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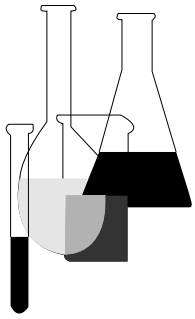
Residue Chemistry Test Guidelines

OPPTS 860.1380
Storage Stability Data



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 860.1380 Storage stability data.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 et seq.).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP test guideline 171–4, Results of Tests on the Amount of Residue Remaining, Including A Description of the Analytical Methods Used (Pesticide Assessment Guidelines, Subdivision 0: Residue Chemistry, EPA Report 540/9–82–023, October 1982). This guideline should be used in conjunction with OPPTS 860.1000, Background.

(b) **Purpose.** These studies are required to validate the stability or rate of decomposition of the total toxic residue (TTR) in or on the raw agricultural commodity (RAC) (or processed commodity) between the time of harvest or sample collection and the final analysis of the residue.

(c) **General.** (1) In most instances samples collected in magnitude of the residue and nature of the residue (metabolism) studies are stored for a period of time prior to their analysis. During this storage period residues of the pesticide and/or its metabolites may be lost by processes such as volatilization or reaction with enzymes. Therefore, in order to be certain that the nature and level of residues that were present on samples at the time of their collection are the same at the time of analysis, controlled studies are needed to assess the effect sample storage has on the TTR. In other words, registrants need to show that pesticide residues are stable during storage of analytical samples or show the degree to which residues are lost during that period.

(2) The term ‘‘storage stability’’ in this document does not address manufacturing use product or end use product storage stability data required under the product chemistry subpart of 40 CFR part 158 or the storage of food commodities under typical commercial conditions, e.g. during the storage and transport of produce prior to its reaching the consumer. Studies addressing the latter are examples of ‘‘reduction of the residue’’ or ‘‘anticipated residue’’ studies that are occasionally required to obtain a more realistic estimate of residues in food at the time of consumption. The purpose of the present document is to address storage of analytical samples, in most cases under frozen conditions. For this reason a better name for the study might be that proposed by the Food and Agriculture Organization of the United Nations (FAO)—Stability of Pesticide Residues in Stored Analytical Samples (see paragraph (h)(4) of this guideline).

(3) Storage stability data will be required in conjunction with most magnitude-of-the-residue studies, e.g. crop field trials, processing studies, or livestock feeding studies. The Agency will make the following excep-

tion: Unless a pesticide/residue of concern is otherwise known to be volatile or labile, storage stability data will not be needed for samples stored frozen for less than 30 days. The judgment as to what constitutes volatile or labile will be based on information such as basic physical properties and the results of metabolism studies.

(4) Storage stability requirements for nature of the residue or metabolism studies are discussed in paragraph (e) of this guideline.

(5) **Other considerations**—(i) **Need for concurrent studies.** (A) It is preferable that storage stability data be obtained as part of a magnitude-of-the-residue study, not independent from it. Placing samples with known residue levels into storage along with the treated commodity samples represents quality assurance similar to verifying the identity of test material. If the treated samples were subjected to erratic storage conditions due to loss of electrical power, the samples with known residue levels could be used as a direct measure of any effects that temperature fluctuations might have on residues. Thus, use of concurrent storage stability samples represents simple good laboratory practice.

(B) The Agency prefers that storage stability studies be conducted concurrently with the corresponding magnitude-of-the-residue study when possible. While this may not be possible for data needed to support completed field trials used for reregistration purposes, it should be possible in conjunction with new magnitude-of-the-residue studies being initiated in support of registration or reregistration. However, concurrent storage stability studies will not be required in many cases (see paragraph (h)(1) of this guideline). Provided that the pesticide residues are found to be stable in the matrices of interest, a storage stability study run in a separate freezer at a different time period will be acceptable if the storage conditions (especially temperature) are similar to those in the corresponding magnitude-of-the-residue study. For pesticides whose residues are known or suspected to be unstable or volatile, concurrent studies may be needed. For such pesticides it is advisable to run a storage stability study in advance of the magnitude-of-the-residue studies to determine proper storage conditions and maximum storage times before treated samples are placed into storage.

(ii) **Representative commodities to be analyzed.** (A) Use of crop grouping is acceptable. If residues are shown to be stable in a given commodity, the residues in other crops of the same group, as listed in 40 CFR 180.41, would be assumed to be stable for the same time period under the same experimental conditions.

