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ASSIGNING VALUES TO NON-DETECTED/NON-QUANTIFIED PESTICIDE RESIDUES IN HUMAN HEALTH FOOD EXPOSURE ASSESSMENTS

Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, DC 20460

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List of Acronyms

ARs	Anticipated Residues
CFR	Code of Federal Regulations
FDA	Food and Drug Administration
FQPA	Food Quality Protection Act
FR	Federal Register
IR-4	Interregional Project #4
HED	Health Effects Division
LLMV	Lower Limit of Method Validation
LOD	Limit of Detection
LOQ	Limit of Quantitation
MAFF	Ministry of Agriculture, Fisheries, and Food
MLE	Maximum Likelihood Estimation
NDs	Nondetects
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
PDP	Pesticide Data Program
PHIs	Preharvest Intervals
RDF	Residue Distribution File
SRD	Successive Random Dilutions
USDA	United States Department of Agriculture

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ASSIGNING VALUES TO NON-DETECTED/NON-QUANTIFIED PESTICIDE RESIDUES

EXECUTIVE SUMMARY

Residue data are used by the EPA's Office of Pesticide Programs (OPP) to support the establishment or reassessment of a pesticide tolerance associated with a particular food use. In some cases, a portion of the measurements of the levels of pesticide residue present on food shows no detection of residues. These "nondetects" (NDs) do not necessarily mean that the pesticide is not present at any level, but simply that any amount of pesticide present is below the level that could be detected or reliably quantified using a particular analytical method.

The primary science policy issue concerning NDs is what value the OPP should assign to them when estimating exposure and risk from a pesticide in food. The reason this is an important issue stems from the new requirements that the Food Quality Protection Act of 1996 (FQPA) impose on EPA. Among other things, FQPA established a stringent health-based standard ("a reasonable certainty of no harm") for pesticide residues in foods to assure protection of the public health, including sensitive populations such as infants and children, from unacceptable pesticide exposure and risks. OPP's goal is to make exposure and risk assessments as accurate and realistic as possible while not underestimating exposure or risk, so that all humans, including infants and children, are fully protected. The specific issues addressed in this paper concern the values the Agency should assign to NDs in order to meet this goal.

In general, the Office of Pesticide Programs recommends use of a default value of ½ the Limit of Detection (LOD) or ½ the Limit of Quantitation (LOQ) for commodities which have been treated but for which no detectable residues are measured. This paper also describes OPP's policy of performing a "sensitivity analysis" to determine the impact of using different assumptions (e.g., assuming NDs = full LOD or full LOQ vs NDs = zero), on the OPP's risk assessment for the pesticide under evaluation. If it is demonstrated through the sensitivity analysis that the default assumptions have no effect on the final OPP risk decision, then there is little reason for OPP to attempt to further refine these default assignments.

If OPP finds that these default assignments do have a significant effect on the risk estimate or risk decision or decides that a more refined risk estimate is needed, a second, more accurate set of statistical methods can be used instead to determine the values or distribution of values for NDs. These statistical methods provide a more accurate way of estimating food exposure and risk than assuming that, for NDs, exposure occurs at ½ LOD or some other single, finite value

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and allowing risk assessors to *impute* a series of values which represent concentrations below the stated detection limit. These methods would generally be used only in situations where the NDs comprise a significant (but less than half) portion of the data set and the rest of the data are normally or lognormally distributed, but exceptions can be considered on a case-by-case basis.

This document was developed from two previous versions entitled *Assigning Values To Nondetected/Nonquantified Pesticide Residues into Human Health Dietary Exposure Assessments* and *A Statistical Method for Incorporating Nondetected Pesticide Residues into Human Health Dietary Exposure Assessments* that were released for public comment in December of 1998 (63 FR 67063-67066). The Agency received comments from various organizations. Each of the commenters offered recommendations for improving the science policy. All comments were extensively evaluated and considered by the Agency. This revised version embodies many of the sentiments and recommendations of the commenters. The public comments, as well as a detailed summary of the Agency's response to the comments are being made available in the Federal Register.

This document was drafted for the purpose of providing non-binding guidance to interested stakeholders regarding the evaluation of non-detects in pesticide risk assessments. It contains OPP's view concerning a reasonable way of addressing this issue. Although OPP will consider this guidance in evaluating risk assessments, this guidance does not bind OPP decisionmakers. Stakeholders remain free to comment on the application of the policy to individual pesticides or on the appropriateness of the policy itself. OPP will carefully take into account all comments that are received.

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I. Introduction

Pesticide manufacturers (i.e., registrants) who petition EPA to establish a tolerance are required to submit data on the level of pesticide residues that remain in or on food. Data on the levels of pesticide residues in food are also available from a number of other sources. Often, instrumentation in the laboratory is not able to detect any residue below the limit of detection (LOD). However, even though the laboratory instrumentation cannot detect a residue, a residue may be present at some level below the LOD, and may still present a potential concern to human health. This paper describes the OPP's policy for assigning values for use in human health exposure and risk assessment to non-detected/non-quantified pesticide residues in food. In general, and as described more fully later in the document, EPA recommends use of a value of ½ the analytical Limit of Detection (LOD), ½ the Limit of Quantitation (LOQ), the (full) Lower Limit of Method Validation (LLMV), or true zero for these non-detected residues.

This document was developed from two previous versions entitled *Assigning Values To Nondetected/Nonquantified Pesticide Residues into Human Health Dietary Exposure Assessments* and *A Statistical Method for Incorporating Nondetected Pesticide Residues into Human Health Dietary Exposure Assessments* that were released for public comment in December of 1998 (63 FR 67063-67066). The Agency received comments from various organizations. Each of the commenters offered recommendations for improving the science policy. All comments were extensively evaluated and considered by the Agency. This revised version embodies many of the sentiments and recommendations of the commenters. The public comments, as well as a detailed summary of the Agency's response to the comments are being made available in the Federal Register.

One issue that arises from use of the aforementioned default assumptions of ½ LOD, ½ LOQ, etc. is whether the Agency's method for assigning finite values to non-detects (NDs) in its risk assessments may either overestimate or underestimate risk depending on the actual distribution of data below the LOD. Specifically, the question arises as to whether OPP's default assumptions regarding the residue values to associate with non-detected or non-quantifiable residues are a significant factor in controlling the risk decision *per se.*

Should there be concern about the effect of OPP's default procedure of assigning one-half the limit of detection/quantification values to treated commodities with non-detected residues on the risk estimate or risk decision, this paper also describes OPP's policy of performing a "sensitivity analysis" to determine the impact of different assumptions (e.g., assuming NDs = LOQ or NDs = zero) on the Agency's risk assessment for the pesticide under evaluation. If it is demonstrated through the sensitivity analysis that the risk estimate or final risk decision is unaffected by the default assumptions, OPP will conclude that the relevant risk estimate is sufficiently "robust" so as not to warrant a more refined estimate of exposure and risk.

In those instances in which the default assignment is critical or decisive in determining

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OPP risk management action or it is simply desired that a more refined risk and exposure estimate which relies to a lesser extent on default assumptions be developed, one of a series of more accurate statistical methods can be used to estimate the values or distribution of values associated with the ND values. Such statistical methods provide a more accurate way of estimating exposure and risk from pesticides in food than assuming that exposure through the ND's occurs at ½ LOD or some other single, finite concentration. These methods are fully described in EPA's *Guidance for Data Quality Assessment: Practical Methods for Data Analysis* originally issued in July, 1996 (EPA/600/R-96-084), which has been peer reviewed by EPA program offices, regional offices, and laboratories (US EPA 1998a. In general, these methods would be used only in situations where the NDs compromise less than half the data set and the rest of the data are normally or lognormally distributed, but exceptions will be considered on a case-by-case basis. It is expected that many of the ND values obtained from this method would be less than ½ the LOD or LOQ but greater than zero.

