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OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Residues in Livestock

INTRODUCTION

1. Residues in Livestock studies are conducted in order to quantify levels of residues in meat, milk, eggs and edible meat by-products, such as fat, liver, kidney of ruminants, following the use of a pesticide product. The situations to which such studies apply include application of a pesticide to raw agricultural commodities (RACs), and the feeding or grazing of such commodities and their by-products by livestock; pesticides that may be directly applied to livestock; and pesticides that are used in livestock premises.

PURPOSE

2. The primary purposes of the Residues in Livestock study are to provide:

- the basis for establishing maximum residue limits (MRLs) and
- for conducting dietary intake assessments for consumer safety.

GENERAL CONSIDERATIONS

3. Residues in Livestock studies provide data on the quantitative transfer of residues to meat, fat, milk, eggs and edible meat by-products. The transfer factor (T_f) is calculated as follows:

$$Tf = \frac{\text{residue level in edible commodity (milk, eggs or tissues)}}{\text{residue level in the diet}}$$

Residues in Livestock studies are typically conducted in ruminants (cattle) and poultry (laying hen). In general, the results of cattle feeding studies may be extrapolated to other domestic animals (ruminants, horses, pigs, rabbits and others) and laying hen feeding studies to other types of poultry (turkey, goose, duck and others).

4. In cases where the metabolic pathways in rodents (typically rats) differ significantly from those in ruminants (typically goats), a pig metabolism study may be required. In such circumstances, if the metabolic pathways in the pig study are different from those in the ruminant study, a pig feeding study should be conducted to provide the necessary information in accordance with the objectives in paragraph 2, unless the expected intake by pigs is not significant. Under certain circumstances livestock feeding studies in ruminants and poultry may be waived (see paragraph 8).

5. In the case of direct application of pesticides to livestock, registrants are advised to consult with their national authority to determine if such uses are regulated as pesticides or veterinary drugs. For pesticides that are directly applied, residue studies using the designated method of application to the

livestock species to be tested (dips, sprays, pour-ons, jetting), designated dosages and withdrawal times are conducted to determine residues levels in edible livestock commodities.

6. When the use of a pesticide in premises such as livestock housings is such that label restrictions cannot preclude the possibility of residues in meat, milk or eggs, residue studies should be conducted reflecting the conditions of maximum exposure. The studies should reflect all possible residue transfer routes such as direct absorption, direct consumption or direct contamination, e.g., contamination of milk from milking equipment.

7. Registrants are encouraged to consult national statutory requirements for animal protection and treatment, for sampling and, in particular, for slaughtering before commencing a study.

Situations in which a study may not be necessary

8. Conventional Residues in Livestock studies are not necessary when residues levels are below the limit of quantitation in feed items from crop field trials that reflect the proposed use of the pesticide (i.e., maximum rate, maximum number of applications, minimum pre-harvest interval), unless the Metabolism in Livestock study shows a potential for significant bioaccumulation of the pesticide in animal commodities. However, when quantifiable residues are present in the feed items, it will be necessary to consider the anticipated dietary burden and the results of the Metabolism in Livestock study.

9. In cases where a metabolism study with dosing at the equivalent of 10X, where 1X is the anticipated dietary burden, results in levels of the residues of concern in all edible commodities which are below the limit of quantitation (LOQ) (typically 0.01 mg/kg), then no quantifiable residues would be anticipated in livestock commodities as a result of the proposed use. In such situations, the metabolism study can also serve as a feeding study. In the absence of a Residues in Livestock study, regulatory authorities would consider the LOQ of a validated analytical method for determining residues in milk, meat and eggs as the basis for setting appropriate MRLs. Registrants are therefore encouraged to develop appropriate methods for enforcement of residues in livestock commodities.

10. In the case of treatment to animal premises, the registrant may present a scientific rationale for not requiring specific studies to be conducted on the basis of data derived from direct animal treatments and general handling and animal husbandry practices. Such a rationale may be accepted, for example, when the formulation type is identical or comparable. In addition, label restrictions may be able to be developed that may preclude the possibility of residues being present in meat, milk or eggs.