(B) Combining of the crop groups in 40 CFR 180.41 into larger groups would generally be acceptable for the purposes of determining stability of residues in storage. For example, leaves of root and tuber vegetables could be combined with leafy vegetables (except Brassica). With re-

gard to how many representative crops need to be analyzed (with residues shown to be stable) before it can be assumed that residues are stable in all crops, the Agency believes that at least five diverse crops need to be tested. If a pesticide is to be applied to all types of crops, suggested crops for a storage stability study are an oilseed (or soybean or nut), a nonoily grain, a leafy vegetable, a root crop, and a fruit or fruiting vegetable. The fruit/fruiting vegetable should be an acidic commodity, such as citrus or tomatoes. Field corn grain is to be considered a nonoily grain as opposed to an oilseed. The crop parts to be examined in these studies are those used for food and feed, in other words, those on which residue data are generated and tolerances established, e.g. wheat grain, wheat forage, and wheat straw.

(C) The guidance on representative crops is directed toward a pesticide that will be applied to all crop groups. Many pesticides are applied to only a portion of these groups. Therefore, the five crops listed above will not always be the most appropriate ones. Since the Agency can not provide guidance for all the possible combinations of crops that might be treated, registrants will need to use judgment as to which representative commodities to use for storage stability studies. One example will be presented here. Suppose a pesticide is to be applied to only cucurbit vegetables and stone fruit. In this case storage stability data should be provided on one crop from each of these groups. Registrants may contact the Agency if questions arise as to which commodities should be tested for a particular combination of treated crops.

(D) If residues are found to be unstable in any representative commodity, additional storage stability studies will normally be required on additional commodities of that group if tolerances are being sought on such crops. Under these circumstances the concept of combining crop groups in 40 CFR 180.41 may no longer be applicable.

(E) There are three major types of crops for which the Agency receives magnitude-of-the-residue data for processed commodities: Oilseeds, grains, and fruits/fruiting vegetables (mainly citrus, apples, and tomatoes). Since some of the processed commodities (e.g. oils, juices) have matrices quite different from the starting RAC, storage stability data are required to support processing studies. If the residues of concern of a particular pesticide have been shown to be stable in the processed commodities from one each of the three types of crops cited above, additional storage stability data will generally not be required on other processed commodities (provided that the storage conditions are similar and samples are not stored longer than those of the representative processed commodities).

(F) As with crops, this guidance on processed commodities is directed toward pesticides applied to all types of crops that have processed commodities in which residues may concentrate. For pesticides that are not applied to all such crops, storage stability data may be needed on proc-

essed commodities other than the three types mentioned above. For example, if a pesticide is to be used on only root crops, storage stability data should be generated on the processed fractions of potatoes or sugar beets.

(G) With respect to animal commodities, storage stability data are normally required to support livestock feeding or dermal treatment studies. The representative commodities to be examined should include muscle (cattle or poultry), liver (cattle or poultry), milk, and eggs. If residues are stable in these matrices, analyses of other tissues (fat, kidney) will not be needed.

(d) **Storage stability requirements for magnitude of residue studies**—(1) **General.** Storage stability data normally are required for each component of the TTR that is measured in the magnitude-of-the-residue studies. In most cases this means all components included in the tolerance expression. The Agency will allow representative components of the residue to be employed when numerous compounds are included in the tolerance on a case-by-case basis. Registrants are advised to contact the Agency when questions arise in this regard.

(2) **Test compounds and analytical methods.** (i) Samples could either be from crops (or livestock) that have been treated with pesticides in the field or from the spiking of control (untreated) samples with known amounts of each analyte. In all cases, the storage stability samples should be analyzed using the same analytical procedure that was employed in the corresponding magnitude-of-the-residue studies. If not, data will be needed to show that the method gives results equivalent to those obtained by the method used in the magnitude-of-the-residue studies.

(ii) The samples used in the storage stability study could also be those obtained from metabolism studies using radiolabeled material. If these are to be used, the residues should be measured using the ''cold'' analytical method that was employed in the magnitude-of-the-residue studies or another method validated for quantitating the TTR. In other words, the storage stability data should not be based on simply counting total radioactivity. (NOTE: The discussion in this paragraph does not refer to the storage stability data needed to support a metabolism study. The latter involves examining the chromatographic profile of all radiolabeled residues as described in paragraph (e) of this guideline.)

