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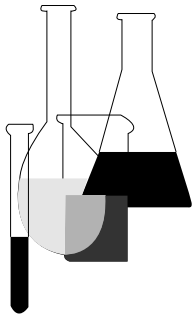
Residue Chemistry Test Guidelines

OPPTS 860.1480
Meat/Milk/Poultry/Eggs



“Public Draft”

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.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ''Environmental Test Methods and Guidelines'' or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 860.1480 Meat/milk/poultry/eggs.

(a) **Scope.**

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

(2) **Background.**

(i) The source material used in developing this harmonized OPPTS test guideline is the OPP guideline 171–4j (see reference in paragraph (g)(1) of this guideline. In addition, paragraphs (g)(2) through (g)(7) provide references for background materials on dietary exposure, dermal treatments and data reporting guidance published subsequently to the OPP guidelines.

(ii) This OPPTS guideline should be used in conjunction with OPPTS guideline 860.1000, Background, which provides general information and overall guidance for the 860 series on Residue Chemistry. Topics discussed in this 860.1480 guideline include: Purpose (paragraph (b)); Data requirements (paragraph (c)); Conduct of studies (paragraph (d)); Guidance procedure for calculating livestock dietary exposure (paragraph (e)); Data reporting format (paragraph (f)); and References (paragraph (g)).

(b) **Purpose.** Whenever pesticide residues are detected in feed items, data on the transfer of residues to meat, milk, poultry, and eggs are required. These studies are also required if a pesticide is to be applied directly to animals. Data from these studies are used to determine which components of the total toxic residue (TTR) are present and at what concentrations secondary residues could result in meat, milk, poultry, and eggs in order to set appropriate tolerances.

(c) **Data requirements.** Data must be submitted to show the level of residues that will result in ruminant meat (muscle), meat byproducts (liver, kidney) and fat, poultry (muscle, fat, liver), eggs, or milk. These data are needed whenever a pesticide is to be applied directly to livestock or residues occur on a livestock feed. Based upon the residue level in the feed item and the residue resulting in meat, milk, poultry, and eggs in these feeding studies, the use must be classified as specified in 40 CFR 180.6(a). Category 1 of section 180.6(a) applies to cases where it is shown that residues will occur in animal products, Category 2 applies if there is a reasonable expectation of residues in animal products, and Category 3 applies if there is no reasonable expectation of residues. Since tolerances for residues in animal products are required when the use is judged to be Category 1 or 2, the animal feeding studies must not only show whether residues transfer, but may also need to serve as a basis for setting appropriate tolerance levels for the animal products.

(d) **Conduct of studies.**

(1) **Feeding Studies.**

(i) In most cases only the parent pesticide should be fed to livestock. However, in those cases where the parent compound comprises only a minor proportion of the residue of concern, it may be acceptable to feed a mixture of parent and plant metabolites. Any registrant considering such dosing should contact the Agency prior to initiation of such a study. In cases where a unique plant metabolite exists (i.e., one that is not formed in livestock), a separate feeding study may be required dosing with that metabolite. The feeding study should include the level of intake expected (1X)(see next paragraph), plus two exaggerated levels of 3X and l0X. The 1X level should represent the worst case estimate of the potential livestock exposure based on the assumption of all components of the feed having tolerance level residues. The exaggerated levels are especially important in judging a Category 3 use, as well as to cover possible future tolerances for the pesticide on additional feed items and allow estimation of whether residue levels in tissues vary linearly with the level in the feed. The dosage levels should be expressed in terms of concentration (ppm) in the total ration (dry weight basis), so that the Agency can relate the dosage to that expected from the proposed use. It is also desirable to express the feeding level in terms of milligrams per kilogram body weight.

(ii) In selecting the dosage levels based on total rations, the petitioner should take into account the proportion in the diet of the feed item bearing the residue and in the case of ruminants the percentage of dry matter in the feed. Table I of OPPTS guideline 860.1000, Background, should be used as a guide in determining the proportion of the diet of the various food items. The correction for percentage of dry matter is explained in more detail in paragraph (e) of this guideline. For example, dried citrus pulp (91% dry matter content) may in some circumstances comprise up to 20 percent of the total ration (dry weight basis) of dairy cattle. If a tolerance of 5 ppm of a given pesticide were proposed on dried citrus pulp, the total diet (dry weight basis) should be fortified at the 1.10 ppm level (i.e., [5.0 ppm/0.91]x[0.20]) to reflect the expected level of intake (1X). If other feed items with tolerances could also be fed in combination with citrus pulp, the contribution from these feed items should also be added in. As noted, two dosages at exaggerated levels are also required, preferably threefold and tenfold or higher where not precluded by toxicity of the pesticide.