CONDUCT OF STUDIES

Livestock Feeding Studies

Nature of the test substance used for dosing

11. The test substance used in the study should be representative of the residue in the crop or feed.

12. Livestock are dosed with the representative component(s) of the residue as defined in the feed, which is derived from crop metabolism, confined rotational crop and processing studies. The residue definition of a pesticide might consist of parent compound plus one or more metabolites, or a single or several metabolites or degradation products. If the parent compound is the major residue in feeds/plants, and when it is metabolised by livestock similarly as in plants, it is appropriate to dose the animals with the parent compound only. If a unique plant metabolite is the predominant residue in the feeds and plants, then

it may be appropriate to dose with the metabolite only. Generally the feeding of mixtures is not recommended and needs a specific rationale.

Application form

13. The test substance(s) should be applied in a suitable form, preferably by capsule to simulate the residue concentrations in feed and to ensure consistent exposure over the duration of the study. If the substance is applied to the feed, it must be thoroughly mixed with the feed and regular analytical checks must be made to ensure the consistency and stability of the chemical in the feed over the study duration.

14. In the case of a study for a feed-through product, if the formulation is specifically designed to change the absorption characteristics within the digestive system, this formulation should be employed in the feeding study.

Dose levels

15. A Residues in Livestock study will normally comprise 3 different dose levels, 1X, 3X and 10X. To determine 1X, the residue contribution of a number of feed items to the overall dietary burden of the animal is estimated. This involves correlating the percentage of the feed item in the total diet of the animal with the highest residue (HR) for the raw agricultural commodities or feed items. For processed commodities and by-products, for example, fruit pulps/pomaces, oilseed meals, cereal fractions, etc., and whole grains or seeds, where the RAC is likely to be blended or originate from a number of sources prior to processing, the supervised trial median residue (STMR) of the commodity may be more representative of the highest residue likely to occur in practice.

16. In some cases a fourth dose level <1X may also be included in the study to reflect a lower livestock dietary burden situation. Factors to consider may include reduction of residues in feeds by processing, use of residue decline information to simulate realistic exposures, or, e.g., consideration of percentage crop treated.

17. The various feed levels in the study are needed to provide information on the relationship between the dose level and the resulting residue concentration in livestock commodities. This information is used as follows:

- When additional uses not foreseen during the conduct of the study will lead to a higher dietary burden than foreseen by the dose calculation for the 1X level, this may require revision of the existing MRLs and of the dietary exposure assessment. If the exposure to the animal is between the dose levels tested, then higher MRLs based on linear interpolation of the residue results between the dose levels tested in the study might be appropriate.

- Where there is no linear relationship between the dose and residue levels, then extrapolation between dose levels should be done with care and extrapolation from ruminants to other livestock animals that are fed significantly different diets may not be valid.

18. The diet estimated/calculated for the maximum dietary burden should reasonably reflect best feeding practices, so as to represent a reasonably balanced diet that is nutritionally suitable for livestock.

19. Feed tables indicating the percentages in livestock diets are given in the OECD Overview of Residue Chemistry Studies Guidance Document (1) and may be subject to updates as more current information becomes available. The concentration in the feed should be expressed as mg/kg on a dry

weight basis. The dose level in livestock is expressed on a mg/kg body weight (bw) basis in addition to reporting the bodyweights of test animals both before and during the duration of feeding.

Calculation of dose levels

20. The feed tables in the Overview of Residue Chemistry Studies Guidance Document include four feedstuff categories: Forages; Cereal Grains and Crop Seeds; Roots and Tubers; and "Plant Byproducts." Livestock feeding data from the United States/Canada, the European Community and Australia have been collated in these tables.

