Department of Pesticide Regulation

Brian R. Leahy
Director

Edmund G. Brown Jr.
Governor

M E M O R A N D U M

TO: Lisa Ross, Ph.D.
Environmental Program Manager
Environmental Monitoring Branch

FROM: Vaneet Aggarwal, Ph.D. *Original signed by*
Environmental Scientist
Environmental Monitoring Branch
916-445-5393

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SUBJECT: EVALUATING ANALYTICAL METHODS FOR COMPLIANCE WITH THE PESTICIDE CONTAMINATION PREVENTION ACT REQUIREMENTS

1.0 Introduction

The Pesticide Contamination Prevention Act (PCPA) of 1985 requires the Department of Pesticide Regulation (DPR) to identify pesticides with the potential to pollute groundwater (GW) and monitor for those pesticides to determine if they have migrated to groundwater (Food and Agricultural Code (FAC) section 13148). If a pesticide is detected in groundwater, DPR is then required to determine whether the detection was the result of agricultural use of that pesticide (FAC section 13149). PCPA requires any finding of a pesticide to result from either:

(a) an analytical chemical method approved by DPR that provides unequivocal identification of a chemical, or

(b) from verification, within 30 days, by a second analytical method or a second analytical laboratory approved by DPR.

This memorandum describes DPR's criteria for determining whether an analytical method is unequivocal or a 2nd method selected for verification is appropriate. Use of these criteria will ensure that pesticide detections in ground water will meet the requirements of the PCPA.

This memorandum presumes that staff who makes this determination has a thorough understanding of the principles of analytical chemistry and the procedures and equipment used in laboratory analyses.

1.1 Purpose

This document describes (a) the criteria for determining if an analytical method is unequivocal, and (b) the criteria for determining if a 2nd analytical method selected for verification is appropriate. This memorandum also describes the determination memorandum review process and documentation requirements.

1.2 Definitions

Gas Chromatography (GC) – An analytical chemical separation method by which a sample is vaporized into a flowing gas stream known as the mobile phase, passed through a capillary column containing a stationary phase and separated into individual components by their relative affinity for the stationary phase.

Mass Spectrometry (MS) – An analytical chemical detection method by which individual chemicals or their characteristic fragments are identified by their mass to charge (m/z) ratios. It can also determine the concentration of a compound in a mixture.

Liquid Chromatography (LC) – An analytical chemical separation method similar to gas chromatography except that the mobile phase is liquid.

2.0 Materials

2.1 Unequivocal Determination Memo Template. Available at: <http://www.cdpr.ca.gov/docs/emon/pubs/em_methd_main.htm>.

2.2 Approved laboratory method. Available at: <http://www.cdpr.ca.gov/docs/emon/pubs/em_methd_main.htm>.

2.3 Method Validation Data, including spike levels, recoveries, storage stability studies, etc. If the written method is not yet available, this information can be obtained from the chemist who developed the method and used as the basis for the determination memorandum.

3.0 Overview of specific and non-specific detection methods

3.1 Unequivocal (specific) detection method: Unequivocal detection methods provide a fingerprint of the molecule by responding to specific structural characteristics of the analyte. Such a highly specific detection method distinguishes the target compound from potential interfering compounds with an extremely high level of confidence. Consequently, additional verification is unnecessary.

The most common specific detection methods are mass spectrometric methods. In general, the eluant from the column is subjected to ionizing conditions. This might include bombardment with electrons or other charged particles. The analyte molecule, if present, is converted to a characteristic precursor ion and charged fragments ("product ions") of certain mass: charge ratios previously determined from a certified reference standard. The mass spectrometer determines the presence and abundance of

these characteristic ions, thereby providing identification and quantification of the original analyte. The method is highly specific, hence "unequivocal," based on (a) matching retention time of the certified reference standard, (b) presence of the precursor ion at the retention time, and/or (c) presence of one or more characteristic product ions. It is preferable, but not required, that the precursor and product ions are also present at the same relative abundance as observed with the certified reference standard under the same operating conditions.

For example, for GC/MS we ionize the molecule using electron impact. This breaks up the pesticide molecule into smaller parts. The stability of those parts is shown by the intensity of each mass on the spectra. The more stable the ion is, the bigger is the mass intensity in the spectra. If we scan using full scan, we should see the molecular ion of the pesticide. Usually the molecular ion is very small, < 10% of the size on the strongest ions. Full scan MS is not very sensitive and is not usually used. When we do the analysis by selected ion monitoring (SIM), we usually do not even look for the molecular ion since it is very small. We look for the largest three ions in the spectra of the pesticide. In many cases we can get some structural information from the three ions we choose. This could be the isotopic ratio of Cl or Br in the pesticide, for example.

