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CONCEPT NOTE

Collection of Data on Pesticides in Maize and Tomato in Africa: Protocol for Africa Pesticide Residue Survey Study

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Abstract
Pesticide use has grown rapidly in West Africa over the past decades. Regulatory capacity has not kept pace with the rapid proliferation of pesticide products and on-farm use. As a result, health and environmental impacts from the growing use of pesticides, despite their potential importance to food safety, remain largely unmonitored, underreported, and poorly understood by key stakeholders. This study protocol was the document for conducting a pesticide survey study to identify the most critically emerging pesticides across the Continent of Africa. Multiple countries were selected in this study to represent the north, east, south, and west regions of Africa. Two food commodities, maize and tomato, were chosen to monitor the pesticide level for food safety. This study protocol describes the fieldwork and laboratory work per the standards of Good Laboratory Practices (GLP) and ISO-17025 and US EPA 860 Residue Chemistry Guidelines but the survey study performed was not considered as a GLP or ISO 17025 study. This is because many steps were not able to be closely monitored per the GLP requirements. This protocol describes the requirements for a pesticide residue study in food collected from local markets. This protocol describes the test commodities, sampling methods, sample transfer/shipping, storage stability, sample analysis, sample disposal, and documentation and record keeping.

Keywords Maximum residue limit · MRL · Food Safety · Pesticide Regulation · Africa · Tomato · Maize · Protocol

Introduction

In Africa where traditional agriculture is widely practiced, pesticides pose serious health hazards and environmental contamination, due to the increasing pesticide use in agricultural production and limited capacity for pesticide residue testing and monitoring (Fuhrimann et al. 2022; Isgren & Andersson 2020). It was reported illegal marketing of pesticides, and poor user applications of pesticide products in Africa (Haggblade et al. 2019). It is generally assumed, but not well described, that pesticide residues on food crops consumed at the local level are a serious cause for concern, and intervention, by international aid organizations. Additionally, it has been difficult to target interventions without understanding if presumed residues are actually exceeding legal maximum residue limits (MRLs), if they are the result of illegal pesticide use (knowingly or unknowingly by farmers), if they are the result of poor product quality or counterfeit products, or if other factors are involved, such as poor efficacy testing and label establishment during the registration of the products. GLP is generally a guideline or a requirement for pesticide residue studies and MRL. Some laboratories were ISO-17025 accredited. Therefore, this multi-country study suggested using the GLP and ISO standards but GLP and ISO were not "a must" depending

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on the laboratory conditions. This project was defined as a "GLP-like" or "ISO-like" study.

Pesticide regulation at national and regional levels in Africa is important to regulate sales of pesticides, ensure consumer protection, and facilitate imports/exports of food crops. With the increasing demand for food, many African countries and regional associations have begun a period of extremely rapid pesticide market growth. Africa is quickly developing a dependency on agricultural pesticides (FAOSTAT 2019). Recently, studies of fraudulent pesticides in Africa suggested that the use of illegal pesticides may lead to significant health concerns due to severe environmental contamination and pesticide residues in food crops (Haggblade et al. 2019, 2021, Theriault et al. 2020). A more recent study assessed concentrations of pesticide residues in fruits and vegetables in Uganda and observed multiple pesticide residues occurred in commonly consumed fruit and vegetables (Ssemugabo et al. 2022). The USDA Interregional Research Project Number 4 (also known as IR-4 Project or IR-4) was established in 1963. Since then, IR-4 has been providing data for pesticide tolerance establishment in the US (IR-4, 2022). IR-4 develops residue and efficacy data for the registration of safe and effective pest management solutions with the U.S. Environmental Protection Agency (EPA). IR-4 also supports the pesticide registration of specialty uses on major crops, such as corn, soybeans, cotton, and etc. (IR-4, 2022). Therefore, in order to help direct future capacity efforts in Africa, it is important to better understand to the best of our ability the actual residues found on locally consumed food crops (type of pesticide, amount, frequency, variability, etc.) and best trace these residues back to the potential sources or causes (is it a regulatory issue, farmer issue, poor product quality issue, etc.?). This project will not answer all these questions at once, but the researchers will work to provide a baseline of data, develop research teams in each African region that can increase surveillance work in the future, and point us in the right direction of the sources of the most hazardous pesticides for future capacity building efforts to mitigate residues on food crops.

