DIETARY EXPOSURE BRANCH

STANDARD EVALUATION PROCEDURE

Residues in Meat, Milk, Poultry and Eggs: Feeding Studies/Feed-throughs

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I. INTRODUCTION

(A) Purpose of the Standard Evaluation Procedure

This Standard Evaluation Procedure is designed to aid Dietary Exposure Branch reviewers in their evaluations of livestock pesticide feeding studies submitted by petitioners/registrants. The document also informs pesticide manufacturers and the public of the considerations involved in review of such data.

(B) Background Information

Livestock feeding studies are required under 40 CFR 158.240 when residues of a pesticide requested for registration under the amended Federal Insecticide, Fungicide, and Rodenticide Act appear in livestock feed. Such residues could be due to treatment of agricultural crops or to intentional addition of the pesticide to the livestock feed (a feed-through). Usually one feeding study can be used to cover both of these situations. The section of the Residue Chemistry Guidelines (Subdivision O) dealing with such studies is 171-4(c)(3).

These animal studies are also needed to set tolerances for residues of pesticides in meat, milk, poultry and eggs under Section 408 of the Federal Food, Drug and Cosmetic Act. Exposure of the public to pesticide residues from ingestion of these raw agricultural commodities can then be estimated.

(C) Objective of Feeding Studies

As noted in 40 CFR 180.6, a conclusion must be made whether finite residues of a pesticide or its "conversion products" will be found in meat, milk, poultry and eggs when animals are "fed agricultural products bearing

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(1) Dermal treatment of livestock is addressed in a separate Standard Evaluation Procedure.

... pesticide residues," when pesticides are used "directly on the animal," or when pesticides are "administered purposely in the feed or drinking water." To make this conclusion livestock feeding studies and/or direct animal treatments (discussed in separate Standard Evaluation Procedure) are required to determine the extent to which the pesticide transfers to meat, milk, poultry and eggs. Such experiments should measure the "total toxic residue" (parent pesticide plus its degradation products, metabolites and impurities of toxicological significance) in these commodities following oral ingestion and/or dermal treatment depending on the proposed uses of the chemical. If the data then show that finite residues are expected from such uses, the studies will be used to determine the appropriate tolerances.

II. INFORMATION TO BE SUPPLIED

The submitted study should include all the information needed to describe completely the actual administration of the pesticide to the animals (i.e., the feeding portion of the study), the analyses of the tissues, milk, and eggs for the residue of concern, and the handling/storage of samples between those two procedures. Appendix 1 lists the basic information required for review of livestock feeding studies. This list represents a shortened version of the Data Reporting Guideline for this type of study (available from NTIS as #PB 89-124606).

Useful Standard Evaluation Procedures and Data Reporting Guidelines related to this document include those on Analytical Methods, Storage Stability, and Qualitative Nature of Residue: Metabolism in Food Animals.

III. THE DATA EVALUATION PROCESS

(A) Determine Need for Study and Dietary Burdens

Ruminant and poultry feeding studies are usually required whenever detectable residues of a pesticide are found on plant parts (such as grain, forage) or byproducts (meals, pomaces) consumed by livestock. [The last two paragraphs of this section describe a situation wherein the feeding study may not be needed when low residues are present on feed items.] Table II of the Residue Chemistry Guidelines (Subdivision O of EPA Pesticide Assessment Guidelines) lists the feed items associated with each crop. Hog feeding studies may also be required if accumulation of residues in tissues is likely.

If detectable residues are observed in feed items, the maximum dietary burdens for cattle, poultry and swine are then determined with the aid of the aforementioned Table II. The latter lists the maximum percentage of various feed items in the diet on a dry weight basis. This percentage is then multiplied by the level of pesticide residue (tolerance in ppm) on the feed item. For example, alfalfa hay bearing 50 ppm of a pesticide would contribute 12.5 ppm to the beef cattle diet (50 ppm X 25% of diet). The reviewer should keep in mind that some feed items can be eliminated due to feeding restrictions on the pesticide labels. Table II of the Guidelines lists which feeds can be so restricted.