For LC MS/MS it is different. Usually we will get the mass of the pesticide plus 1 for the charged species (M + 1). Since the pesticide is not fragmented by electrons, the molecular ion is usually very intense in LC/MS/MS analysis. We use the first quadrupole to isolate the M+1 ion and then in the second quadrupole, we fragment this ion into usually 2 or more product ions. We select the most intense product ions coming from the second quadrupole and we usually do a SIM analysis using the 3rd quadrupole where we only look for the specific masses we should get from fragmenting the pesticide. We then compare the ratio of the intensities of the two product ions to determine if it is the pesticide that we think it is. If the ratio is not too far off, usually < 20% difference, then we have confidence that it is the pesticide that we think it is.

In addition to the chromatographic separation/specific detection analytical methods discussed above, it is also theoretically possible that other analytical methods may be used for unequivocal identification of an analyte. However, it is extremely important to do extensive testing on such methods to confirm they are, in fact, unequivocal (as operationally defined here).

3.2 Nonspecific detection method: All detection methods that respond to a myriad of chemicals and use detectors that cannot distinguish between these different chemicals are considered to be nonspecific. Chemical identification is inferred from

chromatographic retention time by comparing with a known standard. Most conventional nonMS chromatographic methods are nonspecific; they respond to any compound that elutes from the chromatographic column. The only means of identification is the time that a compound needs to traverse the separated column in the chromatograph. The peak assignment is made solely by inference:

Example: calibration runs show that pesticide X elutes at time Y, any peak observed at time Y for any sample is then assumed to be caused by pesticide X.

However, since many chemicals may be present in environmental samples, this identification may be ambiguous if two chemicals in the sample possess a similar retention time.

Common nonspecific GC detection methods include electron capture (EC), thermionic specific detection (TSD), or flame photometric detection (FPD), among others. A common nonspecific LC detection method is ultraviolet absorption (UV). Although these detectors are somewhat specific to detect any compound that they are capable of detecting, but they do not give any other compound specific information. Therefore, such methods do not meet the criteria of unequivocal detection.

4.0 Criteria Used to Determine if an Analytical Method Provides Unequivocal Identification of an Analyte

A method will be deemed unequivocal if it meets one or both of the following criteria:

4.0.1 **Selectivity**: The method is known not to show any significant interference from other chemicals or matrices.

4.0.2 **Structural Analysis**: The method includes combined separation/detection procedures that allow nearly unique identification of a chemical based on its specific structural characteristics (e.g. LC/MS).

4.1 Discussion of criteria

Chromatographic-based techniques are the most common analytical techniques used by the California Department of Food and Agriculture (CDFA) laboratory. These techniques, such as GC and LC, generally have three steps. The first is an isolation of the analyte of interest in the sample by extraction. This step often uses an organic solvent to remove the analyte along with other similar chemicals in the sample. The second step is separation of those chemicals in the extract using a chromatographic column. In this step, the extract is introduced into a GC or LC column, and the

chemicals then elute from the column at characteristic times, called retention times. Each chemical's retention time is dependent on the type of separation column used and the operating conditions. However, two or more chemicals can possess the same retention time under certain conditions. The final step is the detection of the chemicals as they elute from the chromatographic column.

Detection methods that meet the above criteria may be considered to provide unequivocal detection. These criteria are influenced by the operating conditions and the nature of the chemical analyzed. Therefore, decisions will not be solely based on the detection method used, but must be made on a case-by-case basis, often requiring consultation with the laboratory.

4.2 Examples of methods previously used by DPR where case-by-case decisions were made

Several mass spectrometric methods previously approved and used by DPR are listed in Table 1. These methods utilize low resolution (GC-MS and LC-MS) and tandem (LC-MS/MS) mass spectrometry. In all cases shown in Table 1, DPR applied methods that utilized mass spectrometry for target analyte structural elucidation and confirmation. These methods were developed and optimized to eliminate interferences in the analysis and unequivocally identify the target analyte. Basic to all of these confirmatory methods was the measurement of retention time, identification and confirmation of the precursor ion, and the identification of one or more of the product ions in MS/MS analysis.

For the low resolution GC-MS and LC-MS methods, multiple factors were used to eliminate the possible interferences. These factors included measurement of retention time, identification of the precursor ion (e.g., (M-H) or $(M+H)^+$ ions), and identification of two or more ions. Unequivocal determination was achieved when at least one ratio was measured and corresponded to the same ratio of ions found in the certified reference standard and within the preset tolerance of 20% relative percent difference (RPD) (unless it can be shown that there is interference on one or more ions). RPD is a measure of precision. It is often used as a quantitative indicator of quality assurance and quality control for repeated measurements where the outcome is expected to be the same. RPD is calculated as:

$$RPD = \frac{|x1 - x2|}{\frac{(x1 + x2)}{2}} * 100$$

The tandem MS/MS methods are highly specific and multiple sequential factors were also used to eliminate possible interferences. The factors included measurement of retention time, mass filtering and confirmatory identification of the precursor ion (e.g., $(M-H)^-$ or $(M+H)^+$ ions), and second mass filtering step, which segregated the specified product ions that are unique to the target analyte.