(iii) In those instances where no detectable residues (or low levels of residues close to the analytical method's limit of quantitation) are found in field treated commodities, the Agency advises that spiked control samples be employed in the storage stability studies. Related to the latter point, it is suggested that the minimum residue level to be used in storage stability studies be 10× the method's limit of quantitation with the minimum in any case to be 0.1 ppm. This will make it less likely that the stability of the residues can not be ascertained due to highly variable recoveries.

If typical residues observed in the magnitude-of-the-residue studies are much higher than the minimum level suggested above, it is preferable (although not required) for the storage stability study to employ comparable residue levels.

(iv) Analytical methods yielding low and variable recoveries should be avoided when conducting storage stability studies (as well as magnitude-of-the-residue studies). Regardless of the method used, freshly fortified samples should be analyzed at each time point when storage stability samples are removed from storage for analysis. This will allow for correction of observed residue values for the stored samples if recoveries are significantly higher or lower than 100 percent for the freshly fortified samples.

(v) In those instances where the TTR consists of more than one component, i.e. parent compound + metabolites, the storage stability samples may be fortified with the mixture if the analytical method is capable of measuring each component of the residue separately. In those cases where the method converts all residues to a common moiety, spiking with mixtures or using field treated/weathered residues is discouraged. The type of chemical and toxicity involved would determine the acceptability of spiking with a mixture (or using field treated samples) when a common moiety method is employed. For example, with pesticides where similar chronic toxicity concerns exist over numerous components of the residue, spiking with a mixture followed by use of a common moiety method is probably acceptable. On the other hand, it would not be acceptable to use a common moiety method for cholinesterase inhibitors where significant differences in toxicity may occur as the parent compound oxidizes to assorted metabolites. In other words, in the latter case the method would need to detect each of the metabolites separately.

(3) **Sample form.** (i) It is preferred that the form of the commodity (e.g. homogenate, coarse chop, whole commodity, extract) in a storage stability study be the same as that in the corresponding magnitude-of-the-residue study. In some cases the storage stability study may need to reflect storage of more than one of the above forms. For example, if crop field trial samples are stored as homogenates for several months, extracted, and the extracts stored for several weeks prior to final analysis, the storage stability samples should be handled in the same manner.

(ii) If a storage stability study does not reflect the storage of extracts prior to final analysis, the whole study need not be repeated. It would be acceptable to spike extracts of untreated samples, hold them in storage for the same time and under the same conditions as the corresponding extracts in the magnitude of the residue samples, and then analyze them to determine the stability of residues in the extract. To avoid this additional study, registrants are advised to routinely include the storage of extracts

in their storage stability studies unless their standard laboratory practice is to analyze extracts on the same day as they are obtained.

(iii) In some cases magnitude of the residue samples are stored in a whole state, while the storage stability samples are kept as homogenates. (The latter is necessary to ensure the sample can be spiked uniformly.) Provided the residues are found to be stable, the Agency will normally accept such studies since the use of an homogenate in the storage stability study is likely to represent a worse case versus the use of a whole commodity. The homogenization process can release enzymes, acids, and other chemicals that react with the pesticide or its metabolites. If residues are unstable in the homogenate, the Agency will decide on a case-by-case basis whether to correct for loss of residues in the stored whole commodities based on the results of the homogenate or take another course of action (e.g. require field trials to be repeated with the samples stored in a different form and/or analyzed closer to the time of collection.) The factors to be considered in making this decision include the degree of loss observed in the homogenized samples and the current risk status of the pesticide.

(iv) The FAO guidelines (see paragraph (h)(5) of this guideline) state the following:

> If prolonged storage is unavoidable, it is usually preferable to extract the sample, remove most or all of the solvent and store the extracts at a low temperature, preferably at or below –20 °C. This removes the residue from contact with enzymes which might degrade the pesticide and also prevents further possibility of residues being ‘‘bound’’ in the tissue.

While the Agency does not believe this procedure should be the preferred method of storing samples, it is an acceptable alternative to storing whole samples or homogenates provided that the storage stability samples are handled in the same manner.

