr. Wayne's lab were shown that included standalone freezers for short-term storage, walk-in freezer for long-term storage with an alarm system and real-time monitoring to indicate the fluctuations in the system. Griding/milling the sample is crucial to get uniformity and ease in the analysis of residues. The training demonstrated the working of Hobart food chopper, Robot Coupe cryogenic mill, Wiley mill, and shredder to be used for frozen samples.

The reference substance used should be certified of quality for analytical reference standards. The certificate should contain details on storage conditions, purity, expiry date, and GLP compliance. The same should be re-certified for any extended expiry dates. For any standard solution calculations, the purity of the standard solution is taken into calculations to determine the pesticide residue concentration of unknown samples. The containers in which the reagents are used should be labeled correctly including chemical name, the solvent used, storage conditions, preparation, and expiration dates. Analytical in-life inspection forms that test the quality of chemicals, working conditions of the instruments, details of the personnel, and samples were shared with the participants.

There was a question on transporting samples under frozen condition. It was briefed that the sample can be shipped by packing it with wet ice (from field to lab) and use the dry ice for grinding purpose. The participants had questions on material in which specific samples should be stored, how to ship samples in containers, how to record the weight of the frozen sample, how long could the sample be stored under freezer before the analysis, and how long the prepared solvents could be stored in the laboratory. All these questions and clarifications were addressed by Dr. Wayne at the end of the presentation. The participants actively participated in online question and answer sessions by posting their answers in the chat box.

The fourth day of the training workshop covered the extraction and analysis of the samples. The session started with the True or False and Q&A session. The most important terms related to high-end instrument analysis were explained. Methods to detect the efficiency, performance of the instruments were covered. Development of methods specific to the instrument available in the institute was elaborated. Ideas on reference method, working method, making changes to the

available method were briefed. The participants were made aware on the importance of keeping track of the methodology (minor or major). Method validation, instrument performance analysis, sample analysis procedure, worklist, instrument usage data record, calculation sheet and lab records were advised to be used during the conduct of experiment. Data acceptance criteria was covered based on the above factors.

Once the sample is received in good condition, it should be extracted without losing the volatile substances and analyzed to determine the pesticide residue levels. The performance of the analytical instruments that are used for the quantification of residues is determined based on the Limit of Detection (LOD), Limit of Quantification (LOQ), and Lowest Level of Method Validation (LLMV). LOD is the smallest amount of the analyte that can be reliably detected from the background for a particular matrix. LOQ is the smallest amount of analyte that can be quantified with a certain degree of reliability. LLMV is the lowest fortification concentration level at which the method is validated for a particular matrix. Once the reference substances are obtained and properly labeled, the reference method adopted for the experiment is noted. In case of any deviation in the instrumental conditions, the changes/modifications made to the reference method should be well documented and updated. The exact procedure of sample analysis that is validated step-by-step should be documented. A copy of this updated document should be provided to the SD. This document should include details on the working procedure, abstract of the work, extraction procedure, differences from the reference methods, columns/mobile phases used in the case of chromatographic analysis. This would serve as a working method, which is needed to verify the validity of the method for different matrices. If this working method has been used successfully on the test matrix or a similar matrix, the SD may waive the requirement of method validation. Any minor modifications to the working method like changes in sample size, changes in extract volumes, modification of clean-up steps removal of clean-up steps, and optimization of instrument analysis parameters will be approved by the SD. On the other hand, changes in the extraction method or extraction solvent, changes in chemistry at major steps are considered as major changes in the experimental analysis. Once the method is developed, the validation results (in triplicates) should be updated to SD. Recovery results of 70-120% are accepted for method development. If the recovery is outside this range, approval from SD or additional method validation should be performed.

Accuracy of weighing is an important aspect to be noted in the chemical analysis and influences the accuracy of the experiment. The weighing balance should be annually calibrated and checked with standard weights. Frozen samples should not be thawed before weighing. The sample jars are well labeled and should use a double sample id before weighing.

An analytical set in the experiment should contain a minimum one control, one concurrent recovery spike, and field treated samples. A double injection is required for analyzing weathered samples, including control, concurrent spike, and field treated samples in the same analytical set. The double injection is not required for analytical standards, solvent blank, samples of the method validation set. A worklist with details on calibration standards, solvent blank, control sample, concurrent spike samples, field treated samples, solvent blank, and calibration standards should be maintained. Following these, examples of calculation sheet for determining the recovery percentage, LOD/LOQ, and the standard curve was discussed.

Participants sought clarifications on injection of samples in triplicate, use of untreated samples (which was mentioned as control samples in presentation).