The policy for assigning values to non-detectable residues is intended to avoid underestimating exposure to potentially sensitive or highly exposed groups such as infants and children while attempting to approximate actual residue concentrations as closely as possible. Both biological information and empirical residue measurements support EPA's belief that these science policies are consistent with these goals.

The policy paper is divided into several sections. Section I is this introduction. Section II, entitled "Assigning Values to Non-Detected/Non-Quantified Pesticide Residues," provides the rationale for assigning ½ LOD or ½ LOQ to commodities that have been treated with a pesticide, but that show no analytically detectable residues for any or all commodities sampled. The third section, entitled "A Statistical Method for Incorporating Non-detected Pesticide Residues," provides a more accurate, statistically-based method for estimating non-detected pesticide residues than simply assigning a default value of ½ LOD or ½ LOQ to these ND's. Section IV provides a list of references. The Appendix to this document is a sample calculation illustrating one (of many available) methods for calculating LOD or LOQ.

This science policy applies at this time only to exposure to pesticide residues via the food supply and, more specifically, only to the refinement of pesticide exposure from food by calculation of Anticipated Residues (ARs), a risk assessment refinement tool. This policy is not appropriate for, and is not to be used in, the determination of the actual residue level which will be established as the "tolerance" (maximum acceptable residue level) for a pesticide in a particular commodity.

This document was drafted for the purpose of providing non-binding guidance to interested stakeholders regarding the evaluation of non-detects in pesticide risk assessments. It contains OPP's view concerning a reasonable way of addressing this issue. Although OPP will consider this guidance in evaluating risk assessments, this guidance does not bind OPP decisionmakers. Stakeholders remain free to comment on the application of the policy to individual pesticides or on the appropriateness of the policy itself. OPP will carefully take into

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account all comments that are received.

II. Assigning Values to Non-Detected/Non-Quantified Pesticide Residues

A. Definitions

In the discussion of which values to assign to non-detected and/or nonquantified residues, it is important that consistent definitions be employed for the various terms being used to describe these concepts. Over the years, a variety of different practices have arisen due to definitional differences between LOD and LOQ, a lack of distinction between the two, preference for one over the other, the proliferation of several synonymous terms such as "limit of determination" or "limit of sensitivity," and the fact that there are situations in which one is, indeed, more appropriate to use than the other. In many cases, a sample is reported to contain nondetectable residues when, upon further investigation, the proper designation should have been "nonquantifiable," or vice versa. In a number of instances, OPP has noted in residue chemistry submissions that these terms have been inappropriately used, used interchangeably, or used without supporting documentation and/or information concerning their derivation. In OPP's policy, these terms will have the definitions provided below:

Limit of Detection (LOD). LOD is defined as the lowest concentration that can be determined to be statistically different from a blank. This concentration is recommended to be three standard deviations above the measured average difference between the sample and blank signals which corresponds to the 99% confidence level. In practice, detection of an analyte by an instrument is often based on the extent to which the analyte signal exceeds peak-to-peak noise (Keith et al., 1983). Samples that do not bear residues at or above the LOD are referred to as "non-detects" (NDs).

Additional, related concepts are the **Method Detection Limit (MDL)** which refers to the lowest concentration which can be reliably detected in either a sample or a blank and the **Instrument Detection Limit (IDL)** which refers to the smallest signal above background noise than an instrument can reliably detect.

Limit of Quantitation (LOQ). LOQ is defined as the level above which quantitative results may be obtained. The corresponding sample/blank difference is recommended to be 10 standard deviations above the blank which corresponds to the 99% confidence level (Keith et al., 1983) and to an uncertainty of ±30% in the measured value at the LOQ. LOQ is typically used to define the lower limit of the useful range of the measurement technology in use. Samples that do not bear residues at or above the LOQ are often referred to as "nonquantifiable."

Lower Limit of Method Validation (LLMV). There are cases in which a laboratory

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does not stringently determine the LOD and LOQ of a particular substrate/method/ equipment combination but, rather, a "Lower Limit of Method Validation" (LLMV) is reported that could be higher than the true LOQ within the capability of the method. The LLMV is simply the lowest concentration at which the method was validated. In these cases, neither the method limit of first choice (LOD) nor second choice (LOQ) was demonstrated and OPP would normally request that an LOQ be estimated by the study sponsor from the LLMV, chromatograms, and other available information. In general, OPP discourages the determination and use of the LLMV if a consequence of this is that a legitimate LOD or LOQ cannot or will not be determined.

To date, OPP has not issued formal guidance or suggested/recommended procedures, or made available a list of acceptable methodologies for the estimation of LOD and/or LOQ values for pesticide residue analyses. Due in part to the many valid operational definitions of LOD and LOQ and procedures used to estimate these, OPP believes it unwise to prescribe any one specific procedure or protocol as a standard universal requirement for pesticide registration submissions. Nevertheless, in the interest of informing registrants and other data submitters of at least one method for LOD/LOQ determination which has been acceptable in the past, an Appendix to this Science Policy document which illustrates this method is attached. This is a slightly modified form of a procedure used by USDA's IR-4 program and is published, in part, in 40 CFR (as 40 CFR Part 136, Appendix B).

Any reasonable generally recognized statistical procedure may be considered and will be evaluated. It is recommended that registrants and other data submitters fully document the procedures and protocols used to estimate the LOD and/or LOQ for review by OPP.

B. Refining Anticipated Residue Estimates Using ½ LOD or ½ LOQ for Non-detected Residues

Pesticide food risk assessments are initially conducted using conservative assumptions such as tolerance-level residues in crops, maximum theoretical livestock diets, highest field trial residue values, and 100% of the crop being treated. Worst-case assessments using such assumptions may result in an *apparently* unacceptable acute and/or chronic food risk. In such cases, refinement of food exposure assessments to derive more realistic estimates is often warranted. To further refine food exposure, calculations may include use of percent-crop-treated data, more realistic livestock diets, averages of field trial data, statistically-collected monitoring data from the U.S. Department of Agriculture (USDA) or the Food and Drug Administration (FDA), and/or incorporation of residue reduction factors to account for cooking or processing. In some cases, probabilistic analyses of composited or even single serving-size samples may be used. The use of these anticipated residues (AR's) produces more refined exposure estimates which more closely approximate the pesticide residues that humans will actually consume in their diets. The ways in which the data are refined depends on such considerations as what data are available, the relative confidence the Agency has in these data, the residues of toxicological significance, which of these residues are detected by the analytical method(s) used, as well as the

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metabolic profile over time.

Not infrequently, data on anticipated residues contain at least some measurements for which the chemical analyst reported residue concentrations at levels "below the limits of detection or quantitation." The fact that no residues are detected does not necessarily mean there are none. Residues may exist at levels that are too low to detect. If the Agency has information demonstrating that a crop sample *was* treated with the pesticide in question, but residues were *not* analytically detected, there are a number of options available for dealing with these nondetectable values and integrating this information into pesticide food exposure assessments. The two extreme options would be 1) assume that if residues were not detected, that they were not present (i.e., residues concentrations are zero); or 2) assume that if residues were not detected (at some limit of detection), that they were present at *just* below that limit of detection. The first option would lead to the least conservative (i.e., most likely to underestimate the actual average residue level in the ND samples) exposure estimate since the Agency would be assuming nondetectable residues were actually zero; the second option would result in the most conservative (i.e., least likely to underestimate the actual average residue level in those samples) estimate since the Agency would be assuming that nondetectable residues were actually present at *just* below the analytical limit of detection.