(iii) Separate feeding studies are required for a ruminant and poultry whenever residues occur on the feeds of these classes of livestock, or direct animal treatment is proposed. The species of choice for these feeding studies are the cow and chicken. In most cases the results of the cattle feeding study will be used to establish tolerances on goats, hogs, horses and sheep. Data will not be translated from other meat animals to poultry.

However, within the poultry group, data on chickens will usually be accepted in lieu of data on turkeys. Data on residues in milk from dairy cows will usually apply as well to dairy goats. Data on tissue storage in rats, dogs, or other small animals used in toxicology studies will not be accepted in lieu of residue data on livestock.

(iv) In addition to establishing a baseline or blank in a pre-dosing period, control animals should be carried through the experiment with treated animals. This is highly desirable, since values for control animals have been observed to change during feeding studies. The number of animals carried at each treatment level, and as controls, will vary with the circumstances but as a general rule each group in a cattle feeding study should compromise a minimum of three animals. For chicken feeding studies, a minimum of 10 birds per group should be used. It is often advisable to have additional animals on test that can be used to determine the rate of decline of residues on the cessation of dosing, so that if residues above tolerance are found, data on the time necessary for residues to fall to the tolerance level are available.

(v) Animals should be dosed daily for a minimum of 28 days or until residues plateau in milk or eggs if they have not done so in 28 days.

(vi) If a feed-through formulation is specifically designed to change absorption characteristics within the digestive system, this formulation should be employed in the feeding study.

(2) **Direct animal treatment.**

(i) When a pesticide is proposed for direct use on food animals, data are required to show the extent of residues incurred by the use. The experimental treatment should reflect as closely as possible the conditions under which the pesticide will be used commercially. Control animals should be carried along with treated animals. Factors such as whether sheep passing through a dip tank were freshly shorn or unshorn should be considered. Generally, separate studies should be carried out for each species of livestock to be treated. The data should also reflect treatment by several applicators.

(ii) When a pesticide may be applied in more than one type of formulation or by more than one mode of treatment, separate studies reflecting the usage or combination of usages proposed are required. However, data from dips or high pressure wetting sprays on cattle may be accepted in lieu of data from dust treatments but not vice versa. When the use of devices which permit unlimited access (e.g. backrubbers) are proposed, the experiment should be designed to assure the maximum exposure of the animal to the pesticide. Data reflecting exaggerated treatments are desirable.

(iii) If livestock are exposed to the pesticide both in feed and as a direct treatment, the magnitude of the residue study should reflect the level of residues to be expected from the combined exposure scenarios. If separate feeding and direct treatment studies have been conducted, it is normally acceptable to add the residues from these studies to determine the appropriate tolerances. However, this may result in higher than necessary tolerances for animal commodities.

(3) **Agricultural premise use studies.** When the use of pesticides in agricultural buildings are such that restrictions cannot preclude the possibility of residues in meat, milk, poultry or eggs, residue studies should be carried out reflecting the maximum conditions of exposure. Separate studies are required for ruminants (cattles), non-ruminants (swine) and poultry (chickens). The studies should reflect all possible residue transfer routes such as:

(i) Direct absorption (dermal or inhalation) from sprays, mists, or fogs with animals present.

(ii) Direct consumption (e.g. by the animal licking surfaces treated with sugar base baits, pick up of bait granules by poultry, or contamination of feed, feed troughs, or water troughs).

(iii) Direct contamination of milk from deposition on milking equipment, treatment of milk rooms, etc.

(4) **Meat, milk, poultry and egg sampling.**

(i) Milk and egg samples should be taken twice daily. Eggs from birds within a dosage group may be pooled if necessary so that adequate sample weight is available for analysis and retained samples. Milk from animals within a dosage group should not be pooled so that data for individual animals are available. Enough of the pooled daily milk and egg samples should be analyzed (preferably at least twice weekly) to allow for a determination of trends in storage of residues with time. Three unique samples of milk and eggs should be analyzed at each time point for each feeding level. Petitioners are advised to analyze the samples from the highest feeding level first. If no quantifiable residues are observed in all such samples, those from the lower feeding levels do not need to be analyzed.

