नेपाल सरकार
कृषि विकास मन्त्रालय
नीति तथा अन्तराष्ट्रिय सहयोग समन्वय महाशाखा

फोन नं. ४२११६३५
४२११८०८
४२११५३२
४२११६६५
४२११६८७
४२११५१५
फ्याक्स : ४२११५३५

सिंहदरबार, काठमाडौं
नेपाल ।

पत्र संख्या:
च.नं.: नीअसस/७४/७५/१२६

नेपाल सरकार
कृषि विकास मन्त्रालय
सिंहदरबार

मिति : २०७४/१०/२२

श्रीमान् आयोजना निर्देशक ज्यू
व्यावसायिक कृषि तथा व्यापार आयोजना (PACT)
काठमाण्डौ, नेपाल

बिषय : **RBPL को स्विकृत Standard Operating Procedures (SOP)** पठाईएको बारे।

प्रस्तुत बिषयमा तहाँ कार्यालयबाट RBPL प्रयोगशालाको Standard Operating Procedures (SOP) स्विकृति माग भएकोमा तत् बिषयमा नेपाल सरकार सचिवस्तरको मिति २०७४/०९/३० को निर्णयानुसार तहाँबाट पेश भएको SOP मस्यौदा स्विकृत भएको व्यहोरा आदेशअनुसार अनुरोध गर्दछु। तत् सम्बन्धि सक्कल फाईल यसै पत्रका साथ संग्लग्न गरिएको छ।

(विन्दिरा अधिकारी)
कृषि अर्थविज्ञ

नेपाल सरकार
कृषि विकास मन्त्रालय
कृषि विभाग

☎ ५५२१५९७, ५०१०११२(Fax)
E-ail : director@ppdnepal.gov.np
Website: www.ppdnepal.gov.np

बाली संरक्षण निर्देशनालय

हरिहरभवन, ललितपुर

प.सं. ०७४।७५
च.नं. ९६८

मितिः २०७४/०८/१०

श्री व्यवसायिक कृषि तथा व्यापार आयोजना,
सल्लाघारी, महाराजगंज काठमाडौं।

बिषय : प्राविधिक sop को अन्तिम मस्यौदा पठाएको बारे।

उपरोक्त सम्बन्धमा मिति २०७४।०६।३० मा कृषि विभागका श्रीमान महानिर्देशकज्यू संग भएको समन्वय वैठकमा RBPR प्रयोगशालाहरु संचालन गर्न प्राविधिक मापदण्ड (Standard Operating Procedures) तयार गर्नका लागि वाली संरक्षण निर्देशनालयका कार्यक्रम निर्देशक श्री अच्युत प्रसाद ढकालज्यू को संयोजकमा गठन गरिएको प्राविधिक समितिले तयार पारिएको Standard Operating Procedures (SPO) को अन्तीम मस्यौदा यसै पत्रका साथ संलग्न राखी आवश्यक कार्वाहीको लागि पठाइएको व्यहोरा अनुरोध छ।

.....
अच्युत प्रसाद ढकाल
कार्यक्रम निर्देशक

Standard Operating Procedures for Rapid Bioassay of Pesticide Residues Analysis Laboratory

2017

FINAL DRAFT COPY

Submitted by
Technical Committee

Standard Operating Procedures for Rapid Bioassay of Pesticide Residues Laboratory

	Plant Protection Directorate, Lalitpur, Nepal
SOP for Rapid Bioassay of Pesticide Residue Analysis Laboratory	
SOP Number: RBPR/01/2017	Effective Date: , 2017
Version Number and Date: RBPR/01/2017	Review Date:
Superseded Version Number and Date:	
Prepared by:	Date:
Approved by: Signature: Name: Position: Organization: Date:	
Issue date:	

Disclaimer

When using this document please ensure that the version you are using is the most up to date either by checking on Plant Protection Directorate, Department of Agriculture, Ministry of Agricultural Development, Government of Nepal website for any new versions or contact Plant Protection Directorate to confirm the current version. Out of date documents must not be relied upon.