Approach

The study protocol aimed not only to bolster ongoing and future pesticide capacity programs but also to identify residue levels of pesticides in locally-consumed foods across the continent. This attempted to generally trace the source of concerning residues back to the causes and sources, as best as possible within the time constraints of a survey study.

The primary objectives of this study included (1) carrying out a pesticide residue survey study to establish an entry baseline of pesticide levels in food commodities in four regions of Africa, (2) tracing back the cause or sources of residues found on foods, providing information on areas for future intervention to mitigate these residues under future capacity efforts, and (3) strengthening regional networks to support the establishment of regulatory systems for pesticide MRL and to promote pesticide regulatory cooperation and harmonization.

The approach was to start with training and coaching African research partners, reviewing past research and literature, guiding partners to conduct produce sampling and residue testing, and analyzing results to best determine the cause of the residues. This selection pesticide analytical laboratory was built based on recent visits and on-site assessments by the study team, for example, the pesticide testing laboratories in West Africa inspected by Jiang & Haggblade (2019) and Dubois (2019), through which the study team was able to establish scientific collaboration with key scientists working on solving these issues. The experts of the study team visited these laboratories and provided GLP or ISO 17025 training. Prior to the study, each on-ground team was trained for both field sampling and GLP laboratory analysis through one-on-one training between March 2021 and June 2021 (Lehotay & Cook 2015; Jiang et al. 2004). In March 2021, Lehotay provided training for the study teams on the laboratory QuEChERS method (Yu et al. 2020) and more advanced QuEChERSER technique (Lehotay 2006). In each analytical laboratory, method validation was performed to ensure the fortification recoveries were in the acceptable range between 70% and 120%, followed by sample analysis (Jiang et al. 2004). If any analyte was out of the acceptable range, a second validated method was used, for example, from GC-MS/MS to LC-MS/MS. Only valid data were reported.

The study protocol consists of nine sections: (1) Project title, (2) Justification and objectives, (3) Sponsor, (4) Study director, (5) Proposed dates, (6) Guidelines and reference materials, (7) Test system design, (8) Documentation and record keeping, and (9) Personnel and testing facility. The complete study protocol is given in the Supplementary Materials.

Key Components of Study Protocol

Section 1. Project title: This is a concise phrase stating the keywords or phrases summarizing the study. The project title of this study was "Africa Pesticide Residue Survey".

Section 2. Justification and objectives: This section is 1–2 brief and short paragraphs describing the background and objectives of the study. For example, this project was sponsored by the USDA Foreign Agricultural Service (FAS) and U.S. Agency for International Development (USAID)

Table 1 Proposed dates of the survey study

Date	Meaning
Study initiation date	the date the protocol is signed by the study director.
Experimental start date	the first date the test substance is applied to the test system
Experimental termination date	the last date on which data are collected directly from study
Study completion date	the date the final report is signed by the study director

Reference: EPA GLP. 40 CFR 160. Good Laboratory Practice standards (EPA 1989)

for food safety and capacity building in Africa. Therefore, this section of this survey study was an attempt to identify residue levels of pesticides in locally-consumed foods across the African Continent during the COVID-19 pandemic time, to trace the sources of pesticides, and to identify the cause of residues of concern, as best as possible within the time constraints of this project. COVID-19 may have an impact on the study results. In this paragraph, all of the objectives must be listed.

Section 3. Sponsor: The sponsor is the identity who initiates and supports this study. The sponsor was "USDA Foreign Agricultural Service".

Section 4. Study Director (SD): The study director is the single point of control (Randolph et al. 2014). The role of the study director is to take proactive steps to assure that all applicable regulations. If the SD is not available, it would be preferable to assign an individual for immediate assistance and general guidance. This study director of this study was "Wayne Jiang, Michigan State University" and the second contact was "Jason Sandahl, Minor Use Foundation".