(ii) Analyses of a few samples of milk fat is advisable at some point to show how residues partition into that commodity. This information can be used to determine if a specific tolerance value should be specified for milk fat and to calculate dietary risk more accurately.

(iii) Analysis of eggs. The analysis should be conducted on the egg yolk and white combined in one sample. They may also be analyzed separately provided the weights of each are known so that the residue can be calculated on a whole egg basis.

(iv) Animals should be slaughtered within 24 hours of the last dosing and tissue samples taken and frozen as soon as possible. Tissue residue level results from animals slaughtered long after cessation of dosing are not usable in estimating tolerances, and thus if only such samples are analyzed, the feeding study will have to be repeated. The commodities to be analyzed in a feeding study include the following tissues that are used as human food: muscle, fat, liver and, in the case of cattle only, kidney. For dermal uses on poultry or swine, skin should also be analyzed. As noted above for milk and eggs, three unique samples of edible tissues should be analyzed at each dose level to show the variability of residues among different animals. In the case of cattle, this usually means one sample per animal as three cows are generally dosed at each level. For poultry, tissue samples from 3–4 birds may be composited to generate the three ‘‘unique’’ samples for each dosage group.

(v) Dermal treatment of livestock. Animals should be sacrificed within the preslaughter interval (PSI) prescribed on the product label. However, PSI’s longer than 3 days are not considered to be practical by the Agency in most cases. Since it has been observed that residues may not peak in tissues until a week or so after application, additional data reflecting longer PSI’s should be obtained to establish the maximum residue levels for tolerances.

(vi) The components of the residue to be analyzed in tissues, milk and eggs should be those found to constitute the ‘‘total toxic residue’’ in the animal products as determined in the livestock metabolism study described in OPPTS 860.1360. The analytical method should be described in detail or referenced. Fortified samples should be run concurrently with those from the feeding study to validate the method. The required limit of quantitation for the animal products will be related to the toxicity of the compound but should generally be on the order of 0.01–0.05 ppm or less. Requirements for analytical methods are spelled out in detail in OPPTS 860.1340.

(5) **Storage stability data.** Appropriate storage stability data are required on representative livestock commodities as outlined in OPPTS Guideline 860.1380.

(6) **Waiver of livestock feeding studies.** When low residues are present in feed items, registrants should refer to OPPTS Guideline 860.1300, Nature of the residue-plant, livestock, for a possible waiver of conventional livestock feeding studies. In some cases, the livestock metabolism study indicates that a feeding study and meat and milk tolerances are not necessary.

(e) **Guidance procedure for calculating livestock dietary exposure.**

The feed percentages listed for ruminants (i.e., beef and dairy cattle) in the updated Table I of OPPTS Guideline 860.1000, Background, are on

a dry matter basis, while tolerances for these feed items are established on an as-fed basis. Percentages for ruminants in the ‘‘Guide For Estimating Toxic Residues in Animal Feeds or Diets’’ (authored by Dr. L. Harris, 1975, and commonly known as the Harris Guide)(see paragraph (g)(8) of this guideline), and the ‘‘Update of Livestock Feed Consumption’’ [Animal Nutrition, Inc., 1993, referred to in this document as the ANI Report) (see paragraph (g)(9) of this guideline) are also listed on a dry matter basis. Therefore, the correct calculation of ruminant dietary burden includes the conversion of the feed to a dry-matter basis in the diet.

Percentages of the diet for poultry and swine feeds in the Harris Guide and the previous Table II of the earlier guidelines are also on a dry matter basis. However, poultry and swine listings in the updated Table I of OPPTS guideline 860.1000 and the ANI Report are on an as-fed basis since almost all feeds for poultry and swine are in the dry category. Therefore, the dietary burden calculation for poultry and swine using the updated Table I does not require conversion of the feed to a dry-matter basis.

The dietary burden calculation must also handle the situation that arises when the feed item(s) on which there is (are) tolerance(s) for a given chemical do not comprise a complete diet for the animal. For example, pesticide A has tolerances on alfalfa forage (50% of beef cattle diet) and alfalfa hay (25%), but on no other feed items. In this case, there is no information on the feed item(s) which would be used to round out the animal’s diet. If those additional feed items are wet, the residues on an as-fed basis will be diluted more than they would be if the feed items were dry. Errors in the estimate of the dietary burden to the animal could result.