(4) **Sample container.** As with most parameters in a storage stability study, the sample container should be the same as that used for the magnitude of the residue samples. However, the Agency has learned that the standard practice by registrants is to store magnitude of the residue samples in plastic bags (for ease of handling and storing large samples that may not be homogenized) and the storage stability samples in glass jars. (The latter involve smaller, usually homogenized, samples that need to be fortified with the TTR of concern in most cases.) The Agency has reservations about this practice since the containers may differ in their airtightness and pesticides might adsorb differently to the two materials. However, as long as the pesticide is not volatile, studies will not be rejected solely due to the use of different containers.

(5) **Storage conditions.** (i) The Agency recognizes that magnitude of the residue samples almost always require transport from the site of

treatment to the laboratory prior to placement into storage until residue analysis can be performed. Efforts should be made to keep samples cold during transport, e.g. packed with dry ice, and to keep the transport period as short as possible. The storage stability study should then simulate the conditions (temperature, humidity, light) used in the laboratory for storage of magnitude of the residue samples prior to their analysis. Storage temperatures should be –20 °C or lower. For classes of pesticides with known instability, petitioners should consider using even lower temperatures to avoid or at least reduce loss of residues in storage. Samples should also be kept in the dark to eliminate the possibility of photochemical reactions. (While the focus of the present document is on the storage stability study, the Agency wishes to emphasize that efforts should always be made to assure the integrity of magnitude-of-the-residue samples from the time of their collection until being placed into storage in the laboratory. Magnitude-of-the-residue study reports should detail how samples are handled and stored prior to receipt by the laboratory.)

(ii) In older magnitude-of-the-residue studies, the exact storage temperatures may not be known, although samples were kept in a freezer. If such studies are to be used in support of reregistration, the Agency suggests storage stability studies be conducted at two temperatures (e.g. –5 and –20 °C) to address the uncertainty regarding storage temperature of the older samples. Samples stored at the higher temperature should be analyzed first. If residues are stable at that temperature, the samples stored at the lower temperature do not need to be analyzed.

(6) **Frequency of sampling.** (i) The Agency has no strict requirements on the number of sampling intervals that should be examined in a storage stability study. There needs to be a sufficient number of time points to establish that the residues are stable throughout the maximum storage period used for magnitude of the residue samples or to show how much of the residue is lost at various time points if it becomes necessary to correct for such losses. In all cases the sampling points should include zero time to establish the residue levels present at the time samples are placed into storage. The minimum number of sampling times will vary depending upon the stability of the residues and the maximum length of the storage period for the magnitude of the residue samples. For example, if the latter is only a few months, it may be sufficient to examine samples stored that amount of time and some intermediate time (in addition to the zero-time sample) if residues are stable. On the other hand, more time points would be necessary if the samples are stored several years or if residues are observed to decline significantly during the several months of storage.

(ii) The following represent intervals suggested in FAO guidelines (see paragraph (h)(4) of this guideline). These are not intended to be Agency requirements, but possibilities to be considered by registrants. If relatively rapid degradation of residues is likely, sampling intervals such as

0, 14, 28, 56, and 112 days could be chosen. For longer storage periods involving stable residues, intervals of 0, 1, 3, 6, and 12 months are suggested. In any case, the longest storage interval in the magnitude-of-the-residue study needs to be included as discussed in the next section of this document.

(iii) The storage intervals observed in a magnitude-of-the-residue study typically will encompass a wide range. The corresponding storage stability study does not have to include each and every sampling time from the study. The Agency will usually interpolate results when corrections for loss are necessary and the intervals from the two studies do not match.

(iv) The Agency also has no strict requirements with regard to the minimum number of samples per time point for each analyte. Although one stored sample (in addition to the freshly spiked samples) may suffice in many cases, the Agency strongly encourages registrants to have reserve samples in case problems are encountered (e.g. poor recoveries observed in freshly fortified samples or an apparently aberrant result (i.e. the availability of additional samples may provide justification for discarding such a value)). Reserve storage stability samples are also useful if treated samples end up being stored longer than anticipated or additional analyses of treated samples already in storage are requested by the Agency.