Table of contents

ACRONYMS

a.i.	Active ingredient
AChE	Acetylcholinesterase
AR	Analytical reagent
ATCI	Acetylthiocholine Iodine
Ca.	Circa (=Approximately)
Carb	Carbamate insecticide
DCM	Dichloromethane
DTC	Dithiocarbamate fungicides
DTNB	5, 5'dithio-bis-2-nitrobenzoic acid
DoA	Department of Agriculture
g	Gram
GAP	Good Agriculture Practice
GLP	Good Laboratory Practice
ml	Milliliter
MoAD	Ministry of Agricultural Development
nm	Nanometer
OP	Organophosphate insecticide
PBS	Phosphate buffer solution
PPD	Plant Protection Directorate
PPE	Personal Protective Equipments
PY	Pyrethroid insecticide
RBPR	Rapid Bioassay of Pesticide Residue
RO	Reverse osmosis
SOP	Standard Operating Procedures
TARI	Taiwan Agricultural Research Institute
µl	Microliter

CHAPTER I: INTRODUCTION

1. Background

A large quantity of pesticides is being used worldwide to protect agricultural crops from ravages of insect pests, diseases and weeds. The use of pesticides is more common on fruits and vegetable crops not only to save them from pests and diseases but also to protect their market values. Often such fruits and vegetables products are brought to the market for sale within a short period of time after use of pesticides on them. As such, these products have more chances of having excessive pesticide residues as compared to other crops. Since vegetables and fruits are perishable and readily consumed, pesticides residues on those commodities could be detrimental to human and the environment.

It has been estimated that 85% of total pesticides used in Nepal are used in vegetables. Though, the national average of pesticide used is 396 g a.i. per hectare but average pesticide used in vegetable is quite high i.e. 1600 g a.i. per hectare.

Chemical analysis to monitor pesticide residues is time taking, expensive and needs highly qualified and trained manpower and costly equipment. An alternative to chemical pesticide residue analysis is the Rapid Bioassay of Pesticide Residues (RBPR). Thus high residue risk vegetables and fruits production farmer's organizations could also be identified and corrective measures can be taken to practice good agricultural practices (GAP) to bring down the pesticide residue levels in their produce.

RBPR technique has been successfully adopted by many countries like Taiwan, Korea, Philippines, Thailand and Vietnam. The Plant Protection Directorate with logistic support of FAO has brought into operation a rapid pesticide residue testing laboratory in June 18, 2014. The laboratory set up in the premises of the Kalimati Fruits and Vegetables Market Development Committee, Kathmandu, Nepal helps to analyze the amount of pesticide residues found in vegetables and fruits products. Considering the huge success and widely acceptance of this laboratory, PPD with all logistic support from Project for Agriculture Commercialization and Trade opened new six RBPR laboratories in Nepal viz. Birtamod (Jhapa), Nawalpur (Sarlahi), Pokhara (Kaski), Butwal (Rupandehi), Nepalgunj (Banke) and Attaria (Kailali). Realizing the need of Standard Operating Procedure (SOP) for effective functioning of these laboratories, this SOP for RBPR laboratories was prepared by the technical committee headed by Mr. A.P. Dhakal (Annex I).

2. Introduction of Rapid Bioassay of Pesticide Residue (RBPR) Analysis

The RBPR technology is simple, detection is quick, the expenditure is low and the result is clear in toxicology sense. With this technique crop produce which exceeds the pesticide tolerance limits can be segregated and such produce can be withdrawn from the market before reaching to consumers.

It is a technique based on inhibition of acetyl-cholinesterase enzyme which can detect residues of pesticide toxicity of organophosphate (OP) and carbamate (Carb) group of insecticides

based on Taiwan Agricultural Research Institute's (TARI) technology. Acetyl cholinesterase is an enzyme responsible for controlling stimuli in nervous system in animals and human beings.