OPP believes that neither approach reasonably represents reality, particularly in data sets in which many nondetects are present. Rather, biological information and empirical residue measurements indicate that residue data sets (including the NDs) are often lognormally distributed. On a theoretical basis, concentrations of pesticides in food crops might be expected to be a Random-Product process and the Theory of Successive Random Dilutions (SRD) would predict that concentrations of pesticides would be lognormal (Ott, 1995). In addition, a fair amount of empirical evidence for a lognormal distribution of pesticides in foods exists from a recent study by the United Kingdom's Ministry of Agriculture, Fisheries, and Food (MAFF) in which thousands of individual serving sized samples were analyzed for a variety of pesticides and found to follow in most cases a lognormal distribution (MAFF, 1997).

Given the above information, OPP recommends (as an initial step in the exposure assessment process) use of a residue value of ½ LOD (or ½ LOQ if an LOD has not been determined) or the LLMV, as appropriate, for samples with no detectable residues <u>if it is known or believed that these samples have been treated with a pesticide</u> according to the following protocol:

- OPP generally recommends use of a value of zero for the proportion of the data set corresponding to the percentage of the commodities known **not** to be treated with pesticide (see Section II.D.).

- For the remainder of the data points for pesticide-treated commodities, OPP recommends as its preferred approach use of the following assumptions:

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(1) if a valid Limit of Detection (LOD) exists, use ½ LOD as the assigned value for NDs when conducting food exposure and risk assessments;

(2) if an LOD is not available, but a valid Limit of Quantification (LOQ) exists, use ½ LOQ for the NDs;

(3) if neither an LOD nor an LOQ is available, use the Lower Limit of Method Validation (LLMV) for the NDs; and

(4) if both LOD and LOQ are determined and if nonquantifiable residues are detected between the LOQ and LOD, use ½ LOQ for those measurements.

In general, OPP considers that the "replacement" or "substitution" method (replacing treated non-detects with ½ LOD or ½ LOQ) will result in reasonable estimates of risk and exposure if the number of non-detects is small (e.g, 10-15%). The use of ½ LOD or ½ LOQ for nondetectable residues in samples is widely used in the risk assessment community and is advocated by EPA (EPA, 1998a) when the appropriate conditions are met. Registrant's are encouraged to use the substitution method in these instances and OPP would perform sensitivity analyses routinely in these situations only on a case-by-case basis. When the number of non-detects increases to greater than ca. 10-15% (but is still less than 50%) risk assessments should be performed using the replacement method, but the effect of the substituted values should be assessed by performing a sensitivity analysis and verifying that the relevant risk and exposure estimates are not significantly affected. Such an analysis should be included as part of the risk characterization. If it is determined that the effect of this substitution is significant, it may be desirable to use statistical methods developed for censored data (as explained in Section III of this document). When data sets consist of >50% non-detects, the handling of ND's should be considered on a case-by-case basis and no general rule of thumb is possible.

Additional details concerning this procedure and assignments are provided below:

(1) Policy for NDs When a LOD Has Been Properly Determined

The selection of a numerical value to represent NDs in a refined exposure assessment depends on the level of confidence OPP has in the supporting documentation of the various method limits under consideration. For OPP to have a high level of confidence, the claimed LOD should be demonstrated using chromatograms, calculations, and statistics as noted above. Although there are a variety of acceptable techniques which can be used to estimate the LOD or LOQ, one example which would acceptable is shown in the Appendix to this policy paper. The information provided in this attachment is only an illustrative example. Data submitters are free to use any reasonable and scientifically supportable methodology. In any case, and in accordance with OPPTS Test Guidelines - Residue Chemistry 860.1340(c)(2)(iii), the procedures used by a laboratory to determine the LOD and LOQ should be fully explained and/or copies of any

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appropriate publications should be submitted with the analytical method description to the Agency.

OPP recommends that the actual numerical value used to represent ND residues and to be entered into the acute or chronic AR calculation should be ***½ LOD***. Particularly in those cases in which acute food risk is only marginally acceptable and ½ LOD is used for a significant portion of the samples, this assumption should be mentioned in the risk characterization and the use of a sensitivity analysis should be considered (see Section II.C. of this document).

(2) Policy for NDs When Only An LOQ Has Been Properly Determined

If an appropriate LOD has not been properly determined, OPP scientists will examine whether an LOQ has been experimentally and statistically demonstrated and if a given sample with ND residues may be adequately represented by ½ LOQ as demonstrated by chromatograms and other information. *OPP recommends that the actual numerical value to be entered into the AR calculation should be* ***½ LOQ***.

(3) Policy When Neither a LOD Nor LOQ Has Been Properly Determined

If neither the LOD nor the LOQ has been properly determined, the **full LLMV** (lowest concentration at which the method was validated) generally will be used in risk assessment. The rationale for this policy is that the Agency has less confidence in data samples when an LOD or LOQ cannot be statistically determined or reasonably estimated from the data. In general, if a LLMV is reported instead of an LOQ, it is likely that insufficient analyses were performed and a ½ LOQ value could not be calculated with sufficient statistical rigor and precision to be reliably used in a risk assessment. Accordingly, to assure that actual exposure to pesticides in food will not be underestimated using such data, the OPP will use the full LLMV for each ND of a treated sample in this situation. OPP actively discourages a registrant from choosing to use or report a LLMV if this is to be used as a substitute for a properly-determined LOD or LOQ. However, OPP believes that in many cases a *rigorously-determined* LLMV (e.g., one in which numerous determinations were made at levels close to the LOQ and appropriate statistical methodologies can be used) can be used to estimate an LOD or LOQ. In these cases, OPP recommends use of the ½ LOD or ½ LOQ default, as appropriate, in risk assessments.

(4) Policy When Detectable But Non-quantifiable Residues Are Found

If a sample contains detectable, yet nonquantifiable residues, i.e., residues falling between the LOD and the LOQ, OPP recommends that such samples typically be represented numerically in the refined exposure assessment as ***½ LOQ*** *when assessing both acute and chronic risk.* This science policy is consistent with the extensively peer reviewed "OPPTS Test Guidelines Series 875 - Occupational and Residential Exposure" which states that ½ LOQ should be used to represent samples bearing detectable residues between the LOD and LOQ. This is also consistent with the USDA Pesticide Data Program's (PDP) policy for reporting these values: all

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residues detected at >LOD but <LOQ by the PDP program are reported as ½ LOQ.

If information is available indicating that most residue values are just above the LOD or just below the LOQ, a decision will be made on a case-by-case basis regarding the appropriate value to assign to NDs. The rationale for selection of a residue value different from ½ LOQ for these commodities should be explained clearly in the risk characterization. If available and clearly supported by raw data (chromatograms, etc.), the analyst's estimate of the residue between the LOD and the LOQ may, at the discretion of the OPP, be used as a means of further refinement of the estimated exposure. If a significant portion of the residue values was derived via the analyst's estimation of values between the LOD and LOQ, this should be noted in the risk characterization.

C. Sensitivity Analysis

In general, assigning numerical values to NDs as described above is not expected to significantly affect OPP's risk estimate. However, OPP, under certain circumstances, will perform a sensitivity analysis if it is believed that the substitution of ½ LOD, ½ LOQ, or LLMV values for nondetects has significantly affected the outcome of a risk assessment and/or OPP's risk decision. That is, if the OPP risk assessment shows *unacceptable* risks when ½ LOD values are used for nondetects, EPA will attempt to demonstrate that the use of ½ LOD has not *by itself* significantly affected the risk assessment by *re-estimating* risks with zero substituted for ½ LOD or ½ LOQ.

Conversely, if the risk assessment shows *acceptable* risk when ½ LOD values are assigned to nondetects, we will re-estimate the risks, where appropriate, with the full LOD or LOQ substituted for ½ the LOD or LOQ. This latter substitution will never change the estimated exposure (and risk) by more than a factor of 2 (and then only if all crops were considered treated and if all values were ND). If the Agency risk assessment changes substantially as a result of assigning these alternate values, the sensitivity analysis will have demonstrated that the Agency risk assessment is sensitive to assumed concentrations for the nondetects. OPP may then request that additional data and/or an improved analytical method be developed and submitted. To date, the conduct of these sensitivity analyses has not resulted in a significant change in the upper percentiles of estimated acute exposures.