(7) **Length of storage period.** (i) The duration of a storage stability study should normally be equal to or longer than the maximum storage period for the corresponding samples in the magnitude-of-the-residue study. However, for cases in which samples from storage stability studies were stored for shorter intervals than samples from the corresponding magnitude-of-the-residue studies, extrapolation of the storage stability data to longer intervals will be considered on a case-by-case basis when minimal losses have been observed at the shorter storage intervals. Such extrapolation will be considered only in cases where the storage stability data are available for at least 6 months and reflect at least 3 time points in addition to the time-zero point.

(ii) Under some circumstances the Agency may also accept the analyses of retained split samples from field trials as an alternative to the extrapolation described above. In some cases the treated samples from field trials or other magnitude-of-the-residue studies are split into several portions, one portion analyzed quickly, i.e. within 30 days of harvest, and the other portions placed in frozen storage. If analysis of the stored portions after an extended period in the freezer shows the same residue level as the portion analyzed within 30 days of harvest, the Agency will consider using such analyses to support magnitude-of-the-residue studies.

(iii) It should be noted that the extrapolation process and use of split samples discussed in the previous two paragraphs will normally not be

applicable when residues of a pesticide have been found to be unstable in any commodity. The available data on other crops need to show that residues are stable for the Agency to consider these alternatives in support of field trials on a particular crop.

(iv) During reregistration, questions may arise with respect to the need for conducting new crop field trials versus conducting storage stability studies to support old field trials. The decision as to which studies should be conducted will normally be based on which can be completed in a shorter time frame. For example, field trials may be available for a given crop, but the samples were stored 4 years and no storage stability data are available. In this case, in order to expedite reregistration, the Agency would want new crop field trials to be carried out since they could be completed in a much shorter time than a 4–year storage stability study.

(8) **Use of storage stability results.** (i) If a storage stability study shows limited decline of residues during the storage period observed for the corresponding magnitude-of-the-residue study, correction factors will generally be used to determine the residue levels that were present at the time of sample collection in the study. However, if extensive dissipation of residues has occurred during storage, the study may need to be repeated with samples analyzed closer to their time of collection. As a rule of thumb, correction factors will be applied to losses in storage up to 30 percent. Beyond that point, the Agency will consider corrections on a case-by-case basis taking into account factors such as the absolute (parts per million) and relative (percent of TTR) residue levels of the component that is unstable in storage.

(ii) The degree of loss will normally be adjusted or corrected for analytical method recoveries before applying the 30 percent rule of thumb. In other words, the apparent residue level of an analyte after storage should be divided by the analytical method recoveries obtained for freshly fortified samples analyzed at the same time. For example, a storage stability sample was originally prepared by spiking at 1.0 ppm (level confirmed by zero-day analysis after correcting for method recovery of 75 percent on a freshly fortified sample). After a given period of storage, a portion of the sample is analyzed and found to contain only 0.63 ppm (an apparent loss of 37 percent). If the method recoveries for freshly fortified samples analyzed at the same time are 70 percent, the corrected residue level in the stored sample is 0.63 ppm/0.70 = 0.90 ppm. Thus, the corrected degree of loss in storage is 10 percent (or corrected recovery of 90 percent for the stored sample).

(iii) Regardless of the degree of loss in storage, registrants should not report just the corrected results in magnitude-of-the-residue studies. Such adjustments should be left for the Agency to perform. This comment applies to corrections for both storage losses and analytical method recoveries. However, it would be acceptable for registrants to propose correction

factors and report corrected results provided that the uncorrected residues, correction factors, and corrected results are all clearly presented in the report.

(e) **Storage stability requirements for metabolism studies.** (i) The Agency needs to make a determination as to whether sample integrity was maintained during collection, preparation, and storage of samples in plant and livestock metabolism studies. In light of the difficulty of spiking samples before the identity of the residue is known and the length of time needed for metabolism studies, the present Agency position is that storage stability data should not normally be required for samples analyzed within 4–6 months of collection, provided evidence is given that attempts were made to limit degradation of residues by appropriate storage of matrices and extracts during the analytical portion of the study.

(ii) In those cases where a metabolism study can not be completed within 4–6 months of sample collection, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. This can be done by analyses of representative substrates early in the study and at its completion. Such analyses should show that the basic profile of radiolabeled residues has not changed during that time. If changes are observed (e.g. disappearance of a particular HPLC peak or TLC spot), additional analyses or another metabolism study with a shorter collection to analysis interval may be required.