The rapid test is also available for detection of pyrethroid (PY) group of insecticides and dithiocarbamate (DTC) group of fungicides as a GT-test which is widely used in Thailand.

These compounds inhibit acetylcholinesterase, an enzyme critical to the control of nerve impulse transmission from one cell to another. When the enzyme is inhibited, there is overstimulation and then paralysis of the secondary cell. The character, duration, and degree of the resulting physiologic effect are directly related to the amount and rate of enzyme inhibition at certain receptor sites in the central and peripheral nervous systems. Some critical amount of enzyme must be inactivated before the signs and symptoms of poisoning are evident.

2.1 Advantages and limitations of RBPR

2.1.1 Advantages of RBPR

The RBPR technique is more useful for residue analysis in fruits and vegetables products which are highly perishable and need quick testing. It provides quick toxicological indications on plant samples with residue of organophosphates (OP), carbamates (Carb) of insecticides and pyrethroid group of insecticides and DTC fungicides. The technique does not require intensive training of technicians and sophisticated equipments as compared to technical chemical analysis. In RBPR analysis the samples also need not be cleaned to make them free from undesirable impurities which are a time taking process and is required for chemical analysis.

2.1.2 Limitations of RBPR

It is comparatively less precise as compared to chemical residue analysis and is not applicable to all the chemical pesticides. The inhibition of enzyme also cannot distinguish one OP from another OP, OP from carbamate or one carbamate from other carbamate insecticides in AChE test of RBPR analysis for carbamate and OP insecticides. Rapid detection test of synthetic pyrethroid insecticides does not distinguish the exact pyrethroid insecticide. Similarly, rapid detection of dithiocarbamate fungicide cannot distinguish the exact fungicide of dithiocarbamate group.

3. The laboratory and equipments

The laboratory must have adequate space and infrastructure. The laboratory and its facilities shall have minimum chance of contamination and provide personal safety. Separate rooms may be identified for sample receipt and storage, sample preparation, extraction and instrumentation used in the analysis steps.

Receiving samples, their storage and processing for residue analysis is required to be handled only in areas assigned for the respective work. All the basic principles of good laboratory practices (GLP) such as smoking, eating, drinking or application of cosmetics should not be permitted in the working area.

Only small volumes of chemicals/ solvents should be held in the working area and the bulk of the solvents stored separately, away from the main working area.

There should be an ample supply of hand gloves and other personal protective clothing. The laboratory will require reliable and regular supply of electricity and water.

All the instruments, apparatus, glass wares, reagents and chemicals should be of high quality. Adequate supplies of reagents, solvents, glassware, etc., of suitable quality are essential. Balances and automated pipettes/ dispensers and spectrophotometer need to be calibrated regularly. The operating temperatures of refrigerators and freezers should be continually checked.

4. Sampling

The combined and well-mixed aggregate of the primary samples should be taken from a lot. Samples must be transported under appropriate conditions to the laboratory in clean containers and robust packaging. Polythene bags, ventilated if appropriate, are acceptable for most samples.

Samples must be identified clearly and indelibly, in a way that prevents inadvertent loss or confusion of labeling.

Every sample received into the laboratory should be accompanied by complete information on the source of the sample, on the analysis required and on potential hazards associated with the handling of that sample. On receipt, a sample must immediately be assigned a unique identification code which should accompany it through all stages of the analysis to the reporting of the results. Samples should be subject to an appropriate disposal review system and all records should be kept. If samples cannot be analyzed immediately but are to be analyzed quickly, they should be stored at (1 - 5 °C), away from direct sunlight, and analyzed within a few days. The size and quantity of sample are given in Annex II.

5. Record keeping and data management

All the records shall be kept in register and as well as in computer. Recorded data shall be retrieved or used when needed.

6. Training

SOP training will be arranged by the Plant Protection Directorate, Ministry of Agriculture Development, Govt. of Nepal whenever required based on SOP and inputs to training on pesticide risk analysis using RBPR laboratory.

