United States Department of Agriculture
Agricultural Marketing Service, Science and Technology
Pesticide Data Program

SOP No.: PDP-QC-07		Page 1 of 15
Title: Demonstration of Method Performance		
Revision: 9	Replaces: 07/01/07	Effective: 10/01/07

1. Purpose:

To provide USDA/AMS Pesticide Data Program (PDP) a mechanism by which to evaluate a laboratory's ongoing ability to perform analyses when there are additions/changes in pesticides or commodities or analytical methods.

2. Scope:

This standard operating procedure (SOP) shall be followed by all analytical laboratories conducting residue studies for PDP including support laboratories conducting stability or other types of studies that may impact the program.

3. Definitions:

Refer to Glossary

4. Outline of Procedures:

7.1 Evaluation Guidelines
7.2 Scenarios
7.3 Establishment LODs and LOQs
7.4 Verification of LODs
7.5 LOD Check
7.6 Determination of Method Range
7.7 Precision and Accuracy Data Collection
7.8 Quarterly two times (2x) the Limit of Quantitation (LOQ) Spikes
7.9 Method Evaluation Reporting
Attachment 1 – Method Evaluation Flowchart, July 1, 2007
Attachment 2 – Method Evaluation Reporting Forms [Verification of Limits of Detection (LODs),LOD Check, Determination of Method Range, Precision and Accuracy Data Collection], July 1, 2007

5. References:

- USDA/AMS PDP Quality Assurance/Technical Meeting, March 20-22, 2007, Crystal City, VA
- USDA/AMS Combined MDP/PDP Technical-Quality Assurance Meeting, March 27-31, 2006, Richmond, VA
- USDA/AMS PDP Quality Assurance/Technical Meeting, March 22,24, 2005, Manassas, VA
- USDA/AMS PDP Quality Assurance/Technical Meeting, May 18-20, 2004, Fairfax, VA
- USDA/AMS PDP Quality Assurance/Technical Meeting, May 7-9, 2003, Manassas, VA
- USDA/AMS PDP Quality Assurance/Technical Meeting, April 9-11, 2002, Manassas, VA
- USDA/AMS PDP Quality Assurance/Technical Meeting, February 21-22, 2001, Tallahassee, FL
- Chemist Qualification document from Robert Epstein and summarized by Terry Jackson with State participant comments, April 23, 1992
- Validation of Methods Used in the Florida Department of Agriculture and Consumer Services' Chemical Residue Laboratory, Parker, G.A., JAOAC, 74, No. 5, pp. 868-871, 1991
- Quality Assurance Principles for Analytical Laboratories, Garfield, F., AOAC, 1991
- Quality Assurance of Chemical Measurements, Taylor, J.T., Lewis Publishers, 1989
- Evaluation of Analytical Methods Used for Regulation of Foods and Drugs, Horwitz, W., Analytical Chemistry, Vol. 54, No. 1, pp. 67A-76A, 1982

6. Historical Background and Philosophical Overview:

At the start of the Pesticide Data Program, it was recognized that the methods used for analysis must be validated. The problem encountered was the enormous number of combinations of pesticides and commodities the participating laboratories were asked to screen. To help make this a manageable task, two concepts were invented: marker compounds, and commodity groups. Each concept seeks to group pesticides or commodities by common properties and exploits these common properties to reduce the possible combinations to a manageable number. These concepts were introduced in PDP-QC-07 and PDP-QC-02 with mixed results. The interaction of these two SOPs and these two concepts has been marked with considerable confusion. In an effort to reduce the confusion associated with these two SOPs, PDP-QC-02 was archived July 1, 2004.

For PDP-QC-07, two additional concepts are introduced: modules and scenarios. These concepts are outlined in section 7.1. This allows for the systematic and logical application of method evaluation requirements (modules) to several commonly encountered situations (scenarios) in the PDP laboratories while exploiting the properties of marker compounds and commodity groups.

It is assumed that problems will lead to an investigation of causes, such as instrument reproducibility and instrument linearity.