There was a clarification on how much the regression coefficient for the standard curve developed to determine unknown concentration. It was addressed that it should be equal to 0.99 for better accuracy of results. The presentation had series of True or False questions from the presentation in which participants actively participated by answering in the chat box.

The last day of the training workshop focused on the importance of storing the data in digital and paper format for better tracking on the data. The session followed with the set of True or False and MCQ to assess the general idea participants had on the topic. Things that are considered as the raw data including photographs, media, recorded observations from instruments that are directly recorded were elaborated and emphasized on signing the document record. Dos and Don'ts of raw data recording, golden rules and important points to be noted in documentation were taught.

Raw data refers to any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. It may include photographs, microfilm or microfiche copies, computer printouts, magnetic media, including dictated observations, recorded data from automated instruments. All data generated during the conduct of a study, except those that are generated by automated data collection systems, shall be recorded directly, promptly, and legibly in ink. The data should be dated and signed by the person entering the data. Any change in entries shall be made so as not to obscure the original entry, shall indicate the reason for the change with date and sign at the time of change.

In an automated data collection system, the individual responsible for direct data input shall be identified at the time of data input. The raw data shall be sufficiently detailed, accurately recorded, and verified to allow reproducibility of experiments and results.

Rules for Good Documentation:

1) Make notes directly into the logbook or record
2) Affix "loose papers" into notebooks or binders
3) Label data with trial/sample information
4) Sign/date lab records daily as work progresses

Record keeping refers to anything related to the experimental study that should be documented. Both the used and unused data should be kept with the necessary information. In the absence of contemporaneous documentation, a warning letter could be issued by FDA, if the FDA does not have confidence that the final report can accurately and completely describe these operations more than 18 months after the study was conducted. The FDA could also issue a warning letter, if the SD has not noted unforeseen circumstances or deviations that may affect the quality and integrity of nonclinical studies when they occurred and failed to document what corrective actions, if any, were taken at that time. In several cases, deviations that occurred should be noted six months to more than one year later.

All raw data, documentation, records, protocols, specimens, and final reports shall be retained in the archives. It should be securely stored in an orderly manner for easy access. An individual should be identified as responsible for the archives. The documents could be retained based on regulations. Master schedule, copies of protocols, records of QA inspections, training records, equipment records need to be retained for the retention period.

The report submitted as a part of the project should have a cover page, GLP compliance statement signed and dated by the lab research director and analysts, signed and dated QA statement, lab personnel, table of contents, location of raw data, analytical reference substance and summary of the project.

One of the participants had a question on reporting the data with number of significant figures, which was addressed by Dr. Wayne. The participants showed their gratitude to Dr. Wayne for his wonderful presentations and technical knowledge shared. They also thanked the funding agents and APAARI for their support by the end of the lab training.

The workshop attracted 71 participants from the partner countries. The participants engaged in Q&A session to clarify on the difference in methodologies adopted in different countries and regions. Questions asked during each day of training were answered in the chat box by the participants and the right answers were addressed towards the end of the training sessions. Upon successful completion of the training session, the participants were awarded with the certificates by APAARI team. The certificates were also emailed to the participants.

Attachment 1: List of participants in the virtual lab training

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Attachment 2: Lab training evaluation

Twentyeight participants (out of 71) responded to the evaluation survey that was sent to them after the training. The survey outcomes are as follows:

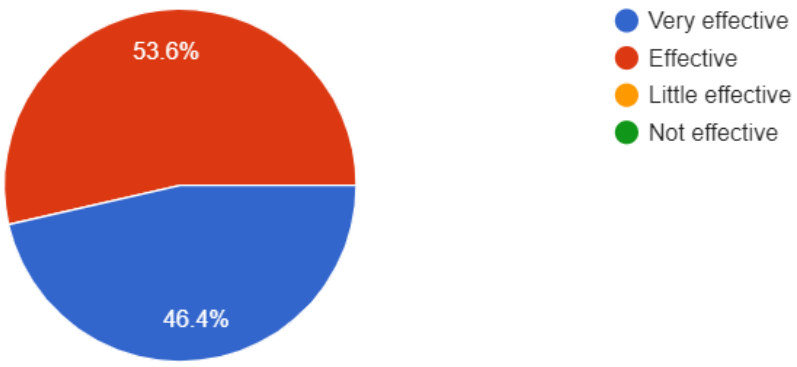
1. **How would you rate the usefulness or quality of this workshop in terms of the following training contents?**