CHAPTER II: AChE TEST FOR ORAGANPHOSPHATE AND CARBAMATE INSECTICIDES

Acetylcholinesterase test is used for detecting two categories of neurotoxic insecticides, organophosphate (OP) and carbamate (Carb). This is an in vitro test for these insecticides. It uses AChE (acetylcholinesterase) obtained from the heads of a special strain of house fly. When the AChE reaction solution is mixed with samples from healthy produce, it slowly turns yellow as the level of 5-thio-2-nitrobenzoateanion increases. If these insecticides are present, the enzymatic reaction slows down or stops. The rate at which color development is inhibited indicates the quantity of chemicals present, as well as their toxicity. The AChE test is very rapid, taking few minutes from sampling to completion of the test. The principle and procedure followed in this test is developed by Taiwan Agricultural Research Institute (TARI) of Taiwan.

1. Materials required

1.1 Equipments and glassware

- Visible spectrophotometer with RBPR programme or equivalent
- Fume hood
- Air conditioner/electric fan
- Voltage stabilizer
- UPS, Battery inverter set
- Computer/Laptop with printer
- Refrigerator, deep freezer, centrifuge,
- Office/lab furniture
- Micro pipettes in different volumes (20 µl, 100 µl, 1000 µl, 1000-5000 µl and pipette tips
- Cuvettes
- Test tubes, test tubes racks and test tube mixer (vortex mixture)
- Vegetable samplers, forceps and rubber cushions
- Other glassware's (measuring cylinders, funnels, beakers, sample bottles, conical flask etc.)
- Parafilms, marker and stop watch etc.
- Knife, forceps, chopping board, scissors
- Wash bottles
- Personal protective equipments (mask, goggle, apron, hand gloves etc.)
- Others

1.2 Chemicals required

1. Reagent:
    - Acetylcholinesterase (AChE)
    - Acetylthiocholine Iodine (ATCI)
    - 5, 5'dithio-bis-2-nitrobenzoic acid (DTNB)
    - Sodium Phosphate Buffer

2. Bromine water (0.4-1%)
3. Ethanol
4. Distilled water

2. Preparation and storage of reagent/chemicals

2.1 Reagent and chemical storage

- The reagent (AChE, ATCI, DTNB) powder should be stored in freezer
- Buffer powder should be stored at below 0°C and needs to be protected from moisture.
- The solution should be prepared immediately prior to use, but can be stored at room temperature for thirty days. It can be dispensed and stored at below 0° C for a long time.
- The reagent powder can keep for two years if stored at a temperature below 0° C and three months if stored at room temperature.
- The reagent solution will keep for a maximum of six months if stored at a temperature below 0°C.
- Bromine ampoule must be kept away from heat, sparks, and flame. Do not store near combustible materials or in direct sunlight. Keep away from contact with oxidizing materials. Store in a cool, dry, well-ventilated area in a tightly closed container in safe place away from incompatible substances and corrosives area.

2.2 Preparation of reagent, storage and use

- AChE ca.30mg should dissolve in 10 ml of distilled water or equivalent or as prescribed in a bottle and stored at below 0°C after dividing into proportion.
- 216 mg of ATCI should dissolve in 10 ml of distilled water or equivalent or as prescribed in a bottle and stored at below 0°C after divide into proportion.
- 19.8 mg of DTNB should be dissolved in 50 ml of distilled water or equivalent or as prescribed in a bottle and stored at below 0°C after divide into proportion.
- Buffer solution should be stored at room temperature.
- One percent bromine concentration should be prepared.
- After preparation of reagent solution, date of preparation and volume shall be clearly written in label.
- Bromine water cannot be used after fading its colour.
- Bromine water should be stored at room temperature.
- Reagent solutions should be brought back to room temperature before using for insecticide assay.
- Reverse osmosis (RO) water, de-ionized water or mineral water should not be used for preparation of reagents. Always use distilled water for preparation of reagents.
- Store reagent solution in sub-package of one ml tube to avoid repeated freezing and thawing.
- Bromine water is easily evaporated so shorten time to open the bottle and tighten the cap after using.
- Make sure the buffer powder is completely dissolved before using.