D. Use of Percent of Crop Treated

Notwithstanding the above discussion, OPP believes it to be appropriate to use "true zeroes" for those ND's which represent untreated crops and OPP continues to support the use of "true zero" for those samples which have not (or are not expected to have been) been treated with the pesticide of interest. Specifically, exposure assessments will generally be performed with the non-treated samples incorporated as "true zeroes."

The Agency will determine which "nondetect" samples should be represented by zero in a ratio directly proportional to the percent of crop not treated. In calculating *average* residues when a

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variety of limits of detection exist, the average residue value calculated will incorporate **a weighted** average of the LODs **from treated commodities in which no residues were detected**[1]. Such a calculation will not incorporate one-half of the overall average LOD from all laboratories. For example, if 70% of a crop is not treated, but 80% of the monitoring samples in a data set is reported as <LOD, then 70% of the samples would be assigned a value of zero, 10% would be designated as ½ LOD, and 20% of the samples would be assigned the reported residue values. If more than one LOD is reported for the samples in the data set, one-half of the weighted average of the LODs would be used. An illustration of this calculation is shown in the above box. Similarly, in those cases where it is necessary to construct an electronic residue file for an acute exposure assessment (and the average residue values are not appropriate), the file should be constructed such that the treated non-detect samples are assigned a weighted average of the LODs in which no residues were detected. An example of how this file would be established is illustrated in the box on the previous page.

Suppose that 30% of apples are treated with a pesticide (and 70% are therefore not treated), but a PDP survey of 5 lb. composite samples shows that 80% (i.e., a total of 240 samples) of the 300 samples collected have ND (not detected or less than detection limit) residues. Three-quarters of those PDP ND values have a limit of detection of 0.05 ppm and one-quarter of the ND values have LOD's of 0.10 ppm. We wish to calculate the *average* residue in apples for use in a chronic pesticide food residue assessment.

Given this information, we would conclude that 70% of the 300 composite apple samples contain no (or zero) residues since they were not treated with pesticide. This means that 210 of the 300 composite samples are true zeroes (70%). From this, it follows that 210 of the 240 ND values (or 87.5% of the ND's) represent true zeroes with the remaining 30 ND values (or 12.5% of the 240 NDs) representing treated apples with residues at less than the detection limit. To calculate residues in these treated samples, we would assign one-half the 0.05 ppm LOD to three quarters of these ND's (representing an expected 22.5 of the 240 ND samples) and one-half the 0.10 ppm LOD to the remaining one-quarter of these ND's (representing an expected 7.5 of the 240 ND samples). The average residue for use in a chronic assessment would therefore be calculated as follows:

$$\frac{(210 \times 0 \text{ ppm}) + (22.5 \times 0.025 \text{ ppm}) + (7.5 \times 0.05 \text{ ppm}) + \Sigma \text{ (all >LOD values)}}{300}$$

If the residue data were to be used instead to establish an electronic residue file for use in an *acute* probabilistic assessment, the file would contain 210 true zeroes and 30 values at 0.0313 ppm (i.e., ½ the weighted LOD), with the remaining 60 values represented by their >LOD measurements (at either ½ LOQ or >LOQ, as appropriate)

E. Considerations Related to Pesticides Having Analytes of Concern

The LOD and/or LOQ is often not established for all residues of toxicological significance. In some situations, the method may, in fact, be incapable of determining the residues at all. This may particularly be true for multi-residue monitoring methods. For example, FDA often reports only residues of the parent compound. USDA's Pesticide Data Program (PDP) often attempts to analyze all residues of toxicological significance; however, there are certain metabolites of

[1] A range of interlaboratory LOD variation of up to 35x has been observed for a single chemical/crop combination in one residue monitoring data set.

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concern that are not sought by PDP due to analytical difficulty or due to the unavailability or expense of analytical standards. As a result, difficulty arises when attempting to sum the residues of multiple analytes of concern because a numerical limit is not available to assign to nondetectable levels of one or more of the residues of concern. Such shortcomings may render one or both sources of monitoring data of limited value to the refinement of pesticide food residue exposure estimates unless metabolism studies and other information can be used to establish a ratio between the concentration of one or more analyte(s) to the concentration of toxicologically significant residues not determined by the method. Decisions on how to use such residue data will be made on a case-by-case basis.

F. Essentially Zero Residues : Use of Zero (or Near Zero) Residue Concentrations in Risk Assessments

A number of instances may arise in which it is appropriate to assume for risk assessment purposes that residue values so closely approach zero that this value (rather than ½ LOD or ½ LOQ) should instead be used in the exposure assessment. Explanation of the rationale and further illustration of situations where this might be appropriate are detailed in HED's SOP 99.6 (8/20/1999) from which the following is excerpted (US EPA 1999):

> ... [I]t may be appropriate in certain cases to judge that the ND values from the monitoring data are "essentially zero," particularly if a substantial portion of the measured residue values is less than the analytical detection limit (and would therefore ordinarily be replaced by ½ LOD). In these instances, it may be appro priate to introduce a value of zero ppm (or near zero) as a residue value (in place of ½ LOD) for the ND measurements in the risk assessment. This judg ement should be made on a case-by-case basis, with the reviewer bringing a wide range of information to bear on proper valuation of the NDs, including the nature of distribution of the values above the detection limit, the perc ent of the crop which is treated, and information on the processing of commodities before sampling;

For example, information from a radiolabel metabolism study or a field trial conducted at an exaggerated rate may be available which indicates that residues of concern are present at levels much lower than ½ LOD. Alternatively, theoretical calculations based on mass balance considerations, for example, may demonstrate for a seed-treatment use that resulting residues in the harvested crop would be expected to be much less than ½ LOD. Other factors, perhaps when considered jointly, might warrant consideration in this evaluation and suggest that resulting residues would be near zero. For example, for a blended processed commodity such as corn oil, it might in some instances be appropriate to assume measured ND values from monitoring studies represent real zero (or near zeroes) concentrations after consideration of the percent of the corn crop which is treated, the range of actual application rates and pre-harvest intervals, and processing/cooking factors. For example, if only 5% of the corn crop is treated, residue field trials on the raw agricultural commodity show average residues of 0.1 ppm, and processing of corn into corn oil has been demonstrated to reduce residues 15-fold, it would be reasonable to

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assume that average residues are less than 0.0003 ppm and thus any ND value in a corn oil monitoring survey could be assumed to be less than this calculated average. In many cases, it may be a variety of factors which, when considered together, would lead the risk assessor to support a decision to replace <LOD measurements with zero (or near zero) values in the risk assessment.

Alternatively, a value of zero may be appropriate to represent "nondetects" for one or more **analytes of concern** provided this decision is supported by such information as metabolism studies, data at shorter preharvest intervals (PHIs), exaggerated rate data, etc. This approach may be appropriate only for certain crops or certain use patterns. On a case-by-case basis, plant or livestock metabolism data, data reflecting exaggerated application rates and/or short PHIs, close examination of the chromatograms, consideration of the analytes determined by the analytical method(s), and other information may be used singly or in conjunction to formulate a weight-of-the-evidence argument in favor of (or against) use of true zero to represent the level of one or more analytes of toxicological concern potentially present in samples denoted as bearing less than LOD/LOQ residues. This procedure could be particularly important for pesticides having several residues of toxicological concern whereby, using the above information, the chemist gains confidence that only a subset of the terminal residues will be present at normal harvest time; zeros could be used for the other analytes of concern. On an international level, a similar approach is used by the Food and Agriculture Organization/World Health Organization's Joint Meeting on Pesticide Residues in the case of pesticides having a chronic toxicological endpoint.