These problems can be avoided, however, if the burden is calculated in terms of the weight (as opposed to concentration) of the pesticide consumed by the animal, and that amount compared with a standard amount of feed consumed by the animal. Using this approach, the following equation, Equation A, is derived for such calculations.

For ruminants, where feed percentages are expressed on a dry matter basis, equation A should be used to calculate the total dietary burden.

$$\left(\begin{matrix}dietary\\burden\end{matrix}\ [DM]\right)(ppm) = \sum_i \frac{(\%\,diet\ [DM])_i}{(\%\,DM)_i}\ x\ (tolerance)_i\left(\frac{mg}{kg}\right) \quad (A)$$

(dietary burden [DM]) (ppm) = estimation of total exposure of a pesticide through feeds on a dry-matter basis, expressed in ppm (mg pesticide per kg feed)

$(\%\text{diet [DM]})_i$ = percentage in the animal diet of commodity i expressed on a dry-matter basis

$(\%[DM])_i$ = dry-matter percentage in feed commodity i

$(tolerance)_i$ (mg/kg) = proposed or existing tolerance expressed in mg/kg (i.e., parts per million, ppm)

The burden thus calculated is on a dry-matter basis. Therefore, ruminant feeding and metabolism studies submitted to the Agency must have their feeding levels calculated on a dry-matter basis. For feeding studies in which the pesticide has been introduced via capsule, the petitioner should report the feed items and intake of each animal so that dietary burden can be calculated on a dry-matter basis.

Also note that Equation A could have been used for poultry and swine in the original livestock feed table (i.e., Table II of the 1982 Residue Chemistry Guidelines). However, as noted above, the feed percentages for poultry and swine in Table 1 of OPPTS Guideline 860.1000 are on an as-fed basis. In that case, no correction will have to be made for percent moisture; the dietary burden for poultry and swine will be simply calculated by Equation B as follows:

$$\begin{pmatrix} dietary \\ burden \end{pmatrix}(ppm) = \sum_i (\%\,diet)_i \; x \; (tolerance)_i \left(\frac{mg}{kg} \right) \quad (B)$$

The dietary burden in this case will be on an as fed basis.

The following sample calculations using both Equations A and B show how dry matter correction(s) can alter the estimated dietary burden.

Scenario 1. All feed items in the selected diet have proposed or established tolerances, and all feed items have low moisture content.

For example, consider the burden for beef cattle to Pesticide B fed the following diet (percentages from Table 1 of OPPTS 860.1000). The dietary burdens are calculated with and without correcting for moisture content using the feed items chosen for the animal's diet which have relatively low moisture contents.

corn grain	80% of diet	88% DM	0.1 ppm tolerance
corn fodder	20% of diet	83% DM	10.0 ppm tolerance

Calculation of the burden by Equation B (i.e., without conversion to a dry matter basis) would give the following:

$$(0.80) \times (0.1 \text{ ppm}) + (0.20) \times (10.0 \text{ ppm}) = 2.1 \text{ ppm}$$

When the adjustment for moisture content is made, a difference of 0.4 ppm is observed using Equation A:

$$\frac{(0.80)}{(0.88)}\ x\ (0.1\ ppm) + \frac{(0.20)}{(0.83)}\ x\ (10.0\ ppm) = 2.5\ ppm$$

Scenario 2. All feed items in the selected diet have established tolerances, and some, or all feed items have a high moisture content.

If wet items are included in the diet (e.g., forages), substantial errors in the estimated ruminant dietary burden could result if the calculations are not corrected for the moisture content. For example, if corn fodder in the above diet is replaced with corn forage,

corn forage	20% of diet	25% DM	10.0 ppm tolerance

without correcting for moisture, the same 2.1 ppm burden would be calculated by Equation B. However, correcting for moisture, the burden calculated by Equation A would be:

$$\frac{(0.80)}{(0.88)}\ x\ (0.1\ ppm) + \frac{(.20)}{(0.25)}\ x\ (10.0\ ppm) = 8.1\ ppm$$

Thus, using only Equation B, the dietary burden for beef cattle would be seriously underestimated.