3. Procedure of insecticide residue assay

Sample preparation, sample processing and sub-sampling to obtain analytical portions should take place before visible deterioration occurs.

3.1 Sampling and extracting

For carbamate

Carry out in two test tubes in parallel

- Cut 1 gram or 4 leaf discs of plant tissue into pieces and put into test tubes.
- Add 1 ml 95% ethanol (AR grade) to test tube.
- Shake test tube 20 seconds in shaker and allow it to stand for exact three minutes.
- Drain the supernatant solution of sample extract to new test tube for incubation/analysis.
- Test tube should be covered with parafilm when longer soaking period is expected to avoid evaporation of solvent.

For organophosphate

Carry out in two test tubes in parallel

- Cut 1 gram or 4 leaf discs of plant tissue into pieces and put into test tubes
- Add 2 ml 95% ethanol (AR grade) to test tube
- Shake test tube 20 seconds in shaker and allow it to stand for exact three minutes.
- Drain the supernatant solution of sample extract to new test tube. Additional standing of 20 minutes required to evaporate excess bromine water.
- Test tube should be covered with parafilm when longer soaking period is expected to avoid evaporation of solvent.

3.2 Incubation/analysis of sample extract:

3.2.1 Control test (for blank)

- Add 3 ml PBS in the 4 ml cuvette.
- Add 20µl AChE solution and 20µl 95% ethanol (AR grade) for blank
- Mix well for 5 seconds and wait till 2.5 minutes and add 100 µl DTNB solution
- Add 20µl ATCI solution at exact 3 minutes and mixing for 5 seconds to start enzyme reaction
- Read absorbance change by spectrophotometer at 412 nm
- Control absorbance difference must be less than 0.014 to continue sample test.

3.2.2 Sample test

- Add 3 ml PBS in the 4 ml cuvette
- Add 20µl AChE solution and 20µl of sample extract
- Mix well for 5 seconds and wait till 2.5 minutes and add 100 µl DTNB solution
- Add 20µl ATCI solution at exact 3 minutes and mixing for 5 seconds to start enzyme reaction
- Read absorbance change by spectrophotometer at 412 nm and compare with control test

- When the inhibition percentage exceeds 35% then repeat the test with the second test tube for confirmation.

3.3 Calculation of inhibition

Compare the reduction of absorbance for the sample to control (blank) test and calculate inhibition percentage as follow:

$$\text{Enzyme inhibition (\%)} = \frac{\text{Abs.change (control or blank)} - \text{Abs.change (sample)}}{\text{Abs.change (control or blank)}} \text{X100}$$

4. Result interpretation

Enzyme inhibition	Result purpose
<35%	Approve for sell and consumption
35%-45%	Quarantine for minimum 2 days. Repeat the test after washing. If inhibition percentage is less than 35%, then allow it for sale.
>45%	Not suggested for consumption purposes.

5. Precautions/suggestions

- The reagent solution needs to mix well and warm to room temperature before use.
- Bromine water should be stored in tight non corrosive glassware.
- Enzyme activity will vary with the temperature; if the room temperature rises then high enzyme activity occurs, so it is recommended for testing at constant temperature environment.
- The enzyme activity range is normal between 0.20-6.0 da/minute for blank/control.
- All cuvette must be uniform and high quality.

6. Cleaning of glassware and materials

- Cleaning of knives, cushions and test tube etc. must be done by water and then by ethanol properly and dried up completely.
- Cleaning of cuvettes
    - Take non-smooth surface of cuvettes in between the fingers.
    - Wash with clean tap water.
    - Wash with distilled water.
    - Wash with alcohol.
    - Invert the tube in no lint tissue paper.
    - Use alcohol to wet again.
    - Dry in the air.
- Those cuvettes and test tubes used in the test with more than 45% inhibition should be discarded.