III. A Statistical Method for Incorporating Non-Detected Pesticide Residues

There may be instances in which a significant portion (e.g., more than 15%) of the residue data set contains non-detectable residues, when a sensitivity analysis reveals an inordinate effect of the ½ LOD or ½ LOQ assumption on the risk decision, or when it is simply decided that a more accurate assessment of residue levels is appropriate. This section of the policy paper describes how residues levels below the LOQ may be estimated using statistical imputation methodologies. Use of such methodologies should produce a more accurate estimate of <LOD and <LOQ residues.

When the appropriate recommended conditions are met (see below), statistical imputation methodologies are useful for predicting the distribution of non-detectable residues below the LOD in cases where some of the residues of the data set are undetectable. Here, statistical imputation refers to imputation procedures for left-censored data[2]. When properly employed, such methods

[2] *Imputation*, in general, can refer to procedures applied to other aspects of pesticide food residue exposure. For example, imputing single-serving residue values from composite samples is on form of imputation (which is perhaps more aptly referred to as a data deconvolution exercise). Similarly, construction of empirical distribution functions (EDFs) is a form of imputation as well that results in data interpolation. In the context of this paper, the term "imputation" refers to imputing (or "data uncensoring") of left-censored data.

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can provide a scientifically sound basis for more accurately estimating pesticide food residue exposure and risk than assuming that exposure occurs at ½ LOD or some other single, finite value. This method is intended to be used chiefly by persons conducting probabilistic human health pesticide food residue exposure assessments for purposes of registration, reregistration, or tolerance assessment of pesticides.

Briefly, the methods described below use the information provided by the uncensored portion of the data (i.e., that portion of the data with >LOD values) and the assumed normal (or transformed normal) distribution of the data[3] to calculate a mean and standard deviation which incorporates the data which lie below the detection limit in the "censored" region of the data. Cohen's method requires that the distribution be normal (or can be made normal) and that there be only a single LOD or LOQ for all analyses of the same commodity. It will result in an estimated mean (i.e. arithmetic average) concentration which incorporates the <LOD values and can be used in a chronic assessment or in an acute assessment for those commodities for which the use of a mean value is appropriate (e.g., blended commodities). By mentioning several specific methods, OPP does not mean to imply that other methods are not appropriate for this task. Whichever method is selected, OPP recommends that the method be adequately supported by both a sufficiently rich data set above the detection limit and a statistically-robust methodology for imputing those values.

Under the methods presented below, those measured values which lie above the LOD but below the LOQ should be considered as being "semi-quantitative." In contrast to the methodology described in Section II of this document, in which ½ LOQ is generally used as a default assumption for all values which lie between the LOD and LOQ, the actual measured "semi-quantitative" value should instead be used when working with methods for censored data.

A. Cohen's Method

Cohen's Method is a technique which can be used to more accurately determine mean residue values from heavily "censored" data sets, i.e., data sets for which a substantial amount of data (e.g., 15-50%) are simply reported as less than a given detection or quantitation limit. Cohen's method is fully described in EPA's *Guidance for Data Quality Assessment: Practical Methods for Data Analysis* originally issued in July 1996 (EPA/ 600/R-96/084) and in several other publications described in the Reference section of this document (U.S. EPA, 1998a). The

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[3] This assumption of normality (or lognormality) should be verified prior to use of these methods. The reference to an "assumed normal distribution" is made to reflect a common statistical convention that one cannot *prove* a given distribution belongs to a hypothesized family of distributions (e.g, normal, lognormal, Poisson, etc.), but rather can only provide sufficient evidence to suggest that the hypothesized distribution is not inconsistent with actual distribution (analogous to either "rejecting" or "failing to reject" a hypothesis). If there is insufficient evidence to demonstrate that a distribution is not normal, then it is reasonable to refer to it as an "assumed normal" distribution.

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EPA publication is available on the Internet at
http://www.epa.gov/r10earth/offices/oea/epaqag9.pdf .

The method is designed to be used only for data points which are part of a parent population which is normally distributed or that can be made normal via transformation. Practically, this means that the parent population should have either a normal or log-normal distribution. Thus, prior to using this method, the existence of a normal (or transformed log-normal) parent population should be demonstrated. It is strongly recommended that the data be graphed on appropriate probability paper and that normality tests (e.g., Shapiro-Wilk) be performed to verify the assumed distribution. Various statistical procedures (with associated examples) which could be used to accomplish this task are available in the document *Guidance for Submission of Probabilistic Human Health Exposure Assessments to the Office of Pesticide Programs* dated 11/4/98 (U.S. EPA, 1998b) which is available on the world wide web in draft format at http://www.epa.gov/fedrgstr/EPA-PEST/1998/November/Day-05/o-p296 65.htm.

Additional recommended criteria for use of Cohen's methodology is that not more than 50% of the data set be censored (ideally, less than 20% should be censored) and/or at least 10 non-censored data points (with 20 or more being strongly desirable) be available. Exceptions to these recommended criteria can be made on a case-by-case basis. However, with respect to the exceptions, it should be remembered that in many cases it is likely that a more refined estimation procedure such as Cohen's method is being using precisely because the insertion of ½ LOD for ND residues resulting in risk above OPP's level of concern while the substitution of 0 ppm for ND's resulted in risks below OPP's level of concern. That is, in many cases Cohen's method will be used because OPP's risk estimate or resulting decision is very sensitive to assumptions about values to assign to ND residues. Thus, OPP is justified in recommending stricter criteria for use of Cohen's method than might normally be used in attempting to estimate a "best" estimate of a mean residue value.

It is important to note that, when using USDA's Pesticide Data Program (PDP) or other monitoring data to calculate an average residue for use in a risk assessment, the percentage of the data set which represents "true zeroes" (i.e., not treated) should be eliminated from the data set before considering whether the procedure in this document is applicable. For example, if 80% of a crop is not treated, but 90% of the PDP values are reported as NDs, the not treated portion of the data should be removed from the data set; the remaining NDs (i.e., 10% of the original sample) would be considered to represent treated commodities which have residues at levels lower than the LOD. Thus, in this case, 50% of the data would be censored (10% of the samples are ND and 10% of the samples are greater than the LOD).

Since Cohen's method is designed for use with a distribution that is normal, the logarithms of the data should be used if the data are log-normally distributed with the resulting mean and standard deviation of the (original) untransformed data back-calculated using the following formulae for the mean and standard deviation respectively:

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$$M_a = \exp(M_L + 0.5\ s_L^2)$$

$$s_a^2 = M_a^2\ [\exp(s_L^2) - 1]$$

where M_a is the arithmetic mean of the original (untransformed) residue values, M_L is the mean of the logarithms of the residue values, s_L is the standard deviation of the logarithms of the residue values, and s_a is the standard deviation of the original (untransformed) residue values.

In general, the criterion that the data be normally (or lognormally) distributed is not expected to present an impediment to the widespread application of this technique. On a theoretical basis, concentrations of pesticides in food crops might be expected to be a Random-Product process and the Theory of Successive Random Dilutions (SRD) would predict that concentrations of pesticides would be lognormal (Ott, 1995). In addition, a fair amount of empirical evidence for a lognormal distribution of pesticides in foods exists from a recent study by the UK's Ministry of Agriculture, Fisheries, and Food (MAFF) in which thousands of individual serving sized samples were analyzed for a variety of pesticides and found to follow in many cases a lognormal distribution (MAFF, 1997).

Briefly, Cohen's technique for censored samples involves the following steps for log-normally distributed data (derived from Perkins, et al., 1990):

1. Determine N = total sample size

2. n = number of quantitated measurements

3. $h = (N-n)/N$

4. Transform the uncensored measurements to logarithms

5. Determine $\ln(LOD) = X_o$

6. Determine $\underline{S}_L^2/(\underline{X}_L - X_o)^2 = \gamma$ where $\underline{X}_L$ and $\underline{S}_L^2$ are the mean and (population) variance of the log transformed detectable data, respectively[4].