Section 5. Proposed dates of project: The definitions of study initiation date, experimental start date, experimental termination date, and study completion date are given in Table 1. This project was a study on local market samples. Pesticide application dates were not available. Therefore, in this study, study initiation date, experimental start date, experimental termination date, and study completion date were set to be 24-May-2021, 24-May-2021, 30-Sep-2021, and 31-Dec-2021, respectively.

Section 6. Guidelines and reference materials: This project shall collect local market samples which may contain various levels of pesticides. However, the application dates were not available. The reference materials refer to the analytical standards used in the analytical laboratories for fortification and quantitation. To determine the levels of pesticide residues, this protocol suggests using the following procedures and standards as study guidelines: Good Laboratory Practices or GLP (EPA 1989; OECD 1992), ISO/IEC 17025, Residue Chemistry Test Guidelines (EPA Methods 860), appropriate protocol and laboratory standard operating procedures (SOP). Reference materials must be valid and obtained from verified vendors with certified purities of the standard pesticides.

Section 7. Test system design: In this protocol, this section includes 7 sub-sections.

(1) Food Crops The study protocol shall specify the targeted food crops, variety, quantity, market location as well as other specific requirements. Two food crops were selected in this study, one was maize and the other was tomato. Maize, called corn in the United States, is a staple food throughout Africa, making up the dominant part of a population's diet worldwide (Trinidad-Calderon et al. 2021). Tomato is a nutritious food rich in antioxidant compounds which play an important role in human health (Collins et al. 2022). A minimum of fifty (50) fresh tomato and fifty (50) maize samples were collected from the markets. Use commercial varieties. Report variety, weight, price, sampling date, GPS location of sampling sites, and photos of samples and markets. Other descriptive information such as source, lot number, storage, etc., was recorded, if available. Organic tomato and maize can be purchased as untreated (control) samples.

(2) Sampling *: Appropriate sampling methodology was a critical part of pesticide studies (Ambrus & Soboleva 2004; Benzing et al. 2021; Codex 1999). The study flow chart is summarized in Fig. 1. The sampling requirements shall be on market selection, chain-of-custody, random sampling, size and timing, and shipping. Sampling was performed depending on local consumption (most samples were collected in more population cities/towns/villages), Fig. 1. Chain-of-Custody forms should be well maintained. Random sampling was preferred for better for monitoring, collecting representative samples, capturing the breadth of residue levels and population, and truly reflecting the exposure to pesticide residues. If permitted, it would be good to collect more information, such as taking photos of the markets and stores, having interviews with vendors, as well as accurately recording appropriate GPS coordinates of samples. Sample size and timing were also important. For fresh tomato, 2.5-3 kg composed of a minimum of 12 tomatoes per sample shall be collected. For maize, 1.5-2 kg of either milled maize flour or whole grain (kernel) shall be sampled. Do not composite samples.

* Each laboratory had one-on-one training on sampling (market selection, chain-of-custody, random sampling, size and timing) prior to the sample collection. For Francophone countries, the training was performed in French.

(3) Sample shipping *: The protocol shall specify the shipping requirements. Samples were shipped or transported