CHAPTER III: DETECTION OF PYRETHROID INSECTICIDES USING PY TEST KIT METHOD

Pyrethoid groups of insecticide are also being used widely in Nepal. This group includes Cypermethrin, Cyfluthrin, Cyhalothrin, Deltamethrin, Esfenvalerate, Fenvalerate, Fenpropathrin. The GT Test invented by Gobthong Thoophom (GT) Thailand is widely used to detect pyrethroid group of insecticides.

1. Equipments and Material required

Micro pipette 100-1000µl, pipette tips, water bath, timer, test tube, rack, column, glass funnel, vortex mixer, forceps, samples, cotton wool, aquarium air pump set.

PY - Reagents:

Blank reagent, EXTRACT-1, EXTRACT-2, PY-1, PY-2, PY-3, PY-4, PY-5, PY-6,

2. Procedure for analysis

2.1. Extraction

- Make a ball of cotton wool at 1.5 cm in diameter and put in funnel. Add 1 ml of DCM (Extract 1) on the cotton wool.
- Use the wet cotton wool to randomly swab on the surface of the plant sample and place on the funnel.
- Rinse the cotton wool with 2 ml of DCM (Extract 1).
- Squeeze the cotton wool to make the solution flow into the test tube.

2.2 Clean up

- Rinse the column with 3 ml of DCM. Let the solution flow down to the cotton wool, then pour the sample extract into the column, allow the solution flow down to the cotton wool, do not allow the column dry at any step. Use a new set of test tube to contain the solution from the column.
- Rinse the column again with 3 ml of DCM. Let all the solution flow down from the column to test tube.
- Evaporate the solution near to dry using the air pumper (0.25-0.5ml)
- Add 1 ml of 50% ethanol (Extract 2) and blow out for 10 seconds then obtain the extract.

23. Testing

Reagents added	Blank tube	Test sample tube	Procedure
	Blank solution 0.25 ml	Sample extract 0.25 ml	
Py - 1	0.25 ml	0.25 ml	Shake the mixed solution and place the tube in the control temperature water bath

			at 40°C for 10 minutes.
Py - 2	0.75 ml	0.75 ml	Shaking
Py - 3	0.10 ml	0.10 ml	Shaking
Py - 4	0.25 ml	0.25 ml	Shaking
Py - 5	0.50 ml	0.50ml	Shaking
Py - 6	0.50ml	0.50ml	Shaking

3. Result and interpretation

Compare the color in the sample tube and the blank tube.

Test samples that has blue color same as or close to blank	Not finding (0)
The test samples that show stronger blue color than blank up to deep purple	Finding residue at safety level (+1 , +2)
Test samples with no color	Finding residue at hazardous level (+3)

CHAPTER IV: DETECTION FOR DITHIOCARBAMATE FUNGICIDE USING DTC KIT METHOD

This rapid test is also as a part of GT test is widely used in Thailand. The instrumentation is uncomplicated and consists of low-technology items. It is used for analysis of Dithiocarbamate fungicides eg. Mancozeb, Maneb, Metiram, Ferbam, Mabam, Nabam, Propineb, Thiram, Zineb, Ziram etc.. Mancozeb, Propineb, Zineb, Metiram are commonly used fungicides in Nepal.

1. Equipments and materials required

Micropipette 10-100µl, 100-1000µl, Loop, pipette tips, cotton wool, test tube, glass funnel, forceps, vortex mixtures, samples.

DTC Reagents: DTC solution, DTC-1, DTC-2, DTC-3, DTC-4, DTC-5

2. Procedure for analysis

2.1 Extraction

- Make a ball of cotton wool at the size of about 0.5 cm in diameter and put in funnel.
- Add 0.5 ml of DTC solution in the test tube, and then drop 0.5 ml on the cotton wool.
- Use the wet cotton wool to randomly swab on the surface of the plant sample.
- Put the cotton wool in same test tube then shake with the use of vortex mixer about 10 seconds.
- Squeeze the cotton wool to get extract and discard the wool.