7. Using appropriate tables (e.g., in US EPA, 1996 or Perkins) with h and γ, find γ

8. $M_L = \underline{X}_L - \gamma(\underline{X}_L - X_o)$

9. $s_L^2 = \underline{S}_L^2 + \gamma(\underline{X}_L - X_o)^2$

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[4] Cohen's paper (Expression 2.5.5) indicates he is using *n* in the denominator, rather than *n*-1. The use of *n* in the denominator is more commonly associated with a population variance formula while the use of *n*-1 is associated with the sample variance formula.

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10. $M_a = \exp(M_L + 0.5\ s_L^2)$
$s_a^2 = M_a^2\ [\exp(s_L^2)-1]$

An example of the use of Cohen's method is provided in the box to the right (derived from Gilbert, 1987, p. 183).

B. Estimation of Specific Values That Lie Below the Detection Limit

Cohen's method is appropriate for use in cases where it is sufficient to calculate a mean residue value and the basic distributional and other requirements are met. In general, the use of a mean value in a risk assessment is appropriate if a chronic analysis is being performed or if it is actually the mean value in an acute analysis which is of interest. In certain instances, it may not be sufficient to simply obtain the mean (and standard deviation) of a data set by use of Cohen's method. For example, it may be desired to perform a Monte-Carlo analysis using data from a market basket survey in which single serving sized samples were analyzed and many NDs were obtained. Or it may be required to insert data from field trials with many ND values into a Monte-Carlo analysis. In these cases, it is the *entire set* of individual residue values (or their estimates) which are desired and not simply the mean or values which are greater than the LOD.

In these cases, it may be possible to use the information provided by the *uncensored* portion of the data to *impute* those values which lie below the detection limit in the "censored"

Concentrations of a pesticide in 10 samples of an agricultural commodity are as follows (in ppm):<0.2, 0.45, 0.60, 0.76, 1.05, 1.12, 1.20, 1.37, 1.69, and 2.06. The LOD is reported at 0.2 ppm. A statistical evaluation (not shown) demonstrates that these values are consistent with a lognormal distribution. The natural logarithms of these concentrations are (respectively) as follows:

ND, -0.7985, -0.5108, -0.2744, 0.04879, 0.1133, 0.1823, 0.3148, 0.5247, and 0.7227

Using this information, the following results would be generated:

1. N = total sample size = 10

2. n = number of quantitated measurements = 9

3. $h = (N-n)/N = (10 - 9)/10 = 0.1$

4. The natural logarithms of these concentrations are (respectively) as follows:
ND, -0.7985, -0.5108, -0.2744, 0.04879, 0.1133, 0.1823, 0.3148, 0.5247, and 0.7227

5. $\ln(0.20) = -1.6094$

6. $\underline{X}_L = 0.03588$; $\underline{S}_L^2 = 0.21193$;

   $\gamma = 0.21193/(0.03588 + 1.6094)^2 = 0.07829$

7. $\gamma = 0.1164$

8. $ML = 0.03588 - 0.1164(0.03588 + 1.6094)$
   $= -0.1556$

9. $s_L^2 = 0.21193 + 0.1164(0.03588 + 1.6094)^2$
   $= 0.5270$

10. $Ma = \exp[-0.1556 + 0.5(0.5270)] = 1.114$

    $s_a^2 = (1.114)^2[\exp(0.5270)-1] = 0.8611$

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region of the data.[5] As in the case with Cohen's method used to calculate a mean residue value, those data which are greater than the detection limit should be *demonstrated* to follow a specific hypothesized distribution; it is this specific distribution which is used to extrapolate residue values into the less than detection limit (or censored) region of the data. The resulting imputed values can then be used directly in a Monte-Carlo assessment.

A variety of ways are available to impute values which lie below the detection limit in the censored region of the data (i.e., imputing of left-censored data), and there is an extensive literature on this topic (see Reference list). OPP is not advocating specific ways in which this statistical imputation can or should be done, but rather simply emphasizing that, whichever methodology is selected, it should be adequately supported. In general, these methods should be used only when it has been demonstrated that the relevant risk estimate [e.g., chronic risk based on mean input values or acute risk based on the distribution of input values and a high-end (output) exposure percentiles] or risk management decision is sensitive to the assumption that ND values are equal to ½ LOD (or ½ LOQ). In general, these techniques will only be used if more than 10-15% of the data are non-detects.

One popular method for this imputation procedure is Helsel's Robust Method (Helsel, 1990; ILSI, 1998). This method can be used to extrapolate a distribution to the region of the censored portion of the data, and hence generate "replacement values" for those measurements that are simply reported as "below detection limit." As stated by Helsel,

> These methods combine observed data above the reporting limit with below-limit values extrapolated, assuming a distributional shape, in order to compute estimates of summary statistics. A distribution is fit to the data above the reporting limit by either MLE or probability plot procedures, but the fitted distributi on is used only to extrapolate a collection of values below the reporting limit.

In general, the fitting of the distribution is done by either Maximum Likelihood Estimation (MLE) procedures or by probability plot procedures (which generally require that there be only one censoring point). Roughly speaking, the MLE procedures use, in a complex iterative mathematical optimization procedure, the reported above detection-limit values and the values of the detection limits to estimate the parameters of the family of distribution (e.g., normal, lognormal) which "maximize the likelihood" of observing the data actually observed. Once the values of the defining parameters have been obtained, they are used to generate replacement, or "fill-in", values for the below detection limit observations. A sample plot in which Helsel's

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[5] It is important that the non-treated NDs be removed from the distribution prior to performing statistical calculations (as discussed under the Cohen's Method section). Of course, one can instead simply substitute ½ LOD or ½ LOQ, as appropriate, for the NDs in a Monte-Carlo analysis.

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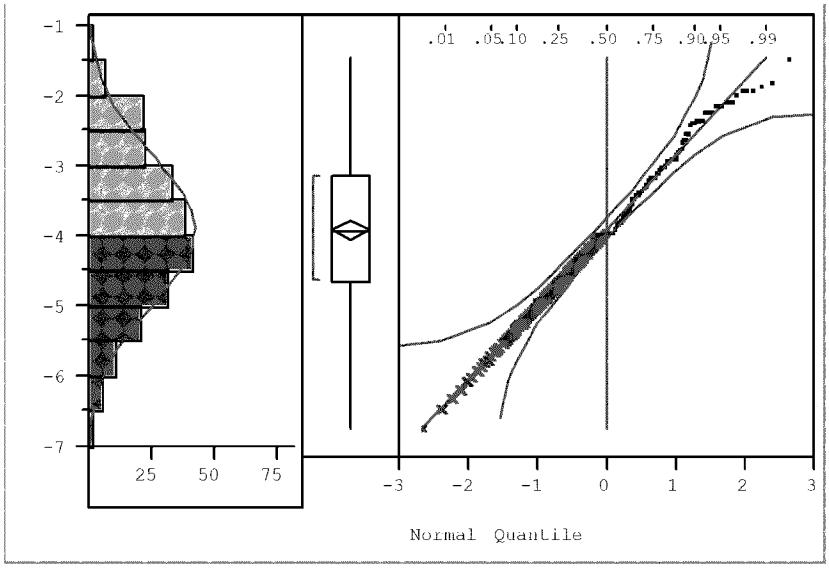
procedure and MLE techniques were used is shown to the right[6]. The plot used recent single-serving data generated by USDA's Pesticide Data Program (PDP).