(f) **Data reporting.** (1) As stated in the Agency report ‘‘Effects of Storage (Storage Stability) on Validity of Pesticide Residue Data’’ (see paragraph (h)(2) of this guideline), reports on storage stability studies should include a detailed description of

* * * the commodities that were stored (whether raw or processed); the test compounds; the experimental design and storage conditions (e.g. freezer temperature, length of storage, type of containers, etc.); residue methods and instrumentation; storage stability results and reporting of the data; statistical analysis; and quality control measures/precautions taken to ensure the validity of these operations, including the dates for each step above.

In light of some of the earlier discussion in this document, it is especially important for registrants to describe how samples are prepared (e.g. coarsely chopped, homogenized) and the containers in which they are placed. Differences between these and the sample preparation/containers used in the corresponding magnitude-of-the-residue studies should be pointed out and data or a rationale provided as to why they should not invalidate the studies. If known, the Master Record Identification (MRID) numbers of the corresponding magnitude-of-the-residue studies should be provided.

(2) The values for individual samples (as opposed to just reporting a mean) should be reported in all cases where multiple samples have been analyzed at a given time point. A suggested tabular format for reporting

the results that incorporates corrections for recoveries in freshly fortified samples follows.

Commodity	Analyte	Residue Level	Storage Period	Fresh Fortification Recovery	Apparent Recovery in Stored Sample	Corrected Recovery in Stored Sample

(3) The values in the second column from the right represent the apparent recovery in the stored samples. These can be divided by the recoveries obtained in the freshly fortified samples to determine the corrected recovery, the measure of the stability of the residue in storage as discussed in the previous section of this document.

(g) **Data reporting format.** The following describes a suggested order and format for a report item by item. However, other formats are also acceptable provided the information described in this paragraph is included.

(1) *Title/cover page.* Title page and additional documentation requirements (i.e. requirements for data submission and procedures for claims of confidentiality of data) if relevant to the study report should precede the content of the study formatted below.

(2) *Table of contents*

(3) *Summary/introduction.* This section should include the following: Purpose, introduction (include summary table of storage validation data), sample preparation and fortification, storage and sampling procedures, analytical procedures, and methods of calculation.

(4) *Materials*—(i) *Test substance.* (A) If fortification is used, describe the test substances (chemical/common/experimental/CAS names, including the determination/check of the purity of the test compounds (parent plus any metabolitess of special concern, all in reference standard form) and preparation of standard solutions).

(B) If weathered residue samples are used, identify the nature and amount of test substances in the sample at zero-time (defined as the beginning of the storage stability testing).

(C) Any and all additional information the petitioner considers appropriate and relevant to provide a complete and thorough description and identification of the test substances used in storage stability validation testing.

(ii) *Test commodity.* (A) Identification of the RACs (crop/type/variety/ botanical name) and the specific crop parts or processed commodity to be used in storage stability testing.

(B) The development stagess, general condition (immature/mature, green/ripe, fresh/dry, etc.) and sizes of the RAC samples used in storage stability testing.

(C) Treatment/preparation of RAC or processed commodity samples prior to storage stability testing (e.g. trimming, cleaning, or other means of residue removal, compositing, subsampling, chopping, extraction, etc., reference to the FDA PAM, Vol. I, sections 141–142 for recommended procedures (see paragraph (h)(6) of this guideline)).

(D) Sample identification number (source of samples, field trial identification number, control or weathered residue sample, coding and labeling information (should be the same as, or cross- referenced to, the sample coding/labeling assigned at harvest).

(E) Any and all additional information the petitioner considers appropriate and relevant to provide a complete and thorough description of the RACs.

(5) *Methods*—(i) *Experimental design.* Number of test commodities, number of test substances, number and magnitude of test levels, number of replicate samples per test compound per test level, number of sampling intervals, representativeness of test commodities to the matrices of concern, etc.

(ii) *Test procedures.* (A) Fortification (spiking) procedure, if used: Detail the manner in which the test compounds was/were introduced to the test substrates.

(B) Storage conditions: Temperature, humidity, lighting, container types/size, crop form (extract/macerate/etc.), sample sizes/weights, duration, etc. should be provided.