2.2 Testing

Reagent s added	Blank tube	Test sample tube	Procedure
	DTC-solution 0.25 ml	Sample extract 0.25 ml	
DTC – 1*	0.25 ml	0.25 ml	Shaking
DTC – 2	20 µl	20 µl	Shaking
DTC – 3	0.25 ml	0.25 ml	Shaking
DTC – 4	0.50 ml	0.50 ml	Shaking
DTC – 5	0.20 ml	0.20 ml	Shaking

* The solvent-1 is toxic for human health, so avoid breathing and leave it evaporated in the ventilated place or fume hood.

3. Result and interpretation

Compare the color in the sample tube and the blank tube.

Evaluation of the results by comparison of color developed	
Test samples with no color/equal to blank	Not finding (0)
The test samples that show stronger blue color than blank up to deep purple	Finding dithiocarbamate at safety level (+1 , +2)
The test samples that show grayish purple to green indicate finding residue at hazardous level.	Finding dithiocarbamate at hazardous level (+3)

CHAPTER V: SAFETY & PRECAUTIONS

- Instructions should be read before using any equipment or chemicals.
- All compounds shall be handled as if they are highly toxic, unless it is known to the contrary.
- All laboratory work must be performed with the proper Personal Protective Equipment (PPE), including goggles or glasses, gloves, lab coat, and closed toed shoes. Additional protection may be necessary, depending on the process.
- All experiments using highly toxic materials shall be clearly labeled as such, using a sign or tag, signed and dated.
- Laboratory space should be well ventilated or must have exhaust fans.
- While working in bromine water (0.4-1%), nitrile gloves, goggles and N-9 masks or equivalents shall be wearing and the activity must be performed in fume hood in well ventilated area.
- While making bromine water solution from pure bromine, canister mask or equivalent, fluorinated rubber gloves or equivalent chemical resistant gloves, splash proof goggles shall be wearing in addition to above mentioned PPE and must be performed in fume hood in well ventilated area by technicians or with guidance of technicians. At least two persons should be present at that time. Keep quantities to a minimum. Keep labeled containers tightly closed and in a cool, dry and well ventilated location.
- Laboratory must have fire extinguisher and first medical aids. Medical emergency number, fire brigade office number shall be clearly displayed.

CHAPTER VI: WASTE DISPOSAL AND MANAGEMENT

- All generators of potentially hazardous wastes must ensure segregation, accurate and complete labeling and safe storage, transport, treatment and disposal of such wastes.
- Wastes should be minimized where possible.
- Waste chemicals and solvents are stored in suitable areas whilst awaiting collection and must not be accumulated.
- Regular disposal from the laboratories must be part of the laboratory program.
- Wastes should be segregated and mixing avoided where possible, as unexpected reactions may occur.
- If you are generating a large amount of one particular type of waste, have a separate residue container for it.
- Ensure the container is not leaking and there is no spillage on the exterior of the container.
- Untrained staff and students are not to handle hazardous wastes and must not be given responsibility for them.
- Personal Protective Equipment should be a consideration when handling chemical waste. Reference should be made to the Material Safety Data Sheet.
- Broken glassware and solid waste obtained from sample preparation should be segregated and disposed separately.
- Liquid waste obtained from analysis shall not be disposed to sewage directly. There must be safety tank to store for it or only after proper dilution of liquid waste will then allow draining in public sewage.