The probability plot procedures (also referred to as "regression on order statistics" or "regression on expected normal scores"), in contrast, can be easily computed with standard statistical software which estimate the intercept and slope (representing the mean and standard deviation, respectively) of a line fit to the data above the detection limit. As stated in Helsel:

> The robust probability plotting method for a single reporting limit can be computedeasily by most commercially available statistics software. Normal scores (NSCORES of Minitab or PROC RANK within SAS, for example) first are computed with all less-thans set to slightly different values all below the reporting limit. Second, a linear regression equation is developed using only the above-limitobservations, where log of concentration is the y variable and normal scores the x variable. Estimates for the below-limit data then are extrapolated using this regression equation for normal scores for the below limit data. Finally, extrapolated values are retransformed into units of concentration, combined with above-limit concentration data, and summary statistics computed.

In either case, the extrapolated below detection limit values can be combined with the actual above detection limit values to produce a discrete data set which can be used as input data in a Monte Carlo probabilistic assessment. Details of this how this could be performed are available in the literature. Briefly, a distribution (e.g., normal, lognormal) which is defined by a mean and standard deviation could be used to impute those values which lie below the detection limit by using the inverse cumulative distribution function, Φ^{-1}, where $\Phi^{-1}(p)$ = z-score and p = n/N+1 (assuming a normal distribution). Any imputed non-detect values used to "fill-in" the distribution (i.e., replace the <LOD values with more appropriate single-valued finite estimates) would be calculated as follows:

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[6] Residue concentrations are plotted as their natural logarithms. The darker portion of the histogram and the large X's in the normal probability plot represent imputed "fill-in" values calculated by MLE methods.

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"Fill-in" value = z-score * SD + mean.

For example, suppose that there are 100 data points of which 98 are above the detection limit and two are below the detection limit. Further supposed that calculation via Cohen's (or any other) method results in an estimated mean of the distribution of 10.0 and a standard deviation of 2.0. The values would then be ranked, and (since there are 100 total values), the first non-detect would occupy the 0.01 quantile and the second non-detect would occupy the 0.02 quantile. The corresponding p values would be calculated as $p_1 = 1/(100+1) = 0.0099$ and $p_2 = 2/(100+1) = 0.0198$ for the first and second ND values, respectively. Using a normal probability table, one would determine that $\Phi^{-1}(p_1) = \Phi^{-1}(0.0099) = -2.33$ and $\Phi^{-1}(p_2) = \Phi^{-1}(0.0198) = -2.06$. The fill-in values associated with these two z scores are -2.33*(2.0) + 10.0 = 5.34 and -2.06*(2.0) + 10.0 = 5.88. Thus, 5.34 and 5.88 would be the two fill-in values associated with the two non-detects.

A publicly available software program is available to implement this and other procedures (UNCENSOR v. 4.0, Newman et al.) and is distributed free of charge. Although OPP has not reviewed or tested this software, registrants and other data submitters and interested parties may be interested in investigating its use.

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APPENDIX 1

To date, OPP has not issued formal guidance or procedures, or made available a list of acceptable methodologies for the estimation of LOD and/or LOQ values for pesticide residue analyses. Due in part to the many valid operational definitions of LOD and LOQ and procedures used to estimate these, OPP believes it unwise to prescribe any one specific procedure or protocol as a standard universal requirement for pesticide registration submissions. Any reasonable, generally recognized procedure consistent with the aims and requirements of regulatory exposure estimation and risk assessment practices of OPP may be considered and will be evaluated.

OPP notes, however, that there may be some confusion in the regulated community with respect to LOD and LOQ issues. This is likely due, in part, to the plethora of definitions for LOD and LOQ, a lack of distinction between the two, organizational preference for one over the other, and the proliferation of several synonymous terms such as "limit of determination" or "limit of sensitivity," In a number of instances OPP has noted in residue chemistry submissions that these terms have been inappropriately used, used interchangeably, or used without supporting documentation and/or information concerning their derivation. In many cases, OPP has found that a sample is reported to contain nondetectable residues when, upon further investigation, the proper designation should have been "nonquantifiable," or vice versa. This confusion over the definition of LOD (or LOQ), and the implicit statistical concepts which are central to its definition, is alluded to by Berthoux (1994)

> The method limit of detection or method detection limit (MDL) is based on the ability of a measurement method to determine an analyte in a sample matrix, regardless of its source of origin. Processing the specimen by dilution, extraction, drying, etc. introduces variability and it is essential that the MDL include this variability.
>
> The MDL is a statistical concept, although it is often thought of as a chemical concept, because it varies from substance to substance and it becomes possible to measure progressively smaller quantities as analytical measurements improve. Nevertheless, the MDL is a statistic that is estimated from the data. As such, it has no scientific meaning until it is operationally defined in terms of a measurement process and a statistical method for analyzing the measurements that are produced. Without a precise statistical definition, one cannot determine a numerical value for the limit of detection or expect different laboratories to be consistent in how they determine the limit of detection.
>
> Many definitions have been published. They may differ in detail, but broadly speaking they are all defined in terms of a multiple of the standard deviation of measurements on blank specimens, or, alternatively, on specimens that have a very low concentration of the analyte of interest. All definitions exhibit the same difficulty with regard as to how the standard deviation of blank specimens is to be estimated. Without a precise statistical definition, one

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cannot determine a scientifically plausible value for the limit of detection, expect different laboratories to be consistent in how they determine the limit of detection,or be scientificallyhonest about declaring that a substance has (or has not) been detected. Beyond the statistical definition, there must be a clear set of operational rules for how this measurement error is to be determined in the laboratory. Most published definitions are weak with respect to these instructions,which must explain how to estimate the variances and what kind and number of blanks to be used.

Given this confusion, and in the interest of informing registrants and other data submitters of one potential method for LOD/LOQ determination which would fully meet relevant scientific and statistical criteria, this Appendix provides an illustrative example of an LOD and LOQ determination. The example provided is a slightly modified form of a procedure used by USDA's IR-4 program and is published, in part, in 40 CFR (as 40 CFR Part 136, Appendix B).

The information provided in this attachment is only illustrative of a technique which a registrant or data submitter may or may not choose to follow. Registrants and data submitters are free to use any reasonable and scientifically and statistically supportable methodology. Regardless of whether this specific example methodology or another separate methodology is chosen, the procedures used by a laboratory to determine the LOD and LOQ should be fully explained and/or copies of any appropriate publications should be submitted with the analytical method description to the Agency. In addition, OPP expects that adequate supporting documentation (e.g, chromatograms, calculations, etc.) would be included in the submission.

ILLUSTRATIVE EXAMPLE

An analyst wishes to determine the LOD and LOQ for a specific method for measurement of a given pesticide in a given crop matrix. This method may be the proposed enforcement analytical method, or simply a method which is used for measurement of residues in crop field trials or market basket surveys. The estimation of the LOQ and LOD of a specific method for a specific pesticide on a specific crop is done in the following two steps.

- The first step is to produce a preliminary estimate of the LOD and LOQ and to verify that a linear relationship between concentration and instrument response exists[7]. These preliminary estimates correspond to what some term the IDL (Instrument Detection Limit) and IQL (Instrument Quantitation Limit), respectively. The matrix of interest will be fortified (spiked) at the estimated LOQ

[7]In general, instrument responses are expected to be linear with respect to analyte concentration, but exceptions do exist. For example, linearity is maintained only in the "linear region" of analyte concentrations and there are certain detectors, instruments, or methods (e.g., FPD detectors operated in the S-mode, certain ion-selective electrodes, or immunoassays) where the expected response is curvilinear Confirmation of linearity in response over the range of concentrations is generally done by visual inspection of the standard curve or calibration plot. In those instances where linearity is suspect, the analyst may wish to perform supplementary statistical tests (e.g., lack of fit test, regression diagnostics and residual analysis, etc.).

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in the next step for the actual estimation of LOD and LOQ of the method.

- The second step is to use the initial estimate of the LOD and LOQ determined in Step 1 to estimate the *method* detection limit and *method* quantitation limit in the matrix of interest.

These two steps are described in detail below.