A flow diagram is attached (*see Attachment 1*) which further clarifies these concepts.

7. Specific Procedures

All required method validation modules (with the exception of precision and accuracy data collection) shall be completed (extracted, analyzed, and reviewed by the TPM or designee) prior to the extraction of any routine analytical sample sets.

7.1 Evaluation Guidelines

7.1.a The following scenarios shall be followed for validation of new methods or changes/additions to existing methods. The following scenarios of changes/additions are possible:

7.1.a.1 Implementing a new method (7.2.a)
7.1.a.2 Changing an analytical method
- **7.1.a.2.a** Extraction (7.2.b.1)
- **7.1.a.2.b** Post-extraction/pre-instrumentation (7.2.b.2)
- **7.1.a.2.c** Instrumentation
  - **7.1.a.2.c.1** New technology (7.2.b.3.a)
    - **7.1.a.2.c.1.a** New LOD
    - **7.1.a.2.c.1.b** Current LOD
  - **7.1.a.2.c.2** Replacement/duplicate (7.2.b.3.b)
- **7.1.a.2.d** Minor Modifications (7.2.b.4.)

7.1.a.3 Adding a new commodity grouping (7.2.c)
7.1.a.4 Adding a new commodity or a processed commodity to an existing commodity group (7.2.d)
7.1.a.5 Adding pesticides related to marker pesticide groups to an existing commodity group (7.2.e). (*see PDP-QC-13 for listing of commodity grouping and marker pesticide groups and applicable PDP-QC-13 addenda for required pesticides*)
7.1.a.6 Adding a new pesticide that is not related to marker pesticide groups to an existing commodity group. (7.2.f)

7.1.b Evaluation takes place through the performance of method evaluation modules. These modules are chosen to meet the requirements of each scenario. The modules are:

- Establishment of LODs and LOQs according to PDP-QC-10 (7.3)
- Verification of LODs (7.4)
- LOD Check (7.5)
- Determination of Method Range (from 1xLOQ to 10xLOQ) (7.6)
- Determination of Method Precision and Accuracy at 2xLOQ (7.7)
- Method Evaluation Reporting (7.9)

7.1.c Section 7.2 of this SOP lists each scenario and the modules that must be performed in that scenario. Sections 7.3 through 7.9 outline the detailed procedures to be followed for each module.

7.1.d This method evaluation framework makes the following assumptions:

7.1.d.1 Commodities are grouped in such a way that assessment of method performance in one commodity in the group can be extended to apply to all commodities in the group.

7.1.d.2 Marker pesticides are chosen to be representative of a broad range of similar pesticides. The assessment of method performance for these pesticides can be extended to apply to similar pesticides.

7.1.d.3 A <u>method</u> can be evaluated using representative commodities and marker pesticides. Once a method is evaluated for these pesticides, it can be extended to other commodities and pesticides.

7.1.d.4 LOD is specific to a <u>pesticide</u> and must be evaluated for every pesticide.

7.1.d.5 Although a method may be extended to other commodities and pesticides, a minimum amount of recovery data must be obtained to confirm this assumption.

7.2 Scenarios

The Technical Program Manager and Quality Assurance Officer will determine which scenario described in the following subsections applies for the analytes/commodities/methods pairings *(see PDP-QC-13 for PDP commodity groupings and marker pesticide groups and applicable PDP-QC-13*

addenda for required pesticides). If local agreement cannot be reached, the PDP Technical Director shall be contacted to determine which modules should be performed.