21. The single feedstuff items in each category are considered to be interchangeable so it is assumed that livestock will not be exposed to more than one item per category at the same time. Feedstuffs that are expected to lead to the highest dietary burden will be selected from each category. Prior to conducting feeding studies, data from crop residue studies will be available. From these studies the highest residue (HR) or the highest average field trial residue level (HAFT) (if information from several field samples per study is available) for the RAC or the supervised trial median residue - processed (STMR-P) for processed commodities originating from more than one source will be used to determine the dietary burden.

22. Differences in regional feeding practices and proposals for good agricultural practice (GAP) can lead to different values for X and therefore the combination of feeding practice and GAP which leads to the highest value of X will be selected as the basis for the lowest dose level. The maximum dietary burden per kg bodyweight is calculated for all livestock species and each region. For detailed examples, see Annex 4 of the OECD Overview of Residue Chemistry Studies Guidance Document.

23. From each livestock group (cattle, sheep, pigs or poultry), the maximum dietary burden is determined and used for the 1X dose calculation. As the feeding studies will be done only once for either ruminants and poultry and/or pigs (where applicable), the highest dietary burden from any one species will be used in the study. For example, if the highest dietary burden is estimated for beef cattle compared to dairy cattle, the test animals in the study will receive the dose calculated for beef cattle, although dairy cattle are used in the study.

Test animals

24. Separate feeding studies should be conducted for a ruminant and poultry whenever residues are likely to occur in the feeds of these classes of livestock. The species of choice for these feeding studies are lactating dairy cows and egg-laying hens.

25. Data on residues in milk from dairy cows will usually equally apply to dairy goats. In most cases the results of the cattle feeding study will be used to establish animal commodity MRLs for goats, pigs, sheep and horses.

26. Within the poultry group, data on chickens will usually be accepted in lieu of data on other livestock poultry, e.g., turkeys, geese and ducks.

27. In the case of feed-through formulations, animals within an appropriate bodyweight range (as would appear on a product label) should be selected to reflect the maximum proposed daily dose of the compound.

Numbers of test animals

28. Ruminants and monogastrics: 1 untreated (control) animal per study and 3 animals per dose group. In the case of bioaccumulating substances (see paragraphs 35-37), the highest dose group will comprise a minimum of 3 additional animals.

29. Hens: 1 untreated (control) animal per dose level (3 to 4 per study) and 9-10 animals per dose group. In the case of bio-accumulating substances, (see paragraphs 36 -38) the highest dose group will comprise a minimum of 9 additional animals.

Use of control animals

30. In addition to establishing a baseline or blank in an acclimatisation period, control animals should be carried through the experiment together with treated or dosed animals. This is highly desirable, as values for control animals have been observed to change during feeding studies. These animals are also necessary to determine whether there are any adverse effects on egg production, milk yield and general health of the animals in the study. The control animals also provide enough sample material to enable appropriate method validation.

Condition of animals

31. The condition of the animals, both during the acclimatisation and dosing phases should be recorded throughout the study period, together with information on the age and individual bodyweights, daily feed consumption (individual or mean group basis), milk production or egg production. Cows should be in their lactation period suitable for commercial milk production and producing an average milk yield. Hens should be in full egg production before dosing commences. It should be noted that if feed consumption is recorded on a mean group basis rather than an individual animal basis, inaccuracies in dosing may occur where some animals may not be consuming as much feed as others and this is not adequately recorded in the study.

32. The physical condition of the animals can provide important information on rates of absorption and depuration of the administered chemical. Any health problems, abnormal behaviour, low feed consumption or unusual treatment of the animals should be reported and the effect of these on the study results should be discussed where relevant.

Duration of study

33. A suitable acclimatisation period prior to the beginning of dosing is recommended. Successful acclimatisation is indicated, for example, by normal feed consumption, body weight stability, or the production of average quantities of milk or eggs.