STEP 1 The analyst derives a standard curve for the method of interest. In this particular instance, the analyst prepares the standard solutions with the following concentrations of the pesticide of interest (all in ppm): 0.005, 0.010, 0.020, 0.050, and 0.100. For each concentration in the sample solution[8], the following instrument responses (measured peak height) are recorded:

Concentration (ppm)	Instrument Response (peak height)
0.100	206,493
0.050	125,162
0.020	58,748
0.010	32,668
0.005	17,552

In order to verify that a linear response is seen throughout the tested range, the instrument response is plotted as a function of injected concentration. The results (and associated statistics) are shown in Figure 1. Note from these results that the instrument response appears to be adequately linear throughout the range of tested concentrations (0.005 ppm to 0.100 ppm), and that the R^2 value from the Summary of Fit box is adequate (0.99003). The standard deviation (as presented in the summary of fit box in Figure 1 as the Root Mean Square Error) is 8986.8.[9] The equation which describes this relationship (provided

[8] All concentrations reported in this attachment are in terms of the solution and not the plant matrix. Subsequent conversions would be necessary to convert concentrations in the solution (e.g, ug/ml) to concentrations in the matrix of interest (e.g., ug/g plant material).

[9] Alternatively, the figures provided by the computer software could instead be calculated using a scientific calculator as follows in abbreviated format (discrepancies between these calculations and those shown in the text are due to rounding):

r = 0.9950
m= 1 973 098.5
b = 15 120.

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in the "Parameter Estimates" box of Figure 1) is as follows:

$$Y = 15120 + 1\ 973\ 098 * (\text{Concentration})$$

where Y is the instrument response (peak height)

The estimated LOD and LOQ are calculated as follows (assuming these values are set to be 3 and 10 standard deviations above the blank response, respectively):

1. The Peak Height at the LOD (Y_{LOD}) is calculated at 3 times the standard deviation while the Peak Height at the LOQ (Y_{LOQ}) is calculated at 10 times the standard deviation

$$Y_{LOD} = 15120 + 3 * (8987) = 42\ 081$$

$$Y_{LOQ} = 15120 + 10 * (8987) = 104\ 990$$

Standard deviation is calculated as follows:

x_i	y_i	yhat	$\lvert y_i\text{-yhat}\rvert$	$(\lvert y_i\text{-yhat}\rvert)^2$
0.100	206,493	212,431	5,938	35,260,680
0.050	125,162	113,774	11,388	129,696,698
0.020	58,748	54,579	4,169	17,380,190
0.010	32,668	34,848	2,180	4,750,400
0.005	17552	24,982	7,440	55,350,470
				Σ = 242,438,439

$$S\ y/x = [242{,}438{,}439/(5-2)]^{1/2}$$
$$8{,}989.6$$

where yi is the observed instrument response and yhat it the predicted instrument response given the "best fit" regression.

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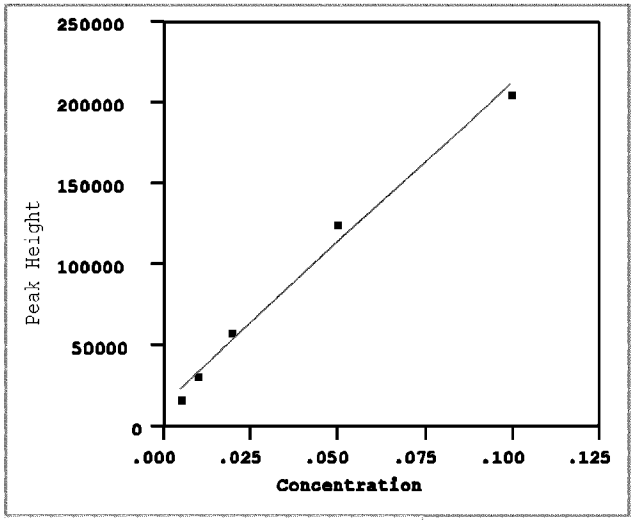

Summary of Fit

RSquare	0.99003
RSquare Adj	0.986707
Root Mean Square Error	8986.837
Mean of Response	88124.6
Observations (or Sum Wgts)	5

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	15119.954	5834.672	2.59	0.0810	-3448.891	33688.799
Concentration	1973098.5	114317.5	17.26	0.0004	1609283.2	2336913.9

Figure 1. Statistical Results using JMP Software

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M. These values (peak height at LOD and peak height and LOQ) are then used to calculate the concentrations associated with these peak heights as follows:

$$Y = 15120 + 1\ 973\ 098 * (\text{Concentration})$$

Rearranging,

$$\text{Concentration} = (Y - 15\ 120) / 1\ 973\ 098$$

Therefore,

$$LOD = Y_{LOD} - 15120 / 1\ 973\ 098 = (42\ 081 - 15\ 120)/1\ 973\ 098 = 0.014 \text{ ppm} \Rightarrow 0.014$$

$$LOQ = Y_{LOQ} - 15\ 120/1\ 973\ 098 = (104\ 990 - 15\ 120)/ 1\ 973\ 098 = 0.046 \text{ ppm} \Rightarrow 0.05$$

Thus, the initial estimated LOD and LOQ are 0.014 ppm and 0.046 ppm, respectively which correspond to the IDL and IQL.

Again, these estimated LODs (or IDL) and LOQs (or IQLs) are expressed in terms of the solution concentration and not in terms of the matrix concentration. At this stage, the solution concentration (ug/mL solution) should be converted to the effective concentration in the matrix (e.g., ug/g of matrix).

STEP 2

With the initial estimate of LOD (or IDL) and LOQ (or IQL) obtained and linearity verified, STEP 2 involves estimating the LOQ and LOD in spiked matrix samples. This procedure uses the estimated instrumental LOQ and the procedure detailed in 40 CFR Part 136, Appendix B and in the Handbook of Environmental Analysis to provide a better estimate of LOQ and verify that method recoveries are acceptable[10].

The method calls for the analysis of 7 or more (n) untreated control samples spiked at the estimated LOQ. The standard deviation of these samples is measured and the LOD and LOQ are determined as follows:

$$LOD = t_{0.99} \times S$$

$$LOQ = 3 \times LOD$$

[10] The following information is derived and obtained from a November 4, 1999 IR-4 memo.

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where t = one-tailed t-statistic at the 99% confidence level for n-1 replicates

S = Standard Deviation of n samples spikes at the estimated LOQ

The following is a set of t values for use in the above equation:

# of Replicates (n)	Degrees of Freedom (n-1)	$t_{0.99}$	# of Replicates (n)	Degrees of Freedom (n-1)	$t_{0.99}$
3	2	6.965	13	12	2.681
4	3	4.541	14	13	2.650
5	4	3.747	15	14	2.624
6	5	3.365	16	15	2.602
7	6	3.143	17	16	2.583
8	7	2.998	18	17	2.567
9	8	2.896	19	18	2.552
10	9	2.821	20	19	2.539
11	10	2.764	21	20	2.528
12	11	2.718	22	21	2.518

In this example, the analyst prepared 7 untreated control samples spiked at the above estimated LOQ of 0.05 ppm. The following results were obtained:

Concentration Detected (ppm)	% Recovery
0.0397	79.4%
0.0403	80.6%
0.0400	80%
0.036	72.0%
0.0498	99.6%
0.0379	75.8%
0.0388	77.6%

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Average Concentration: 0.0404 ppm Standard Deviation: 0.0044 ppm **Average Recovery: 80.7%**

Given that recoveries are adequate at the LOQ (average = 80.7%, range = 72.0-99.6%), the LOD and LOQ for the method are estimated as follows:

LOD= $t_{0.99}$ x S (for 7-1 = 6 degrees of freedom)

= 3.365 x 0.0044 ppm

= 0.0148 ppm

LOQ = 3 x LOD

= 3 * 0.0148 ppm

= 0.0444 ppm

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