7.2.a New method implementation – Proceed with:

- Establishment of LODs and LOQs (7.3)
- Verification of LODs/LOQs for all compounds (7.4)
- Determination of Method Range for marker compounds (7.6)
- Precision and Accuracy Data Collection for all compounds (7.7)
- Method Evaluation Reporting (7.9)

7.2.b Method changes

7.2.b.1 Major Extraction Change - Examples would be using a different solvent, solid phase extraction (SPE) sorbent bed, or a new technique such as microwave extraction or supercritical fluid extraction. Proceed with:

- Establishment of LODs and LOQs (7.3)
- Verification of LODs/LOQs for all compounds (7.4)
- Determination of Method Range for marker compounds (7.6)
- Precision and Accuracy Data Collection for all compounds (7.7)
- Method Evaluation Reporting (7.9)

7.2.b.2 Major changes in post-extraction/pre-instrumentation procedures (cleanup) - Proceed with:

- Verification of LODs/LOQs for all compounds (7.4)
- Determination of Method Range for marker compounds (7.6)
- Precision and Accuracy Data Collection for all compounds (7.7)
- Method Evaluation Reporting (7.9)

7.2.b.3 Instrumentation Changes

7.2.b.3.a New technology

7.2.b.3.a.1 New LOD - Proceed with:

- Establishment of LODs/LOQs for all compounds (7.3)
- Verification of LODs/LOQs for all compounds (7.4)
- Method Evaluation Reporting (7.9)

NOTE: The laboratory shall use best professional judgment to determine if Precision and Accuracy Data Collection (subsection 7.7) is necessary.

7.2.b.3.a.2 Current LOD - Proceed with:
- LOD Check (7.5.b)
- Method Evaluation Reporting (7.9)

NOTE: The laboratory shall use best professional judgment to determine if Precision and Accuracy Data Collection (subsection 7.7) is necessary.

7.2.b.3.b Replacement/duplicate - Proceed with:
- LOD Check (7.5.b)
- Method Evaluation Reporting (7.9)

7.2.b.4 Minor modifications of existing method - The approval process for modifications is defined in SOP PDP-QC-05. The Technical Program Manager and Quality Assurance Officer will determine which portions of the following sections will be completed. *This is dependent upon the extent of modification. If local agreement cannot be reached, the PDP Technical Director shall be contacted to determine which sections should be performed.* Proceed with:
- Establishment of LODs and LOQs (7.3)
- Verification of LODs and LOQs (7.4)
- Determination of Method Range (7.6)
- Precision and Accuracy Data Collection (7.7)
- Method Evaluation Reporting (7.9)

7.2.c Adding a new commodity group: Proceed with:
- Verification of established LODs/LOQs for all required pesticides in the new commodity (7.4)
- Determination of Method Range for the marker pesticides (7.6)
- Precision and Accuracy Data Collection for all required analytes (7.7)
- Method Evaluation Reporting (7.9)

7.2.d Adding a raw agricultural commodity or processed commodity (i.e., canned/frozen/dried/ juice) to an existing commodity group. Proceed with:
- Verification of established LODs/LOQs for all required pesticides (7.4)
- Precision and Accuracy Data Collection (2 points) for all required pesticides (7.7)

- Method Evaluation Reporting (7.9)

  NOTE: The laboratory shall use best professional judgment to determine if additional validation is necessary based on matrix behavior.

7.2.e Adding pesticides related to the marker pesticide groups to an existing commodity group – Proceed with:

- Establishment of LODs and LOQs for each pesticide added (7.3)
- Verification of LODs/LOQs for each pesticide added (7.4)
- Precision and Accuracy Data Collection for each pesticide added (7.7)
- Method Evaluation Reporting (7.9)

7.2.f Adding pesticides that are not related to the marker pesticide groups to an existing commodity group: (For example, the addition of imidacloprid analyzed by the same multiresidue procedure. The new pesticide may then become a marker pesticide for similar pesticides that are later added.) Proceed with:

- Establishment of LODs and LOQs for each pesticide added (7.3)
- Verification of LODs and LOQs for each pesticide added (7.4)
- Determination of Method Range for compound(s) that are to become marker(s) (7.6)
- Precision and Accuracy Data Collection for each pesticide added (7.7)
- Method Evaluation Reporting (7.9)

7.3 Establishment of LODs and LOQs

7.3.a See SOP PDP-QC-10, LOD and LOQ for Chromatographic Methods.