34. Once acclimatized, 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.

35. In cases where quantifiable residues are present in the ruminant and laying hen matrices sampled (milk, meat, fat or eggs) after the terminal dose at the nominal 1X dose level, some authorities call for depuration information to determine when residue levels in commodities will decline to the LOQ of the enforcement method. A depuration phase subsequent to the dosing phase (as ancillary to the feeding study, see paragraph 37) or, a separate depuration study after the feeding study in non-lactating animals in situations where milk is determined to be a major elimination pathway for the chemical (see paragraph 38), may be conducted. Authorities may also consider proposals projecting the time course of depuration based

on available studies and other relevant information. Registrants are encouraged to consult with regulatory authorities that require depuration studies.

Depuration studies

36. A depuration phase conducted with the highest dose group is sufficient to cover all feeding levels associated with GAP, as the objective of the depuration phase is to provide information on the decline rate. At least three time points following cessation of dosing at the highest dose level should be included, i.e. practical zero withdrawal and three other time points, with at least one ruminant and three hens to be slaughtered per time point. An adequate number of time points should be chosen to be able to estimate a half-life of depuration in meat/fat, milk or eggs. It is difficult to prescribe the time taken to demonstrate adequate depuration for any chemical, as this will depend on the defined residue and its properties in tissues, milk and eggs. Registrants are advised to consult with their national authority before designing a study.

37. In some circumstances, such as the cases of compounds that preferentially accumulate in fat as opposed to milk, registrants may consider conducting a separate depuration study using beef rather than lactating cattle, as the rates of depuration may be different where milk becomes an additional route of elimination for the chemical. Typically, three animals should be included at each depuration time point. In such cases, registrants are encouraged to consult with their national authority for advice on study design.

Fat-soluble chemicals and additional considerations

38. The designation of a residue as either 'fat-soluble' or not 'fat-soluble' is important for trading purposes and compliance with relevant standards. Fat-solubility is a property of the residue, the combination of the pesticide and its metabolites, degradates and related compounds to which the MRL or STMR apply and is primarily assessed on the basis of the partitioning of the defined residue between muscle and fat observed in the metabolism and livestock residue studies. Sampling protocols for animal commodities depend on whether a residue is fat-soluble or not[1].

39. The considerations applied to the designation of a residue definition as fat-soluble or not for meat and fat is utilised in the design of any livestock feeding study. Data generated in a livestock study (radiolabelled or transfer) need to adequately demonstrate that consideration of the fat-solubility of the chemical and/or metabolites has been taken into account. If the study is not adequately designed, and appropriate samples have not been taken, then it is difficult to determine whether a residue should be designated as fat-soluble or not.

40. For residues that are considered to be fat-soluble, Residues in Livestock studies (excluding poultry) should provide information on the residue levels likely to occur in fat depots when directions for use of the pesticide are followed. In such circumstances, different fat types should be analysed separately, because pooling of fat depots could lead to an underestimate of the residue level. For each fat depot, the fat description should include:

- the nature of the fat (e.g., perirenal, mesenterial, subcutaneous),
- location in the animal body (if more than one possibility), and
- lipid content (rendered or extracted fat may be assumed as 100% lipid) or data on lipid content from literature.

[1] Reference: FAO Manual (2002), page 52

Sampling

41. Detailed information on samples to be taken from ruminants, hens and pigs are given in Tables A, B and C of paragraph 64 and in the paragraphs following the tables. Tissues to be analysed should include, as a minimum, skeletal muscle, perirenal fat, subcutaneous fat or backfat, liver and kidney. Individual animal residue data should be reported. In the case of fat-soluble chemicals, fat depots should not be pooled, but analysed separately.

Direct Animal Treatment

42. When a pesticide is proposed for direct use on food animals, data are used to show the extent of residues incurred by the use. Direct uses include products that may be applied as back-line treatments, sprays, dips, pour-ons, dusts, dust-bags, back-rubbers, ear-tags and by jetting. The experimental treatment should reflect as closely as possible the conditions under which the pesticide will be used commercially, paying particular attention to the proposed dosing regime, normal animal husbandry practices, animal gender and animal maturity. All factors that might contribute to the variability of residue levels in animal commodities should be considered and taken into account in the planning and conduct of trials.