Reviewers are reminded that the percent of livestock diet figures in Table II of the Guidelines are expressed on a dry weight basis. Therefore, for feed items with a high moisture content such as fresh grass, the residue levels should be corrected to a dry weight basis before utilizing the % of the diet figure.

If tolerances exist for several feed items that could be fed in combination, the contributions from the various feeds must be added. The reviewer should consider various combinations of the feeds to arrive at a "sensible" diet having the maximum dietary burden of pesticide. In doing so, the sum of the % contributions must of course be ≤100%. Also, the term "sensible" means that the mixture of feeds should be practical. For example, although apple pomace and corn silage each may comprise up to 50% of the lamb diet, they would not be fed in combination as 100% of the diet since both are roughages. About 30-50% of the lamb diet usually consists of grain. Some guidance for livestock diets is provided in Morrison's "Feeds and Feeding" (Reference 17 in Appendix 3) and Section 4 (Feed Substitution Tables) of the "Guide for Estimating Toxic Residues in Animal Feeds or Diets" (Reference 16 in Appendix 3). The reviewer could also contact USDA and/or workers at State Agricultural Experiment Stations for additional information on feed composition for livestock (see Reference 20 in Appendix 3).

Having arrived at the maximum dietary burden associated with a sensible combination of feed items, the reviewer has determined the 1X feeding level, a "worst case estimate of the potential livestock exposure." As noted in the Guidelines, the feeding study should include the 1X level as well as exaggerated levels (preferably 3X and 10X).

When low residues are detected on feed items, the reviewer should consider the anticipated dietary burdens and the results of the radiolabeled metabolism study when determining whether feeding studies are necessary. For example, if the dietary burden (1X) is expected to be 0.01 ppm and the ingestion of 10 ppm radiolabeled compound resulted in <0.1 ppm total radioactivity in all edible tissues/milk/eggs, a feeding study would not be necessary. The metabolism study in this case indicates that maximum expected residues in animal commodities will be on the order of 0.1 ppb (assuming linear relationship between dose and residues). In this case the metabolism study also serves as a feeding study and indicates that tolerances are not needed for meat/milk/poultry/eggs [Category 3 of 40 CFR 180.6(a)].

Reviewers should use caution when making the above extrapolations from results of metabolism studies to expected residues in tissues. The waiving of feeding studies should be limited to cases where the extrapolation involves total radioactivity in tissues and milk/eggs in the range of 0.1-0.2 ppm or less. When total radioactivity in tissues, milk, or eggs in metabolism studies is in the range of 1 ppm or higher, feeding studies will usually be necessary regardless of the residue level determined by the extrapolation. This is to take into account the fact that metabolism studies are generally run with a goat for only three days, while feeding studies typically use cattle and last 28-30 days.

(B) Read Report and Identify Data Gaps

Next, the reviewer reads the study to determine whether the information listed in Appendix 1 has been submitted. Any omissions which are significant enough to prevent a complete examination of the study should be noted in the review. Such data gaps must be clearly identified so the registrant can be informed by the Product Manager of the need for additional data and/or details on the conduct of the study.

(C) Assess the Appropriateness and Adequacy of the Data

The reviewer then considers the adequacy of the supplied data/information. In doing so, the reviewer should keep in mind the following major points:

- Was the proper material (parent and/or metabolites) fed at sufficient levels?

- Were feeds analyzed and consumption monitored to ensure ingestion of the claimed levels in the diet?

- Was the duration of dosing adequate (i.e., did residues plateau in milk and/or eggs) and sacrifice accomplished within 24 hours of the final dose?

- Were the proper tissues and r.a.c.'s (milk/eggs) sampled for both control and treated animals?

- Did the registrant provide storage stability data to show residues would not degrade between sample collection and analysis?

- Was the total toxic residue determined in tissues, milk and eggs using a validated analytical method?