7.4 Verification of LODs and LOQs

7.4.a All calculated or established LODs must be verified by fortifying duplicate blank commodities at approximately the LOD level and subjecting them to the analytical method for each type of chromatography system (e.g., GC or LC) and detector system (e.g., ECD, FPD, TOF, MSD, MS/MS) used in the analysis of PDP samples.

7.4.b For laboratories that analyze multiple commodities in the same group and have the same LOD across all commodities, the most difficult commodity in the group may be used.

7.4.c Verification consists of the observation of detectable peaks in the chromatogram at three times the current noise level (run within the last three months). Variability is expected to be high. Therefore, recoveries can be reported as present or not present. If detectable peaks are not observed, the LOD must be re-estimated and the verification repeated.

7.4.d Prepare summary form(s) of the acquired data *(see Attachment 2).*

7.5 LOD Check

7.5.a The LOD for each reported compound shall be checked, at least yearly, by extraction of an LOD spike.

7.5.b The sensitivity shall be checked when instrument systems change (e.g, new instruments and/or detector) by injecting an LOD standard. If the calibration standard is in matrix, the LOD standard must be in matrix.

7.5.c LODs may be raised for analytes in an individual sample set at the discretion of Technical Program Manager.

7.5.d LODs may not be lowered without verification subject to the analytical method, Technical Program Manager approval, and QA review.

7.5.e All LOD check data generated shall be maintained and housed by the laboratory.

7.5.f Prepare summary form(s) of the acquired data *(see Attachment 2).*

7.6 Determination of Method Range

7.6.a Fortified samples are to be run through the entire analytical method on the primary injector and detector system. If additional types of chromatography systems (e.g., GC versus LC) and/or detector systems (e.g., FPD versus MSD) combinations are to be used for quantification, they must be likewise evaluated.

7.6.b Fortify samples in triplicate at approximately LOQ, 5xLOQ, and 10xLOQ for each marker or single analysis PDP analyte. Process these fortified samples through the entire analytical method. A reagent and matrix blank shall be subjected to the analytical method along with the fortified analytes.

7.6.c For each data point, calculate the Percent Recovery compared to known standards to three significant figures if greater than 100% or to two significant figures if less than 100%.

7.6.d Calculate the mean Percent Recovery (%R) and Coefficient of Variation (%CV) for each level. A definition of Horwitz expected intralaboratory and interlaboratory %CVs may be found in SOP PDP-Glossary. The appropriate values may be used as a guideline when evaluating data.

7.6.e Prepare summary form(s) of the acquired data by analyte, level, and commodity group *(see Attachment2)*. Refer to SOP PDP-QC-04 for PDP acceptance criteria of this acquired data.

7.7 Precision and Accuracy Data Collection

7.7.a The precision and accuracy data collection shall be compiled from the commodity groupings as specified by USDA/AMS. Each marker, single analysis, new or other required PDP analyte shall be spiked at 2xLOQ and evaluated using a minimum of seven data points, with at least two points from each commodity in the group analyzed in a particular laboratory.

7.7.b The required data points shall be obtained from:

7.7.b.1 2xLOQ data points completed after Determination of Method Range.

and/or

7.7.b.2 Data points from matrix spikes analyzed concurrently with samples.

These two options provide slightly different data. The second option is preferable since it provides information about the repeatability of the method over time. The first option is permitted when running concurrent spikes would extend the data collection over more than six months and/or concurrent spikes would make the size of sample sets unmanageable.

7.7.c For each data point, calculate the Percent Recovery compared to known standards to three significant figures if greater than 100% or to two significant figures if less than 100%.

7.7.d Calculate the mean Percent Recovery (%R) and Coefficient of Variation (%CV) for each pesticide using the seven data points. A definition of Horwitz expected intralaboratory and interlaboratory %CVs may be found in SOP PDP-Glossary. The appropriate values may be used as a guideline when evaluating data.

7.7.e Prepare summary form(s) of the acquired data (*see Attachment 2*). Refer to SOP PDP-QC-04 for PDP acceptance criteria.

7.8 Quarterly 2xLOQ Spikes

7.8.a All reported compounds (markers, required, and any other compounds reported by the laboratory) shall be spiked at least quarterly at 2x LOQ for each commodity.