43. Different treatments and products comprise many formulation types, including wettable powders, suspension concentrates, emulsifiable concentrates, ready-to-use liquids and dusts. When a pesticide is applied in more than one type of formulation or by more than one mode of treatment, separate studies reflecting the proposed use or combination of uses should be conducted.

44. In general, separate studies should be carried out for each species of livestock to be treated. The types of factors that should be considered in the conduct of a cattle study or pig study may be different from the types of factors that should be considered in the conduct of a sheep study. For example, factors such as whether sheep passing through a dip or spray were freshly shorn or unshorn (i.e., 'off-shears', 'short-wool', 'long-wool') should be considered, together with animal type or breed and environmental conditions.

Treatment regime and dosing

45. Trials should be planned to ensure that animals receive the highest exposure to the pesticide allowed by the proposed use, i.e., the maximum treatment regime. This means:

- the longest exposure time (maximum time in the dip or spray, 'thorough coverage', or
- the maximum amount of material per animal based on the bodyweights of the animals (pour-ons, back-line treatments, dusts), or
- free access of animals to the material, plus correct placement and recharging (back-rubbers and dust-bags).

46. For multiple treatments, the shortest recommended interval between treatments should be used, as well as the maximum number of re-treatments in the 'season' or 'per year.'

47. For administration of the active substance to individual animals, the dose should be expressed on a bodyweight basis. If the dose is intended to be on a body area basis, it should be expressed both on a body weight basis and on a body area basis. Products applied without dilution should have the concentration of active ingredient at or near the top end of the product specification. Concentrations in dips should be at the maximum permissible concentration in relation to the proposed directions for use on the

label. This concentration should be maintained for the trial. The first group of animals that enter the dip should be retained for re-dipping and animals from that group should be analysed as part of the trial. Due account needs to be taken, where applicable, of recharging dips and sprays in accordance with proposed label instructions, use of correct nozzle types, and pressure and required delivery rate of sprays.

Selection of test animals

48. Animals should be selected with attention to those factors that are known to be important, such as milk yield or breed of animal. It is important that a representative sample of animals be taken from the relevant target population to account for animal variability. It has been suggested to include high yielding cattle at an early stage of lactation and low yielding cattle at a late stage of lactation in a residue depletion study. This should guarantee that at least some of the normal inter-animal variability is included in the study.

Numbers of test and control animals

49. For a residue study in tissues, sufficient data are generally provided from residue data from the target tissue of twenty animals, with five animals being slaughtered at each of four evenly distributed time points An exception to this is the proposed use of low volume products on sheep, where six animals should be used at each time point. Provision should, however, be made for the loss of animals during a trial. At least one untreated control animal should be slaughtered at or before the initial time point after treatment.

50. For a residue study in milk, sufficient data will be provided by twenty animals with milk collected from all animals at evenly spaced time points

51. Treated and control animals should be run and/or housed separately to avoid cross-contamination. Animals, especially cattle, frequently groom and lick each other. Treated animals should be run together in an enclosure that is large enough to allow normal animal behaviour.

Duration of study

52. The duration of the study is determined by the number of slaughter intervals needed to adequately demonstrate depletion of residues. Slaughter intervals should be selected to demonstrate the time and duration of maximum residues and their subsequent depletion. Animals should be sacrificed within the pre-slaughter interval (PSI) proposed on the product label, however it is advisable to check with the relevant national authority to determine the length of the PSI that may be practical and design the study accordingly. Since it has been observed that residues may not peak in tissues until a week or so after application, additional data reflecting longer PSIs should be obtained to establish the MRLs. As described in the feeding studies section above, additional slaughter intervals (in addition to setting the PSI) to demonstrate depuration may also be needed for compounds that are fat-soluble.

Sampling

53. Detailed information on samples to be taken from ruminants, poultry and pigs are given in Tables A, B and C of paragraph 64 and in the paragraphs following the tables. Tissues to be analysed should include, as a minimum, skeletal muscle, perirenal fat, subcutaneous fat or backfat, liver and kidney. Special care should be taken to ensure that residues on the skin or wool do not contaminate the tissue samples during sample collection. Individual animal residue data should be reported. In the case of fat-soluble chemicals fat depots should not be pooled, but analysed separately. However, if there is insufficient backfat for analysis, the backfat should be supplemented with other subcutaneous fats, preferably brisket fat, and its source reported in the study.