The unequivocal determination criteria shown in Table 1 are not unique and are used widely in pesticide analytical methods that utilize mass spectrometry as demonstrated in peer-reviewed scientific literature. The criteria provide unequivocal determination and should continue to apply to new mass spectrometric methods that are used by DPR.

Table 1. Mass spectrometric methods previously identified by DPR as unequivocal identification

Source	Instrument	Unequivocal Determination Criteria
US Geological Survey, Method O-2132-99 (Selected Herbicides)	GC-MS	• Retention time (within ± 6 sec) • Precursor ion • 2-3 product ions • Ratio (<20% RPD) of product ions in sample to same product ions in certified reference standards
US Geological Survey, Method O-2134-00 (Selected Herbicides)	LC-MS	• Retention time (within ± 6 sec) • Precursor ion • 2 product ions • Ratio (<20% RPD) of product ions in sample to same product ions in certified reference standards
Enseco, Method LN-CAL-3058 (Selected Carbamates)	LC-MS/MS	• Retention time • Precursor ion • 1 product ion
PTRL West, Method 1000Wymsms 7b (Selected Herbicides)	LC-MS/MS	• Retention time • Precursor ion • 1-2 product ions
CA Dept of Food and Agriculture, Method EM 37.6 (Selected Herbicides)	LC-MS/MS	• Retention time • Precursor ion • 1-2 product ions
CA Dept of Food and Agriculture, Method EMON-SM-13.0 (Imidacloprid and degradates)	LC-MS/MS	• Retention time • Precursor ion • Ratio (<20% RPD) of product ion to precursor ion in sample to same product ion to precursor ion in certified reference standard

4.3 **Notes** :

Note 1: An unequivocal detection method only minimizes the errors caused by interferences; it does not solve the problem of sample contamination. Proper quality control procedures can help minimize the risk of that error.

Note 2: Even though these detection methods provide the capability to identify a chemical, it does not imply that they will be able to do so unequivocally under all operating conditions or for all chemicals. Take mass spectrometry as an example: one can either acquire a whole mass spectrum, or scan selected mass ranges, or just look at one or more selected mass values. The less information one gathers (for example, looking at just one mass spectra or selected mass values), the larger is the possibility of an erroneous identification (false positive or false negative). In identifying a chemical spectroscopically, it is as important to show that there are no peaks where there shouldn't be any as it is to show that there are peaks where there should be.

5.0 Criteria for Determining the Suitability of a Second Analytical Method Used to Verify Detection Results from a NonSpecific Analytical Method

DPR is required to verify the results of a nonspecific analytical method by analyzing the sample using a second analytical method that is either unequivocal or, if nonspecific, significantly different than the first method. If the analytical procedures of the second method vary only slightly from the first method, it is likely that an erroneous identification using the first method would also occur in the second method.

The minimum changes needed to approve the second method depend on the specificity of both methods.

A second method will be deemed suitable if it meets the following criteria:

5.0.1 If the first detection method is non-specific but quantitative, there must be a significant change in the detector and the separation procedure in the second detection method. Or,

5.0.2 If the first method is qualitative or semi-quantitative, the second method must be quantitative. Or,

5.0.3 The second method must be unequivocal.

5.1 **Discussion of Criteria**

5.1.1 Significant Change in Detector and Separation Procedure

If the first and the second methods are nonspecific then, the second method should be based on separation and detection processes that are as different from the first method as feasible.

A significant change in detector means a change in detection principle (for example, for GC, a change from a flame photometric detector [FPD] to a conductivity detector). A significant change in the separation procedure is either a change in separation principle (from GC to HPLC, for example) or a change in the separation conditions (i.e., using a different type of column), as long as this change will alter the sequence in which the compounds are eluted from the column.

Example:

A first method using GC separation and a flame photometric detector could use as a second method:

- a GC with a significantly different column and a nitrogen-phosphorus detector (changing separation conditions and detector). Or
- a HPLC separation with a UV-detector (changing separation principle and detector).

5.1.2 Significant Change in the Ability to Quantify Results

Special consideration has to be given to qualitative or semi-quantitative methods typically used for screening. Qualitative methods yield only detected / not detected results while semi-quantitative methods indicate the order of magnitude for the concentration of the identified chemical. Samples identified as positive using either of these types of analytical methods must be verified by a second analytical method that can quantify the results. In this case, the qualitative screen is considered to be the first method. The quantitative method is then selected based on the above criteria for a second method. A second quantitative method (i.e., a third analysis method) is required only when verification is needed not only for the identity of the compound but also for its concentration.