(C) Sampling: Describe the sampling procedure at zero time and at regular intervals thereafter. The duration of study should correspond to the length of storage of the field trial samples collected for residue analysis;

(D) Dates of sample preparation (maceration/extraction/etc.), spiking or determining the type/amount of weathered residue (zero time), periodic sampling intervals, end of storage, and residue analyses should be provided.

(E) Methods of residue analysis: (*1*) Title/designation/date and source (PAM, Vol. II; scientific literature; company reports, etc.), or cross-ref-

erence the analytical method section of submission if same methods used) should be submitted.

(*2*) Discuss any deviations (in reagents, procedures, instrumentation, operating parameters, etc.) from the Analytical Methods used for residue analysis of field trial samples or processed commodities if same methods is/are used.

(*3*) Detail the principles and stepwise procedures (extraction/clean-up, derivatization, determination), including any modifications made, chemical species determined, confirmatory techniques used, if any, etc., extraction efficiency (if pertinent).

(*4*) Instrumentation and operating parameters (make/model, type/specificity of detectors, columns (packing materials, size), carrier gases, flow rates, temperatures, voltage, limit of detection and sensitivity, calibration procedures, etc.) should be provided.

(*5*) Reagents or procedural steps requiring special precautions (to avoid safety or health hazards) should be explained.

(*6*) Time required for analysis (to carry a sample/set completely through the analytical procedure, including the determinative step) should be submitted.

(*7*) Procedures for calculating residue levels and percent recoveries (detail) should be reported.

(*8*) Any other information the petitioner considers appropriate and relevant to provide a thorough description of the analytical methodology and the means of calculating the residue results should be provided.

(6) *Results/discussion*—(i) *Residue results.* Raw data, dilution factorss, peak heights/areas, method correction factors applied, formula(e)/standard curves used, ppm theoretical/found, recovery levels (range), percent recovery vs. length of storage (dissipation data), appropriateness of length of storage study, etc. should be provided.

(ii) *Statistical treatments.* Describe tests applied to the raw data.

(iii) *Quality control.* Report the control measures/precautions followed to ensure the fidelity of storage stability validations.

(iv) *Other.* Any additional information the petitioner considers appropriate and relevant to provide a complete and thorough description of storage stability validation results should be provided.

(7) *Conclusion.* Discuss conclusions that may be drawn regarding the stability of the test compounds in the test matrices as a function of storage time.

(8) *Certification.* Certification of authenticity by the Study Director (including signature, typed name, title, affiliation, address, telephone number, date) should be provided.

(9) *Tables/figures.* (i) Tabless of raw data from storage stability validation testing and a summary table of residue levels in stored samples as a function of commodity and storage time should be submitted.

(ii) Graphs, figures, flowcharts, etc. (as relevant) may be included.

(10) *References.*

(11) *Appendixes.* (i) Representative chromatograms, spectra, etc. should be provided.

(ii) Reprints of methods and other studies cited (unless physically located elsewhere in the overall data submission, in which case cross- referencing will suffice) should be submitted.

(iii) Include any relevant material not fitting in any of the other sections of this report.

(h) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Environmental Protection Agency. Pesticide Reregistration Rejection Rate Analysis, Residue Chemistry, EPA Report 738–R–92–0001 (1992).

(2) Environmental Protection Agency, 1987, Pesticide Assessment Guidelines, Subdivision O, Position Document, Effects of Storage (Storage Stability) on Validity of Pesticide Residue Data, EPA Report 540/09–88–002.

(3) Environmental Protection Agency. Pesticide Reregistration Rejection Rate Analysis – Residue Chemistry; Follow–up Guidance for: Generating Storage Stability Data; Submission of Raw Data; Maximum Theoretical Concentration Factors; Flowchart Diagrams. EPA Report 737–R–93–001 (February 1993).

(4) United Nations Food and Agricultural Organization (FAO). Stability of Pesticide Residues in Stored Analytical Samples. 1994 draft prepared by Codex Committee on Pesticide Residues Working Group on Methods of Analysis and Sampling.

(5) United Nations Food and Agricultural Organization (FAO). Guidelines on Pesticide Residue Trials to Provide Data for the Registration of Pesticides and the Establishment of Maximum Residue Limits—Part 1—Plants and Plant Products (1986).

(6) Pesticide Analytical Manual. Food and Drug Administration. Volume I. (1994). Available from the National Technical Information Service, Springfield, VA.