Total respondents - 28				
	Excellent	**Good**	**Average**	**Weak**
Good Laboratory Practices (GLP), General Information, definitions	24	4		
Standard Operating Procedures (SOP)	20	8		
Amendments and Deviations	14	13	1	
QA inspection checklist – facility inspection	18	10		
Sample handling				
Sample handling, processing, and short-term and long-term storage	19	9		
Reference substances	15	13		
Sample extraction	12	15	1	
Instrument analysis	13	14	1	
Raw data and records, and quality control	16	11	1	
QA inspection checklist raw data and ASR inspection				
Electronic data	14	14		
Reporting	14	14		
Archives	10	18		

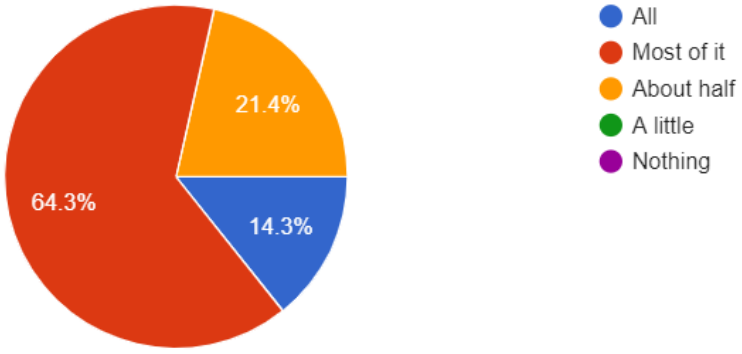
2. **How would you rate the following processes and logistical matters of the training?**

	Excellent	**Good**	**Average**	**Weak**
Discussion	17	11		
Agenda and flow	13	15		
Facilitation and feedback	20	7	1	
Online facilities	13	14		1
Training organization	19	8	1	
Pre-training communication	12	15	1	

3. **How effective was the online interaction in this training?**



4. **How much of what you learned (new knowledge and skills) are you planning to use in your work?**



5. **What are the key abilities (knowledge and/or skills) that you acquired in this training that will enable you to work more effectively?**

- Protocols and equipment standard operating proceedures (SOPs) (5 respondents)
- Sample handling (4 respondents)
- Pesticide spraying in the fields (4 respondents)
- Storage protocols and conditions (3 respondents)
- Laboratory testing (3 respondents)
- Data management (3 respondents)
- GLPs in pesticide residue analysis (3 respondents)
- Sequence of procedure in GLP activities (2 respondents)
- Pesticide residue analysis (2 respondents)
- Effective communication
- Understanding of Functional Capacity
- Concept on trial design for MRL development
- Pesticide mitigation by selection of pesticides with same efficacy with shorter PHI
- Extraction procedure
- Labelling
- Report requirement
- importance of documentation in GLP
- Reference method
- Working method
- Sample shipping
- Sample check
- Sample extraction
- Lab management

Furthermore, the respondents provided the following feedback:

- "It helps me control the error in each stage"
- "It helped me take GLP seriously. It is often ignored or compromised. This training has further emphasized the importance of GLPs."
- "All. Because this is my field."
- "Almost all we learned from this program on laboratory training could be applied for our laboratory such GLP."
- "Planning and designing the study under Indonesian condition, coordinating with all stakeholders participating in this work, train the field study member from practicing to reporting start from spraying up to sending the samples to the lab, archiving the lab data and reporting"

6. **What functional training can be integrated in these proposed technical topics (if any)?**

    - Quality management (control and assurance from sampling up to data generation) (5 respondents)
    - Safety measures handling pesticides in both laboratory and field level (2 respondents)
    - Pesticide laboratory
    - Social interaction
    - Measurement of uncertainity
    - Lab information management system
    - Capacity to navigate complexity
    - Capacity for reflection and learning
    - Collaboration
    - Demonstration of different chromatographic instruments
    - Pesticide application
    - International harmonization of pesticide residues
    - Data recording and maintenance
    - Pesticide calculate for spray in the fields
    - Field applications for pre-harvest interval trails
    - Extraction, specially matrix calibration
    - Method validation and estimation of the uncertainty of measurement
    - Details on the actual supervised pesticide residue trial
    - Food safety with lab management

Furthermore, the following comments were provided by the respondents:

- "If there are more videos in the seminars, it will help visualize and focus more"
- "More real examples should be provided"

7. **What other technical issues would you like to learn about more in future project webinars?**