7.8.b The laboratory may choose to rotate spikes on a regular basis as long as the requirements in subsection 7.8.a are met.

7.8.c The spike results shall be reported to MPO via RDE (the preferred option) or in Excel spreadsheets. Results shall also be addressed in the semi-annual QA Reports submitted to MPO.

7.9 Method Evaluation Reporting

7.9.a The methodology, method evaluation records, summary form(s), chromatograms, and any other supporting data generated during PDP-QC-07 evaluation shall be maintained by the laboratory.

7.9.b Once the laboratory has completed the applicable modules:

- Verification of LODs and LOQs (7.4)
- LOD Check (7.5)
- Determination of Method Range (7.6)
- Precision and Accuracy Data Collection (7.7)

The summary form(s) and a brief narrative describing the method and results shall be sent to the PDP Technical Director together with a cover memo detailing the submission (state which scenario(s) and module(s) that the submission is intended to represent). Submission of complete method evaluation studies is preferred; however, some laboratories have chosen to submit the modules in a piecemeal fashion. If a complete set of modules is not submitted it should be clearly stated. The laboratory shall clearly identify any data that is only intended to be used for confirmation. Otherwise, MPO will evaluate the data as if quantitation will be performed on the instrument/analyte combination. If previous method validation data are used, the laboratory shall be responsible for clearly identifying the data used and the rationale for their use in the narrative. For example, if a previously validated commodity returns and the laboratory has not made any method changes and will be using the same

instrumentation, the laboratory shall submit a letter to MPO explaining how the previous validation data will be used.

After receipt by the PDP Technical Director, method validation data packages undergo a multi-tiered review by MPO. Details of this review process are specified in SOP PDP-ADMIN-06A, subsection 5.3. Once the MPO review of the method validation package is complete, a Letter of Concurrence will be sent identifying the status of the instrument/detector results for the commodity/analyte pairing (e.g, validated, not validated, incomplete). If the data are deemed incomplete by MPO, the Letter of Concurrence will identify the deficiency and include a request for the remaining data.

<u>Example narrative for a complete data package:</u>

> Enclosed is the complete method validation summary of all compounds we are screening for in commodity "y" to support the addition of the commodity to the 2007 PDP program. The specific scenario used in validation was 7.2.a, New Method Implementation. Required modules included establishment and verification of LODs and LOQs, determination of method range, and precision and accuracy data collection for GC/MSD, GC/FPD, GC/XSD, and LC/MS/MS instrumentation. For compound "a", GC/FPD is the primary detection system and LC/MS/MS data is intended for confirmation purposes only. The following analytes were dropped during method development due to difficulty in analysis (e.g., solubility, poor chromatography, sensitivity, and/or loss in SPE cleanup): compound "b", compound "c", and compound "d". If there are questions about this submission please contact: XXXXXX. All references to this submission should use QA# ###-####.

<u>Example narrative for a partial data package:</u>

> Enclosed is the partial method validation summary of all compounds we are screening for in commodity "y" to support the addition of the commodity to the 2007 PDP program. The specific scenario used in validation was 7.2.d, adding a raw agricultural commodity to an existing commodity group. Required modules included establishment and verification of LODs and LOQs and precision and accuracy data collection for GC/MSD, GC/FPD, GC/XSD, and LC/MS/MS instrumentation. Determination of method range data for LC/MS/MS compounds was also required because these compounds were previously validated using GC/MSD and HPLC instrumentation in the 2005 method validation for commodity 'z" in this PDP Commodity Group. Precision and accuracy data is being collected concurrently with sample analysis and will be submitted when the requisite number of data points are obtained. For compound "a", GC/FPD is the primary detection system and LC/MS/MS data is intended for

confirmation purposes only. The following analytes were dropped during method development due to difficulty in analysis (e.g., solubility, poor chromatography, sensitivity, and/or loss in SPE cleanup): compound "b", compound "c", and compound "d". If there are questions about this submission please contact: XXXXXX. All references to this submission should use QA# ###-####.