For a more detailed list of points the reviewer should consider, refer to Appendix 2. In addition to the latter, the technical guidance available in reviewer aid materials such as the Residue Chemistry Guidelines and sources listed in Appendix 3 should be utilized.

Having considered all the points involved (Appendix 2) the reviewer writes a summary of the study clearly outlining any (1) significant data gaps/omissions [as noted above in (B)] and (2) deficiencies in the reported data (such as insufficient levels fed; toxic metabolites not measured; feeding terminated before residues plateaued in milk or eggs).

(D) <u>Make a Regulatory Determination</u>

As noted under the Introduction, a determination must be made as to whether finite residues of the pesticide and its "conversion products" will be found in tissues/milk/eggs and, if found, what tolerances are appropriate to cover such residues.

If data gaps/deficiencies prevent such a determination, the reviewer so indicates and outlines what corrective steps need to be taken. If the study is adequate, the reviewer categorizes the proposed use of the pesticide under 40 CFR 180.6(a).

For the use to be placed under Category 3 of 180.6(a) (i.e., no reasonable expectation of finite residues), no detectable residues should be incurred after

feeding the pesticide at 10X or more the expected dietary burden. In that case no tolerances are required for the pesticide in animal products (meat, milk, eggs, etc.).

The presence of detectable residues from the 10X or lower feeding levels means the use falls under Categories 1 or 2 of 180.6(a) ("finite residues will actually be incurred" or "there is a reasonable expectation of finite residues"). In that case the reviewer must determine what tolerance levels are appropriate. This generally involves using the maximum residues found from the 1X feeding level (method sensitivity used when no detectable residues found in the 1X study). If no animals were fed at the 1X level, the results from the feeding level closest to the maximum anticipated dietary burden are utilized. In that case the calculation assumes residues are proportional to dietary burden. For example, if cows ingesting 4.0 ppm of a pesticide produce milk containing 0.2 ppm residues, the appropriate tolerance for a 2.0 ppm dietary burden would be 0.1 ppm. If the anticipated burden falls roughly in between two levels employed in the study, calculations are made with both levels and the tolerances based on the higher of the two calculated or extrapolated values. It should be noted that the tolerances are almost always set to one significant figure (e.g., 0.01, 0.2, 0.5 ppm) for those ≤1 ppm. On occasion, fractional values greater than 1 ppm may be acceptable, although whole numbers are still preferred.

If dermal uses of the pesticide are registered, the tolerances must cover both the feed and dermal exposure. For this purpose it is generally assumed that residues from oral ingestion and dermal treatments are additive.

Although separate tolerances are established for meat, fat and meat byproducts (latter include liver and kidney), they are often set at the same numerical value. However, if residues concentrate in fat, the latter may receive a higher tolerance. Likewise, when milk residues are found to partition mostly into the milk fat, the tolerance is generally set on the latter with the equivalent whole milk value (1/25X milk fat tolerance) expressed parenthetically (e.g., "milk fat [reflecting 0.02 ppm in whole milk].....0.5 ppm"). If liver or kidney contains significantly higher levels than other tissues, a separate tolerance is established for that organ. The meat byproduct tolerance is then qualified to show the separation of that tissue as in the following example: meat byproducts (except kidney) ..... 0.1 ppm; kidney ..... 2 ppm.

In most cases tolerances are also established for the meat, fat and meat byproducts of goats, horses and sheep at the same levels as cattle. (Note that Table II of the Residue Chemistry Guidelines does not describe diets for these animals. For tolerance purposes their diets are assumed to be the same as cattle.) Unless a separate swine study has been conducted, the cattle data are also utilized to set tolerances on hog meat, fat and meat byproducts. However, the hog diet is based on the swine information in Table II (i.e., not assumed to be the same as the cattle diet).

Having completed the various calculations discussed above, the reviewer states whether the meat/milk/egg tolerances (existing or proposed depending on the type of registration action) are appropriate. If they are not, the proper ones are listed so the Product Manager can inform the registrant of the requirements for revised tolerances (i.e., a new tolerance petition or a revised Section F in a pending petition).