    - Sample extraction and clean up processes for GC, GC/MS, LC, LC/MS (3 respondents)
    - Calculation of the pesticides used to spray in the fields (2 respondents)
    - Method validation (2 respondents)
    - Analytical instrumentation (2 respondents)
    - Any other topic covering GLP
    - Instrument trouble shootings
    - Sample spiking, recoveries, equipment standardization
    - Lab and field workflow – practical and hands on training on field trials and smart ways for data retrieval and results presentations
    - Software like quant
    - Even more about the lab results analysis and reporting
    - Discuss the analysis of highly polar pesticides, as well as how to preserve them
    - The dose of pesticide apply in the fields
    - Pesticide and chemical compatibility
    - Method of analysis of residues from herbicides and insecticides
    - Optimization
    - Estimation of the uncertainty of measurement
    - Lab ISO and quality control of food safety
    - How to estimate MRL from residue data
    - Determination of residues of bismerthiazol, petroleum spray oil

Furthermore, the participants also commented s follows:

- "Include more practical aspects in the training"
- "Please arrange several webinars for compatible time periods to several countries. India, Sri Lanka, Bangladesh that can take place at the same time. Otherwise we missed most important parts because we can not stay at office at night."

8. What are you planning to change/improve after this training?

- "Process in field and lab"
- "Use the lesson learn in this training to improve my work"
- "Being a social scientist, I will extend support to our laboratory staff to enhance their functional capacity to make working arrangements in field more effectively."
- "Sample handling"
- "Labs procedures, SOPs, Documentation, Sample handling and storage"
- "Archiving the information, SOPs modifications."
- "Improve analytical analysis skill"
- "Better compliance with SOPs"
- "All the lab & filed training to be applied for MRL development"
- "I think that's fine"
- "Sample collection and preservation"
- "Calculated the dose of pesticide spray in the fields"
- "Record keeping"
- "I have more improve my skill and knowledge about analysis an pesticide residue and field trial"
- "To consider GLP requirements in setting up a residue laboratory; adopt GLP in current laboratory quality management system"
- "Strengthening SOP's and GLP's in the laboratory and keeping well maintained records."
- "Take the lesson learn to do practise in the fields"
- "The planning to improve after this training such as standard operating procedures and reference method"
- "Please arrange several webinars for compatible time periods to several countries. India, Sri Lanka, Bangladesh we can have same time. Other wise we missed most important parts because we can not stay at office at night. Please arrange video programmes on field, also we want more on metabolic pathways."
- "SOP development , QM, SM and ISO17025"
- "What should be done in Food Safety, especially in identification of critical control points and its management."
- "Reporting of test results"
- "Incorporate principles of GLP in our lab works"
- "Get more knowledge about lab management"
- "Apply the knowledge/skill to improve quality of efficacy trials, PHI field trials and establish residue data on new compounds/crops of specialty to set up IT/MRL"
- "Compilate SOP, manage data of the lab"

9. What other capacity gaps on this recently trained topic are you facing that you would like to learn more about in future training?

- Analytical instrumentation (2 respondents)
- Goal of work
- Pesticide spraying caculate
- Functional capacity
- Auditing skills, report writing, equipment standardisation
- More focus on group discussion for compilation report on GLP study
- Concept on how residue data can be used for MRL development under local conditions

- Working on GC-MS/MS triple quad
- MRL development
- Equipment manufacturers are increasingly modern and with lower detection limits to suit demanding markets, but our purchasing power is limited.
- I would like to learn more about field based trainings
- Laboratory testing
- Sample extraction and derivatization (2 respondents)
- Guidelines on GLP in residue analysis (including resources & infrastructure requirements)
- Manufacturing mass production techniques
- Protocol, calibrating balances, calibration pipette
- Metabolic pathways, specially metabolism inside plants
- Clean up method
- Actual and basic GLP
- More detail on lab training under GLP standards

Two respondents also commented as follows:

- "Since this is a multi component study, is it possible to have training on determining variables that significantly influence the data quality and how to obtain optimum condition to generate the best valid data?"
- "Prepare group discussion"

10. Do you have other comments how we can improve the project's future trainings and/or interactions?

- "Order of data record"
- "The training is very fully to the new lesson for me"
- "Add some social quizzes to make it more interactive"
- "There is need to arrange on hand training in the GLP certified labs"
- "Group discussion on trial designs and pesticides mitigation through selection of the pesticides with shorter PHI, ways of knowledge dissipation to farmers and field workers"
- "Of course instead of online, by actually watching/observing things will be far better."
- "I think that's fine"
- "Although virtual trainings have certain drawbacks, overall training was very effective and informative."
- "This training are very useful interesting"
- "Make more practical oriented rather than theoretical"
- "Outlines of topics under discussion if possible be shared before webinar or the agenda so that the viewer can study topics a few days earlier. this can help in the discussion section of webinars."
- "The training its good for me for do practice"
- "Please arrange several webinars compatible with time"
- "More webinars related to this for more familiarization of details"
- "Pesticides residue and biotechnology"
- "How to estimate MRL/IT from a real residue data"
- "Improve laboratory management capacity"