Example narrative for a previously validated returning commodity with no method, analyte, or instrumentation changes:

In 2007, Commodity "y" returned to the 2007 PDP program. This commodity was previously validated in 2005 and there have been no changes to the method, target analytes, and instrumentation since then. Therefore, the 2005 validation data submitted on Month, Day, Year, is still applicable. If there are questions about this submission please contact: XXXXXX. All references to this submission should use QA# ###-####.

An example format for the submission follows:

Title
Summary to include purpose, results, data anomalies.
Methods
Sample Preparation (example):

- 50g homogenized sample extracted with 100 ml ACN by gently mixing
- 5ml extract purified by a C-18 SPE cartridge, eluted with MeOH, and concentrated to 5 mls
- 1 ml eluate further purified by florisil SPE and eluted with 5 mls 50:50 hexane/acetone
- Eluate dried down to 0.5 ml, re-suspended in acetone, and filtered
- Derivatizaton accomplished by reaction with dansyl chloride.

Analysis (example):

- Instrument GC/HPLC/detector
- Column (DB-)
- Post-column derivatization (where applicable).

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ELECTRONICALLY REPRODUCED SIGNATURES

- Added requirement that all required method validation modules (with the exception of precision and accuracy data collection) be completed prior to the extraction of any routine analytical sample sets to Section 7
- Specified mean percent recovery shall be calculated and reported for demonstration of method range (Section 7.6.d) and precision and accuracy data collection (Section 7.7d)

Revision 8

- Specified LOD verification must be performed for each type of analytical system used in subsection 7.4
- Added LOD verification procedures for laboratories that analyze multiple commodities in the same group and have the same LOD across all commodities to subsection 7.4.b
- Changed responsibility for maintaining LOD check data from the QAU to the laboratory in subsection 7.5.e
- Changed semi-annual 2x LOQ spike requirement to quarterly in subsection 7.8
- Clarified method evaluation reporting requirements in subsection 7.9 and added narrative examples