Scenario 3. Not all feed items in the selected diet have established tolerances.

Similar underestimation of an animal's dietary burden can occur if the available feed items do not comprise a complete diet. Using the example of Pesticide A for beef cattle,

alfalfa forage	50% of diet	35% DM	2.0 ppm tolerance
alfalfa hay	25% of diet	89% DM	8.0 ppm tolerance

the dietary burden, if calculated using Equation B without conversion to a dry matter basis, follows:

$$(0.50)\ \times\ (2.0\ \text{ppm})\ +\ (0.25)\ \times\ (8.0\ \text{ppm})\ =\ 3.0\ \text{ppm}$$

Using Equation A, the dietary burden is calculated as follows:

$$\frac{(0.50)}{(0.35)}\ x\ (2.0\ \ ppm) + \frac{(0.25)}{(0.89)}\ x\ (8.0\ \ ppm) = 5.1\ ppm$$

This latter number represents a worst-case scenario; thus, the burden cannot be more than 5.1 ppm.

(f) **Data reporting format.** The following format is suggested for the report: (Note: Material which would best be presented in a table or figure is indicated by an asterisk.)

(1) **Cover page.**

Title page and additional documentation requirements (i.e., requirements for data submission and statement of data confidentiality claims) if relevant to the study report, should precede the content of the study formatted below. These current requirements are described in PR Notice 86-5 published by the Office of Pesticide Programs on July 29, 1986 (see reference in paragraph (g)(9).

(2) **Table of contents.**

The table of contents should provide page numbers on which are found the essential elements of the study, to include the following: Introduction and Summary, Materials, Methods, Results and Discussion, Conclusions, Tables/Figures (flowsheets, etc.), Certification, References, and Appendices. The requirements of each of these sections are discussed below.

(3) **Introduction and summary.**

(i) This section should provide background and historical perspective for the study. It should include:

(A) Registration history;

(B) Proposed use of the pesticide;

(C) Purpose of the study; and

(D) Summary of the results.

(ii) The summary of the experiment should include:

(A) A discussion of any unusual problems encountered and how these were resolved;

(B) A discussion of any deviation from the experiment's protocol and the effect this may have had on the results; and

(C) A brief description of the study's findings addressing such questions as;

(1) Do residues transfer,

(2) Preferential accumulation in certain organs,

(3) Highest residues, and

(4) When did residues plateau?

(iii) A comparison of the results to those of the animal metabolism studies would also be useful.

(4) **Materials.**

(i) Test substance.

(A) The pesticidal active ingredient and/or its metabolites which are fed should be identified by:

(1) Chemical name;

(2) Common name (ANSI, BSI, 1S0);

(3) Company developmental name/number; and

(4) Chemical Abstracts Service (CAS) number.

(B) The source and purity of each compound should be specified.

(C) Chemical structures* of these compounds are also desired.

(D) The rationale for feeding compounds other than parent pesticide.

(ii) Test facilities.

(A) The animals' housing should be described. Factors to consider include:

(1) Sizes of enclosures;

(2) Individual versus group housing;

(3) Food and water containers;

(4) Temperature;

(5) Lighting; and

(6) Waste handling.

(iii) Test animals.

(A) A description of the test animals should include:

(1) Species;

(2) Breed;

(3) Age;

(4) Weight; and

(5) Health status.

(B) The number of animals per feeding level must be specified.

(C) The mode of identification should be noted (e.g., ear tags).

(D) Body weights* and egg/milk production* should be reported for both the acclimation and dosing periods.

(E) Any health problems, abnormal behavior, or unusual treatment of animals should be reported and the effect of these on study results discussed.

(iv) Feed.

(A) The animals' diet during acclimation and the dosing period should be described as to both:

(1) The types of feed (e.g., corn grain, layers mash, alfalfa pellets) and liquids; and

(2) The quantities provided (i.e., specific amounts or ad libitum).

(B) Feed consumption* (dry weight) should be reported on an individual or treatment group basis throughout the study.

(5) **Methods.**

(i) Dosing.

(A) The preparation of the dose should be described (mixing with feed or concentrate ration, gelatin capsule, bolus, etc.). The ppm (mg/kg feed) level of the test material in the total diet (dry weight basis) is needed. The remanded doses are 1X, 3X and 1OX the anticipated dietary intakes from proposed usages of the pesticide. The calculation of these dietary burdens based on Table I of OPPTS guideline 860.1000 and the procedure in paragraph (e) of this guideline should be explained. The petitioner should consider possible future uses of the pesticide when determining the dosages to be fed. Dosing schemes other than 1X, 3X, and IOX are acceptable provided a satisfactory rationale is given.