CHAPTER VII: DISTRIBUTION AND STORAGE OF DOCUMENT

The SOP will be distributed as below:

- Joint Secretary, Food Security, Agricultural Business and Environment Division, Ministry of Agricultural Development.
- Director General, Department of Agriculture, Ministry of Agricultural Development.
- Program Director, Plant Protection Directorate, Department of Agriculture, Ministry of Agricultural Development.
- In-charges, RBPR Laboratories.
- The original approved paper SOP will be placed in the approved SOP folder and will be held by the Program Director, Plant Protection Directorate, Department of Agriculture, Ministry of Agricultural Development.
- An electronic copy of the SOP will be held in the Plant Protection Directorate, Ministry of Agriculture Development, Govt. of Nepal and can be located on their website. Once a new version of the SOP has been approved, the master SOP spreadsheet should be updated to reflect correct version numbers and review dates.

Annex I

SOP preparation technical committee

Technical committee was formed according to decision of coordination meeting with Director General, Department of Agriculture held in 2074/06/31 BS (October 17, 2017) at PACT Office.

1. Mr. Achyut Prasad Dhakal, Program Director, Plant Protection Directorate: Coordinator
2. Mr. Mahesh Chandra Acharya, Senior Monitoring and Evaluation Officer, PACT: Member
3. Mr. Ram Krishna Subedi, Senior Plant Protection Officer, PPD: Member
4. Mr. Man Bahadur Kshetri, Senior Plant Protection Officer, PPD/Incharge RBPR Lab, Kalimati: Member
4. Mr. Drona Budhathoki, Plant Protection Officer, PPD: Member
5. Mr. Rajiv Das Rajbhandari, Senior Plant Protection Officer, PPD: Member Secretary

Annex II

Sample quantity of vegetable requirement for RBPR

S.N.	Crop	Sample quantity for one sample			Sample no	Remarks
		No.	Part	Wt.		
1. Cole crops						
1.1	Cauliflower, Cabbage, Broccoli, Chinese cabbage, etc	2	Head	not less than 250g	One sample for less than 15 quintal and two sample for more than 15 quintal.	The size of sample must be equivalent to the size of maximum number of size of edible products.
1.2	Turnip, Radish etc.	2 -6	Root with or without leaf	not less than 250g	"	"
2. Fruit crops						
2.1	Brinjal, Sweet pepper	4-6	Fruit	not less than 250g	"	"
2.2	Tomato, Okra, Hot pepper, garden pea	6-20	Fruit	not less than 250g	"	"
3. Cucurbits						
3.1	Pumpkin	1-2*	Fruit	more than 500g	"	*If bigger size than weight basis
3.2	Squash, bottle gourd, Sponge gourd, cucumber Chayote etc	2-3	Fruit	not less than 500g	"	The size of sample must be equivalent to the size of maximum number of size of edible products.
3.3	Bitter gourd,	3-8	Fruit	not less	"	"

S.N.	Crop	Sample quantity for one sample			Sample no	Remarks
		No.	Part	Wt.		
	pointed gourd, etc.			than 250g		
4. Leafy vegetable						
4.1	Small: Spinach, Garden cress, Amaranth, Mustard etc	10-25	Leaves/ Shoot /whole plant with or without root	not less than 250g	"	"
4.2	Medium to big: Broad Leaf mustard, Curly mustard, garden lettuce, spring onion, green garlic, leafy cucurbits	5-6	Leaves/ shoot/ Whole plant with or without root	not less than 250g	"	"
5.	Beans (Yard long bean, cowpea, Broad bean, butter bean, sword bean etc.)	15-25	Whole pod	not less than 250g	"	"
6.	Carrot, Taro	5-10	Root/ rhizome	not less than 250g	One sample for less than 30 quintal and two sample for more than 30 quintal.	"
7	Potato, onion	5-10	Tuber/ bulb	not less than 250g		"
8	Garlic, ginger, etc.	5-10	bulb, rhizome	Not less than 250g		"
9	Drum stick	5-15	Pod	not less		"

S.N.	Crop	Sample quantity for one sample			Sample no	Remarks
		No.	Part	Wt.		
				than 250g		
10	Others edible crop			not less than 250g		As per required and recommended by committee