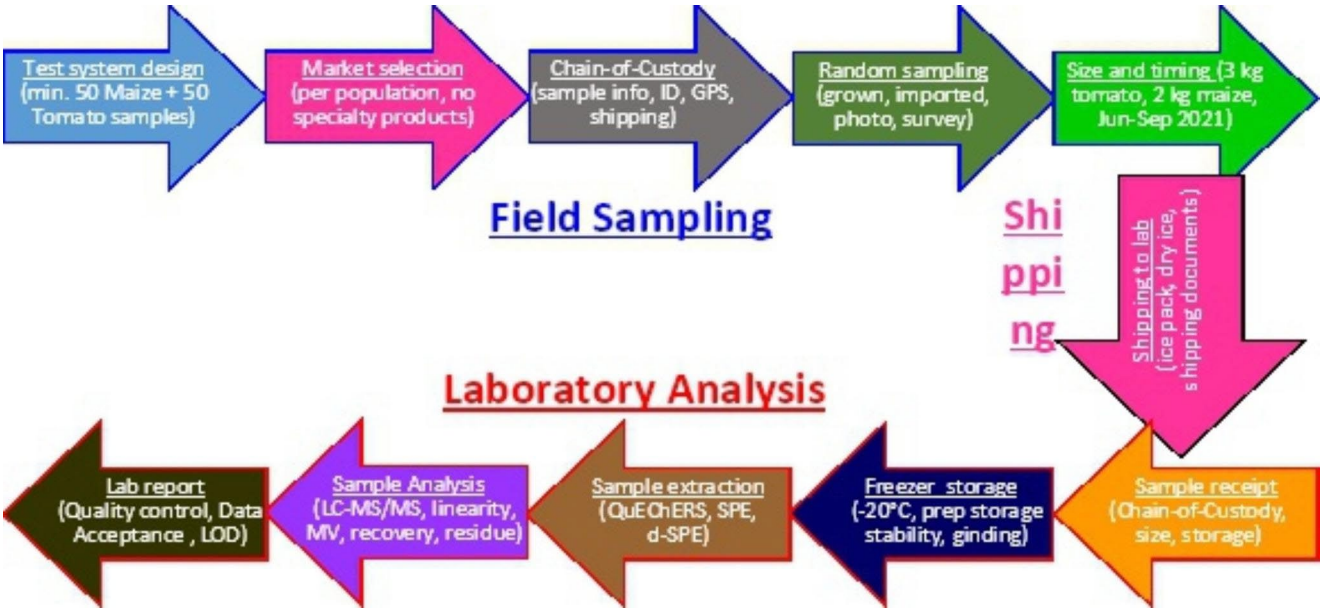

Fig. 1 Study flowchart

to the analytical laboratories in a timely manner. General requirements were to use double sample bags secured with a tape or tie, to insert chain-of-custody forms between bag layers, to send samples to the lab in coolers with ice packs.

(4) Storage Stability Samples The residue behavior in food crops generally follows the first-order degradation kinetics during storage at room temperature (Lu et al. 2014; Zikankuba et al. 2019). The dissipation of pesticides is temperature dependent, i.e., a slower degradation at a lower temperature such as under freezing conditions. This protocol required that as soon as possible after receipt of samples, a minimum of six ground subsamples of the crop shall be fortified with a fortification standard at 0.1 mg/kg (each of the pesticides) for controls. If analysis of treated/control samples was completed within 30 days of sample collection, analysis of storage fortification samples was not required. The study director may use the recoveries of the storage stability spikes to determine whether the field samples were valid if an unexpected incident (such as freezer failure) had happened in the testing facility.

(5) Sample analysis **: A Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach was used for determining pesticide residues (Lehotay 2006; Yu et al. 2020). An alternative method was the AOAC Official Method 2007.01. Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry (Lehotay 2006). The analytical reference substances must be obtained from Thermo Fisher Scientific, Sigma-Aldrich, Dr. Ehrenstorfer, or other reputable sources with ISO 17025 accreditation. Depending upon the availability of grinding equipment, all samples shall be processed and homogenized using Robot Coups, Hobart Food Choppers, Stephan grinders, or other proper grinder equipment with dry ice or liquid nitrogen. Ground samples shall be stored in freezers at -20 °C before analysis.

Method validation (MV) was required by the protocol (Codex 2019). The steps were as follows. Frozen samples were weighed out and extracted with acetonitrile per the QuEChERS methods. The appropriate cleanup methods (such as SPE and d-SPE) were optional depending on the samples. Spike samples of each MV level were analyzed in triplicates and acceptable spike recovery ranges were 70 – 120%. The analytical sequences should contain a control, field samples, spike sample(s), blank, and calibration standards (5 or more level points of calibrations) beginning with a calibration standard and ending with a calibration standard. The linearity requirement was $R^2 \geq 0.985$.