6.0 Special Considerations/Potential Problems

6.1 Methods should be specific and sensitive enough to meet the study objectives (for example, the Environmental Monitoring Branch (EMB) typically requests a reporting limit of 0.05 ppb for ground water samples).

6.2 It is possible for some compounds to undergo structural rearrangement / degradation and co-elute during the analytical process. For example, CDFA chemists (Hsu, 2010) did not fully understand whether isoiprodione arose from rearrangement of iprodione in the injector and/or on-column degradation. Due to this uncertainty, iprodione did not qualify for unequivocal detection designation, and further research was recommended (Aggarwal, 2011b).

6.3 Stereoisomerism is another potential problem. This is a special case and may require the use of special methods, for example, the use of chiral columns.

7.0 Procedure for method review and determination

7.1 **Interim Determination**
If there is an urgent need to begin monitoring before the approved written analytical method is available, an interim determination may be prepared according to the following procedures:

7.1.1 Obtain the following data from the chemist:
a. Instrument used
b. Retention time
c. Precursor ion
d. Specific product ion(s), and/or
e. Ratio of product ions in sample to same product ions in certified reference standards

7.1.2 Prepare an interim memo that includes a description of the data reviewed, a discussion of the findings, a recommendation whether the method appears to be unequivocal or suitable as a second method, and, as needed, concerns or caveats about the data provided by the laboratory.

7.1.3 Request a review by an EMB scientist who is familiar with analytical chemistry principles and procedures. If the scientist agrees with the reviewer's findings and recommendation, the reviewer will forward the interim memorandum to the Ground Water Unit supervisor and Environmental Program Manager I for their review.

7.1.4 If the data reviewed indicate that the analytical method is unequivocal or is an appropriate 2nd method, the Environmental Program Manager I will allow monitoring to begin prior to the completion of the written method. A final determination will be prepared when the approved written analytical method becomes available.

7.2 **Final Determination**

7.2.1 Review the approved written method

a. Only explicit operating instructions contained in a written and approved method together with the supporting data of the method validation will provide enough information to make a final determination.

b. If there are errors or omissions in the final written method, return the written method to the EMB lab liaison with comments. The review will not be continued until the written method meets EMB approval.

7.2.2 Prepare a memorandum that includes the following:

a. Background: See Unequivocal Determination Memorandum Template and examples on the external Web site (Aggarwal, 2011 a, b).

b. Issue: Explanation of the compound and the method being determined.

c. Discussion and Recommendation: Discussion of the specific criteria exhibited to define the method as unequivocal.

7.2.3 Submit the draft memo for review as follows:

a. First review: EMB scientist with education and / or training in analytical chemistry.

b. Second review: EMB Ground Water Unit supervisor and Environmental Program Manager I.

c. Final review: EMB Chief.

7.3 File Maintenance

7.3.1 Signed hard copies will be maintained in the Ground Water Unit filing system.

7.3.2 Electronic copies are posted to <http://www.cdpr.ca.gov/docs/emon/pubs/em_methd_main.htm?filter=grndwater and maintained> on Groundwater internal network drives.

APPROVED: *Original signed by* Date: *July 10, 2012*
Lisa Quagliaroli
Senior Environmental Scientist

APPROVED: *Original signed by* Date: *July 10, 2012*
Lisa Ross, Ph.D.
Environmental Program Manager

APPROVED: *Original signed by* Date: *July 11, 2012*
David Duncan
Environmental Program Manager

8.0 REFERENCES

Aggarwal, V. 2011a. Memorandum to L. Quagliaroli, "Determination if California Department of Food and Agriculture's Chlorothalonil method (EMON-SM-05-020) meets the "Unequivocal Detection" criteria." Available on-line: <http://www.cdpr.ca.gov/docs/emon/pubs/anl_methds/emon-sm-05-020_memo.pdf>.

Aggarwal, V. 2011b. Memorandum to L. Quagliaroli, "Determination if California Department of Food and Agriculture's Azoxystrobin, Azoxystrobin Acid, Azoxystrobin Z-metabolite, Dicloran, Iprodione, Isoiprodione, Vinclozalin and 3,5-Dichloroaniline method (EMON-SM-05-018) meets the "Unequivocal Detection" criteria." Available on-line: <http://www.cdpr.ca.gov/docs/emon/pubs/anl_methds/memo_2281.pdf>.

Hsu, J. 2010. California Department of Food and Agriculture method (EMON-SM-05-018): "Determination of Azoxystrobin, Azoxystrobin Acid, Azoxystrobin Z-metabolite, Dicloran, Iprodione, Isoiprodione, Vinclozalin and 3,5-Dichloroaniline in Well Water." Available on-line: <http://www.cdpr.ca.gov/docs/emon/pubs/anl_methds/emon-sm-05-018.pdf>.