IV. REVIEWER AIDS

There are a large number and variety of source materials that are available to assist the data reviewer in the evaluation process. A listing of some of the more useful references that reside within the Branch is provided in Appendix 3 to this document.

-APPENDIX 1-

INFORMATION REQUESTED OF PETITIONER FOR LIVESTOCK FEEDING STUDIES

1. Identity, source, and purity of material (pesticide and/or metabolites) fed to animals.

2. Identity of animals and number per feeding level.

3. Housing and health of animals and feeding/milking schedules during acclimation and dosing periods.

4. Composition of diet (feeds, concentrates, water/liquids, etc.) and feed consumption prior to and during dosing.

5. Mode of administration of pesticide/metabolites (gelatin capsule, spiked feed, etc.).

6. Dosage levels in parts per million in the total ration (dry weight basis).

7. Frequency of dosing and dates of initial and final doses.

8. Dates of dose preparation, storage conditions until administration, and analyses of spiked feeds to determine actual feeding levels.

9. Sampling dates for milk and eggs and compositing techniques (if applicable).

10. Body weights and egg/milk production prior to and during dosing period.

11. Mode and date of sacrifice (time in hours from final dose of pesticide) and organs sampled (compositing of latter if applicable).

12. Conditions and length of sample storage (including shipping if applicable) prior to extraction/analysis.

13. Detailed description of analytical method and chemical species determined.

14. Recovery data demonstrating validity of analytical method.

15. Dates of sample extraction and analysis of extract (also storage conditions of extract if applicable).

16. Data demonstrating stability of residues under observed storage of samples and extracts (if applicable).

17. Measured residue levels in muscle, fat, liver, kidney (except poultry), milk, and eggs.

18. Representative raw data and chromatograms of control, spiked, and treated samples supporting reported residues and recoveries.

19. Names/addresses of organizations/personnel involved in the feeding and analytical portions of the study.

20. Quality assurance procedures - measures/precautions to ensure fidelity of feeding study and analyses (such as animal identification, proper labeling/coding of samples, record keeping procedures, high quality equipment and reagents, etc.).

- APPENDIX 2 -

POINTS TO CONSIDER IN EVALUATING FEEDING STUDIES

Administration of Pesticide to Animals

- Was the material fed clearly identified as to its chemical structure(s) and purity? Is there any impurity of concern (such as hexachlorobenzene)?

- Was the proper mixture (parent pesticide and/or metabolites) fed? The material fed should resemble the composition of aged residues in feed items. However, as long as plant and animal metabolites of the pesticide are the same, we usually accept administration of only parent compound. In some cases where the identification of parts of the plant residue is not known or a large number of metabolites is formed, the registrant may feed plants bearing weathered residues of the pesticide.

- Were the proper animals administered the pesticide? For ruminants and poultry the preferred species are cattle and chickens. Healthy animals should be chosen with dairy cows in mid-lactation (producing average milk yields) and chickens in full lay (producing eggs on most days). A swine study may be required if there is a likelihood for accumulation in tissues or if the pig (or other non-ruminant such as the rat) metabolism is significantly different from cattle and poultry.

- Did each feeding level have an adequate number of animals? For cattle and poultry there should be at least 3 and 10 animals per feeding level, respectively. (Ten birds allow compositing 3-4 tissues/eggs to produce 3 unique samples.) We also prefer that cattle and poultry feeding studies employ 3 and 10 control animals, respectively.

- Did feed consumption, body weights, and milk/egg production decrease drastically after dosing started? If so, animals did not receive an adequate acclimation period or the pesticide is producing deleterious effects. If the effects continue for the entire study, TOX should be alerted as to the possible hazard of residues in feed to livestock.

- How was the pesticide dosage expressed? Dosages should be given as concentration (ppm) in the total ration (dry weight basis). Expression as mg/kg body weight is acceptable provided enough information (total mg dose or body weight; feed consumption) is available to enable calculation of the ppm in the total ration.