(B) The date of dose preparation should be specified along with the storage conditions prior to its administration.

(C) A brief description of the method used to analyze spiked feeds and the results of such analyses should be presented. These analyses

should demonstrate that the pesticide was stable in the feed or dosing material throughout its entire storage period.

(D) The frequency of dosing should be reported if the test material is not incorporated into the total diet or feed.

(E) The dates of the initial and final doses (or the total length of the dosing period) should be indicated.

(ii) Sample collection.

(A) The collection of milk and eggs should be described with any differences from normal practice explained. Any compositing or pooling of samples ought to be noted, although milk from animals within a dosage group should not be pooled. Compositing the AM and PM milk from each individual cow in the ratio of production is acceptable.

(B) The collection dates* for those samples which are analyzed for the residue of concern should be reported.

(C) The mode of sacrifice and the time interval in hours between the latter and the administration of the last dose should be specified. An explanation of intervals longer than 24 hours should be presented along with a discussion of their effect on residues.

(D) The tissues taken after sacrifice, their type (e.g., thigh muscle, omental fat, etc.), and their weights should be listed. Combining of samples from different animals should be noted (usually acceptable for poultry, but not ruminants).

(iii) Sample handling and storage stability.

(A) The storage and handling of tissues, eggs and milk between sample collection and analysis should be described. Factors to consider are:

(1) Sample preparation (e.g., chopping) prior to storage;

(2) Containers;

(3) How quickly the samples are put into storage;

(4) Storage temperature;

(5) Length of storage (dates of collection, shipping, analysis, etc.); and

(6) Mode of shipping, if applicable.

(B) Evidence should be presented showing that the storage did not affect the results of the study. Preferably, this is obtained by concurrently spiking control samples and storing them under the same conditions as samples from treated animals. For guidance in this area refer to OPPTS

guideline 860.1380, Storage stability. If such information is provided in another section of the overall data package, the study may be referenced.

(iv) Analysis of samples.

(A) A detailed description of the analytical method employed to measure residues should be provided along with a statement as to which chemical species were measured (parent pesticide, metabolites). When the method has been submitted as a separate report in the total data package (as is often the case), it may simply be referenced. See OPPTS guideline 860.1340, Residue analytical method for assistance on how to describe methodology.

(B) Recovery data should be obtained concurrently with the residue analyses to validate the method and establish its sensitivity (lowest reliable quantitation limit). The experimental design of these validation studies should be described including:

(1) Identity of the test compounds and substrates (tissues, milk, and eggs).

(2) Magnitudes of fortification levels;

(3) Number of replicates per test compound per level; etc;

(C) Dates* of sample fortification, extraction, and analysis of extracts should be listed. If extracts are not analyzed on the day of preparation, storage conditions should be described.

(D) Raw data* such as sample weights, final volumes of extracts, and peak heights/areas should be furnished for control, fortified (including those for storage stability data) and treated samples to support reported residue values and recoveries. Analytical responses of standards (calibration curves*) are also needed. See also OPPTS guideline 860.1000 for more guidance on raw data.

(E) Representative chromatograms* should be supplied for control, fortified, and treated samples of each matrix (milk, eggs, each edible tissue, etc.) along with a few sample calculations of residue levels and percent recoveries using the raw data.

(6) **Results and discussion.**

(i) Recovery percentages* (all values, not just averages or ranges) for the pesticide and/or its metabolites should be reported for tissues, milk, and eggs fortified with these compounds.

(ii) Storage stability data* showing the behavior of residues as a function of time in tissues, milk and eggs should be submitted or referenced. Storage duration and temperature of these samples should be specified.

(iii) Levels* of the ‘‘total toxic residue’’ should be reported for each tissue for each feeding level including control (untreated) samples. The tissues recommended for analysis include muscle, fat, liver and kidney (latter not required for poultry). The individual values should be listed for all samples (not merely averages or ranges). It should be clearly indicated whether or not residues have been corrected for recoveries. If the parent pesticide and its metabolites are measured separately, the residues of each should be reported.