** Each laboratory had training on sample processing, extraction, and analysis prior to the sample analyses. For Francophone countries, the training was performed in French.

(6) Quality Control Each laboratory participating in this pesticide survey study shall have its own quality system. Besides the routine activities and quality control procedures, the laboratories must follow the lab SOP, and the requirements for data quality in this study must be met (Table 2).

Table 2 Data acceptance criteria

Sample/Parameter	Requirement
Fortification Sample	
Method validation fortification recovery	70 – 120%
Concurrent spike fortification recovery	70 – 120%
Storage stability fortification recovery	70 – 120%
Calibration	
Linearity (coefficient of determination)	$R^2 \geq 0.985$
Number of calibration points	5 or more
Weight of 1/X	optional
Matrix-matching standards	optional
Days	
From sample extraction to analysis	≤ 14 days
If analysis completed in 30 days of collection	Analysis of storage stability samples (SS) is not required
If analysis not completed within in 30 days	Analysis of SS is required
If analysis not completed within in 30 days	Days of SS must be longer than days of sample storage
Multiple analysis	
Calibration standards	Beginning and ending with an analytical set
MV samples, SS samples	Multiple analysis is optional
Market samples	Double injections or multiple analysis is required
If multiple analysis	Difference between multiple analyses ≤ 15%
If multiple analysis	Average all valid results
If contamination in control sample is found	
Residue in control	< LOD
Freezer storage	
Freezer temperature	– 20 °C
Keep field samples in freezer	min 1 year (after submission of lab report)
Keep storage stability samples in freezer	min. 2 years (preferred 5 years) after submission of final report

Contact the Study Director if the data obtained are out of the acceptable ranges.

(7) Sample Disposal What samples are required to be retained and how long is the freezer storage? Per US EPA, "samples … need be retained only as long as the quality of the preparation affords evaluation" (EPA Interpretation 2017). In this study, the study director decided that a minimum of 100 g or all (if less than 100 g) of each of the remaining frozen treated and untreated crop samples shall be retained for at least 12 months after submission of the laboratory report. Long term fortified storage study samples shall be retained for a period of 2 years and, as appropriate, preferably 5 years after submission of the final report.

Section 8. Documentation and record keeping: All operations, data, and observations appropriate to this study should be recorded directly and promptly into the pre-prepared sampling forms. At a minimum, the following raw data must be collected and maintained:

- Names of all personnel conducting specific research functions.
- Amendments and deviations from protocol or SOP.
- Communication records.
- Sample collection site information, including sampling form, photos, and GPS coordinates.
- Chain-of-Custody forms (COC) and shipping documents.
- Sample and standard storage conditions including temperatures.
- Data regarding calibration and use of application equipment.
- MV data, SS data, and Sample analysis data with calibration curves and calculation sheets.
- All chromatograms, including those that are not reported.
- Instrument maintenance pages, and laboratory notebook.
- Working Method and/or standard operating procedures.

Section 9. Personnel and test facility: The proposed study was an international project working on pesticide residues in food across the African Continent simultaneously. This assignment was completed in the middle of the COVID-19 global pandemic. The sponsor and study director were located in the U.S. while nine pesticide analytical laboratories were located in eight countries. Before each laboratory started the study, a local coordinator was identified to overlook and coordinate the activities in that country. The coordinator helped to identify a lab director who was responsible for the overall conduct of the study for both field sample collection and lab sample analysis. Quality assurance shall be an independent unit, but was not specified in this protocol since the infrastructures of these African laboratories were not equal and had own quality systems. The protocol was prepared at the beginning of the study while the Personnel and test facilities were being determined.

Conclusion

All participating laboratories were trained with the EPA GLP standards and this project was considered as a "GLP-like" or "ISO-like" study because the quality assurance did not meet the requirements for GLP or ISO 17,025. The

fieldwork and laboratory analysis were successfully completed by December 2021. This protocol can be used as a good reference and study guide for pesticide survey studies, laboratory capacity building, and MRL residue studies.

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