- Were adequate levels administered? The study should include the 1X level (max dietary burden) and 3 and 10 times that level. The higher levels allow a determination of whether residues are proportional to intake.

- What modes of administering the pesticide were employed? For cattle the pesticide may be mixed with a concentrate ration or administered in a gelatin capsule. For chickens it is convenient to fortify the entire feed at the appropriate ppm.

- Were spiked feeds analyzed to check for proper mixing and to confirm the intended dose?

- If not used on the day of preparation, was spiked feed stored properly? Were data provided to show the level of pesticide was not affected by such storage?

- If the total diet was not spiked with pesticide (i.e., dose in gelatin capsule or in concentrate ration), was total feed consumption data provided to calculate ppm in diet?

- Were animals administered the chemical <u>daily</u> and until residues <u>plateaued</u> in milk or eggs (<u>or</u> for 28 days if no detectable residues in milk/eggs)? Note that Branch policy was clarified on 4/13/89 to state that dosing should continue at least four weeks even if residues plateau earlier in milk or eggs.

<u>Sample Collection and Analysis</u>

- What sampling procedure was utilized for milk and eggs? Milk and eggs should be collected twice daily and analyzed frequently enough (preferably at least twice weekly) to determine trends in total residues with time. Milk samples from different cows should not be pooled, although up to 3 eggs per composite sample is acceptable. Analyses should be conducted on whole milk and eggs (yolk + white). Several milk samples should also be analyzed to determine how residues partition into milk fat. However, information on the latter may be available in the metabolism study.

- Were animals sacrificed within 24 hours of ingestion of the final dose? Waiting periods longer than this are usually not acceptable for setting tolerances to cover residues incurred by ingestion of pesticide treated feeds. (Note: This may not be the case for determining tolerances to cover <u>dermal</u> treatment of livestock. We allow pre-slaughter intervals of up to three days for such uses. Refer to the separate SEP for details.)

- Which organs were analyzed? Were samples composited? For cattle and swine, analyses should be conducted on muscle, fat, liver and kidney. For poultry, samples should include muscle (leg and breast), liver and fat. Tissues from different cows or hogs should not be combined. Pooling of poultry tissues and eggs is acceptable provided that at least three unique samples are analyzed per feed level (i.e., composites of 3-4 tissues if 10 hens per level).

- Were all samples frozen as soon as possible after collection/sacrifice? Is storage stability data available reflecting intervals from collection to extraction and from extraction to residue quantitation? Refer to separate Standard Evaluation Procedure on Storage Stability for more details.

- Was the total toxic residue measured by a validated method? Has the method been tested by EPA and published in the FDA Pesticide Analytical Manual? Parent compound plus all metabolites and impurities of toxicological concern should be determined in milk/eggs/tissues. Recoveries should be $\geq$ 70%. For more details on analytical methodology refer to the Standard Evaluation Procedure (SEP) on that topic. For background on how we determine what comprises the "total toxic residue" refer to the SEP on Qualitative Nature of Residue: Metabolism in Food Animals.

- Was the method sufficiently sensitive? The sensitivity should be 0.01-0.05 ppm or less.

- Were control values less than the method sensitivity? If samples from untreated animals are found to contain apparent residues, the validity of the study is questionable.

- Did the submitter provide raw data and chromatograms to support the reported residue levels? Representative chromatograms should be presented for control, fortified and treated samples of each commodity. Actual values should be reported for each sample rather than an average for an entire group.

- Was there reasonable agreement between samples from the same feeding level? If one value is considerably higher than the others, can it be discarded as an outlier?

Other Considerations

- Are the results of the cold feeding study consistent with the radiolabeled metabolism study? The levels of the residue of concern should be similar provided comparable doses and pre-slaughter intervals were employed. However, variations may occur due to the shorter dosing period usually employed in the metabolism study.

- Does the proposed feed through use involve a formulation designed to change absorption characteristics within the digestive system? If so, a separate study for the feed through will be required.

- Has a Registration Standard been issued for this chemical and, if so, is it being used in evaluation of the feeding study?