(iv) Residues* in milk and eggs should be listed for each feeding level including controls along with the dates of sample collection. As with tissue residues, the values for each sample should be reported (not just ranges or means).

(v) Discussion should be presented as to whether the data indicate that residues of the pesticide transfer to tissues, milk and eggs. If so, when did residues plateau in milk and eggs? Do they preferentially accumulate in certain tissues? Are the results consistent with the ^{14}C metabolism studies?

(7) **Conclusions.**

A conclusion must be reached as to whether residues of the pesticide transfer from feed items to meat, milk, poultry and eggs. If so, the extent of transfer should be discussed. The results can be summarized by a table* showing either the ranges or maximum residues in type each of sample for each feeding level. Such a table could then be used to determine appropriate tolerances each time additional feed items are registered.

(8) **Tables and figures.**

Note: This section need only include those tables or figures not included in Sections (4) through (7)..

(i) The following data should be presented in tabular form:

(A) Vital statistics of the test animals throughout the study including body weights, egg or milk production, and feed consumption.

(B) Dates of sample collection, fortification, extraction, and analysis.

(C) Raw data such as responses of standards, sample weights, final volumes of extract, volumes of aliquots injected, and peak heights/areas for all control, spiked (including storage stability) and treated samples.

(D) Recoveries of parent compound and/or its metabolites from tissues, milk and eggs.

(E) Residues of parent pesticide and/or its metabolites in storage stability samples as a function of time.

(F) Levels of the ‘‘total toxic residue’’ in tissues, milk and eggs from both treated and untreated (control) animals.

(ii) The following should be presented as figures:

(A) Chemical structures and names of compounds which are fed to test animals and of those which are measured in tissues, milk and eggs.

(B) Reproductions of representative chromatograms (gas, high liquid performance, thin layer, etc.) for control, fortified and treated samples and of any other graphic data (e.g., mass spectra, calibration curves, plot of egg/milk residues as a function of time, plot of residues versus time for storage stability samples, etc.) essential to the study.

(9) **Certification.**

Certification of authenticity by the Study Director (including signature, typed name, title, affiliation, address, telephone number and date).

(10) **References.**

Any references cited in the report should be included here.

(11) **Appendices.**

Reproductions of published reports that support the submitted study may also be included here if, in the registrant’s opinion, it will increase the efficiency of its review by the Agency.

(g) **References.**

The source material for this guideline was taken directly from the following set of documents.

(1) U.S. Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry. EPA Report No. 540/9–82–023, October, 1982, (Available from National Technical Information Service, Springfield, VA)

(2) U.S. Environmental Protection Agency, Residues in Meat, Milk, Poultry, and Eggs: Dermal Treatments, EPA Report No. 540/09–88–092, 1988.

(3) U.S. 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 No. 737–R–93–001, February, 1993.

(4) U.S. Environmental Protection Agency, Pesticide Reregistration Rejection Rate Analysis – Residue Chemistry; Follow-up Guidance for: Updated Livestock Feeds Tables; Aspirated Grain Fractions (Grain Dust); A Tolerance Perspective; Calculating Livestock Dietary Exposure; Number and Location of Domestic Crop Field Trials. EPA Report No. 737–K–94–001, June, 1994.

(5) U.S. Environmental Protection Agency, Pesticide Reregistration Rejection Rate Analysis – Residue Chemistry; EPA Report No. 738–R–92–001, June, 1992.

(6) U.S. Environmental Protection Agency, FIFRA Accelerated Re-registration – Phase 3 Technical Guidance. EPA Report No. 540/09–90–078. (Available from National Technical Information Service, Springfield, VA).

(7) U.S. Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, Series 171–4; Addendum No. 8 on Data Reporting, Residues in Meat, Milk, Poultry, and Eggs: Livestock Feeding Studies, EPA Report No. 540/09–89–010. (Available from National Technical Informatiion Service, Springfield, VA).

(8) Harris, L., Guide for Estimating Toxic Residues in Animal Feeds or Diets, 1975.

(9) Update of Livestock Feed Consumption, Animal Nutrition, Inc., 1993 (available from National Technical Information Service, order # PB94–107877).

(10) U.S. Environmental Protection Agency, Pesticide Registration Notice PR 86–5, Standard Format for Data Submitted under the FIFRA and Certain Provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA), May 3, l986.