Revision 7

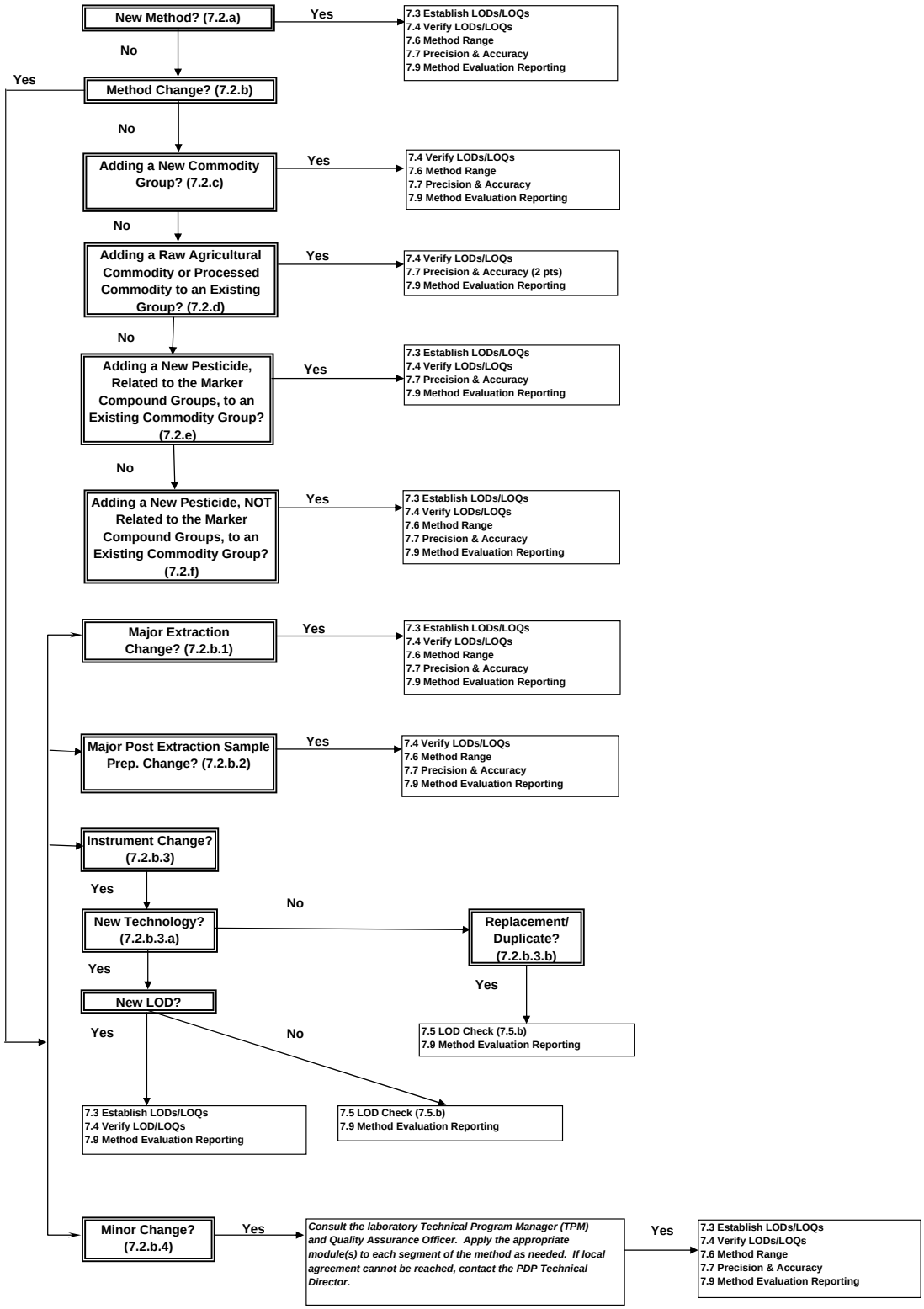
- Deleted subsection 7.6, Consistent Instrumental Response and all references. Renumbered remaining subsections
- Deleted references to most difficult commodity currently analyzed by the laboratory from subsections 7.2.a, 7.2.b, 7.2.e, and 7.6.b
- Deleted requirements for precision and accuracy data collection from subsections 7.2.b.3.a.1 and 7.2.b.3.a.2 and added note to the subsections indicating that the laboratory shall use best professional judgment to determine if precision and accuracy data collection is necessary
- Clarified requirements for adding a commodity to an existing group in subsection 7.2.d and added note to the subsection indicating that the laboratory shall use best professional judgment to determine if additional validation is necessary
- Clarified requirement that each instrument shall have the LOD/LOQ verified in subsection 7.4.a
- Clarified requirement that each instrument shall have an annual LOD/LOQ check in subsection 7.5.a

- Added requirements that LOD check must be in matrix if standards are in matrix to subsection 7.5.b
- Clarified requirement that each type of system used for quantification shall have determination of method range evaluated in subsection 7.6.a
- Dropped requirement to treat the fortified samples for the determination of method range as a set of nine samples from subsection 7.6.b
- Removed outdated reference from subsections 7.7.a
- Added semi-annual 2x LOQ requirements to subsection 7.8

Revision 6

- Changed scope to remove references to archived SOP PDP-QC-02
- Changed title of subsection 7.7 from “Determination of Method Performance” to “Determination of Method Range”
- Added “Changing commodities or” to subsections 7.1.a.3 and 7.1.a.4
- Removed requirement for performing consistent instrumental response module after changes to post-extraction/pre-instrumentation procedures from subsection 7.2.b.2
- Clarified method evaluation reporting requirements in subsection 7.9

Method Evaluation Flowchart



USDA, AMS Pesticide Data Program
Verification of Limits of Detection (LODs)

Commodity: _____
Date: _____
Lab: _____

Pesticide/Compound	Amt Spk Units =	LOD Spike Recovered (yes/no or +/-)	
		Spike 1	Spike 2