- Is the pesticide undergoing Special Review? If so, has any relevant information been submitted under that process?

- Is there any other unpublished (such as data submitted in earlier petitions, Section 18 and 24(c) requests) or published information (such as Codex/FAO Monographs) known to us about feeding of this pesticide to livestock? If so, this information should be consistent with the results of the new study.

- APPENDIX 3 -

REVIEWER AIDS MATERIALS

Following is a listing of some of the more useful source materials within the Dietary Exposure Branch that could prove helpful in reviewing livestock feeding studies:

(1) Federal Food, Drug, and Cosmetic Act, as amended, § 408-409.

(2) Federal Insecticide, Fungicide, and Rodenticide Act, as amended.

(3) Subdivision O [Residue Chemistry] of the Pesticide Assessment Guidelines, § 171-3 and § 171-4, prepared by OPTS/EPA, 1982.

(4) Subdivision D [Product Chemistry] of the Pesticide Assessment Guidelines, prepared by OPTS/EPA, 1982.

(5) Code of Federal Regulations [40 CFR 158, 180, 185, and 186], General Services Administration, Washington, D.C., updated annually.

(6) Pesticide Chemical News Guide, R. E. Duggan, editor, Food Chemical News, Inc., Washington, D.C., 1982, updated monthly.

(7) "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry," Anal. Chem. 52, 2242-2248 (1980).

(8) Acceptable Common Names and Chemical Names for the Ingredient Statement on Pesticide Labels, 4th ed., C.R. Blalock, et al., editors, OPP/EPA, 1979, available from National Technical Information Service, Springfield, VA.

(9) Farm Chemicals Handbook, Meister Publishing Co., Willoughby, OH, updated annually.

(10) Nanogen Index: A Dictionary of Pesticides and Chemical Pollutants, K. Packer, editor, Nanogens International, Freedom, CA, 1975 (updated periodically by supplements).

(11) F.D.A. Pesticide Analytical Manual, Volumes I and II, available from the National Technical Information Service, Springfield, VA.

(12) Guidelines on Supervised Studies to Provide Data on the Nature and Amount of Pesticide Residues in Products of Animal Origin, Codex Committee on Pesticide Residues, draft dated Nov. 5, 1984.

(13) Foods and Food Production Encyclopedia, D. M. Considine and G. D. Considine, editors, Van Nostrand Reinhold Company, NY (1982).

(14) Food and Feed Crops of the United States, J. R. Magness, G. M. Markle, and C. C. Compton, Rutgers University, N.J., 1971.

(15) McGraw-Hill Encyclopedia of Food, Agriculture & Nutrition, D. N. Lapedes, editor-in-chief, McGraw-Hill Book Company, N.Y., 1977.

(16) "Guide for Estimating Toxic Residues in Animal Feeds or Diets" prepared for the E.P.A. by Lorin E. Harris, available from National Technical Information Service, Springfield, VA.

(17) Feeds and Feeding, Abridged: The Essentials of the Feeding, Care, and Management of Farm Animals, Including Poultry, F. B. Morrison, 9th ed., The Morrison Publishing Co., Ithaca, NY, 1958.

(18) Statistical Methods Applied to Experiments in Agriculture and Biology, 7th ed., G. W. Snedecor, Iowa State College Press, 1980.

(19) Registration Standards on various individual pesticides, prepared by OPTS/EPA, (several issued each fiscal year).

(20) Directory of Professional Workers in State Agricultural Experiment Stations and Other Cooperating State Institutions, published by U.S. Dept. of Agriculture.

(21) Various reference texts and journal publications of a scientific or agricultural nature, including FAO/Codex Monographs; Residue Reviews; Analytical Chemistry; Journal of Agricultural and Food Chemistry; Journal of the Association of Official Analytical Chemists.

(22) Dietary Exposure Branch files: petition and registration files; reviewer aids; policies; subject files; reading files; cultural practices files (livestock feeds, cattle, poultry, swine); et al.