Situations Involving Both Direct Treatment and Livestock Feeding

54. There may be specific situations where data are needed to simulate exposure from direct application of a product to livestock in addition to exposure through feeding of treated crops. In such cases, 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 MRLs for animal commodities.

55. In the case of studies conducted in support of plague locust control, a combined study may be conducted whereby animals are exposed to a direct treatment at rates typical of application of a proposed product, while also being grazed on an area exposed to the product at proposed rates. The combined study encompasses two types of treatments in one study and provides a realistic exposure situation compared to one where the results of two separate studies are added. Registrants are encouraged to contact their national authorities before deciding to conduct a specific combined scenario study.

Agricultural Premise Use Studies

56. When the use of pesticides in agricultural buildings is such that label restrictions cannot preclude the possibility of residues in meat, milk or eggs, residue studies should be carried out reflecting the maximum conditions of exposure. In the case of treatment to animal premises, the applicant may present a scientific rationale for not requiring specific studies to be conducted on the basis of data derived from direct animal treatments and general handling and animal husbandry practices.

57. In many cases, it may not be practical to remove animals from their housing while treatment takes place. An exception would be milking sheds. The study should be conducted using the species and animal housing situation which gives the greatest potential for animal exposure. Separate studies should be conducted for ruminants (cattle), non-ruminants (swine) and poultry (chickens). The studies should reflect all possible residue transfer routes such as:

- Direct absorption (dermal or inhalation) from sprays, mists, or fogs with animals present.
- 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).
- Direct contamination of milk from deposition on milking equipment, treatment of milk rooms, etc.

58. In two separate premises, or in two isolated areas of the same premises, the treatment should be applied at the highest treatment rate, and at 1.5 to 2 times that rate, using the proposed methods as indicated on the label. In a third separate area, animals should be kept as control animals. The animals in all three areas should be of the same breed and sex and of the same general age, weight and body condition. In the study, adequate details of the nature of the housing and application of the treatment should be reported.

59. Where multiple treatments are proposed, the trials should be carried out accordingly and the animals slaughtered or eggs/milk collected after all treatments are completed.

Sampling of milk and eggs

60. Before commencing treatment, milk or eggs should be taken from all animals in order to determine residues in control samples. Upon initiation of dosing, milk and egg samples should be collected at least twice a week (i.e., every 3-4 days). On the days of sampling, milk should be collected twice a day at milking (morning and afternoon). If one of those samplings occurs the same time of day as dosing, the samples should be collected prior to administration of the dose. Samples should be taken first from the control group and then from the treated animals.

Pooling of samples

61. Milk should be collected twice daily from each cow. The two samples from an individual cow may be pooled to generate a representative sample for analysis. However, milk from separate cows should not be combined.

62. Eggs should also be collected twice daily. Any excrement adhering to eggs should be removed. Eggs from hens within a dosage group may be pooled if necessary so that adequate sample weights are available for analysis and retained samples. Three unique samples of eggs should be analysed at each time point.

Slaughter and sampling of meat and edible tissues

63. Ruminants should be slaughtered within 24 hours of administering the final dose; the exception being livestock used in residue depletion studies. In a direct treatment study, the first slaughter interval or zero withdrawal is considered to be 8 to 12 hours after the last treatment. Hens should be slaughtered within 6 hours of administering the final dose (zero withdrawal). When slaughtering animals, it should be ensured that the tissue samples are not contaminated by blood, urine, feces or other body fluids.

64. Details of samples to be taken are given in the tables below.

A. Ruminants

Sample Material	Sampling Method	Analytical Sample Preparation	Weight/unit (homogenised) Laboratory Sample
Meat	Collect approx. equal pieces of loin, flank or hind-leg (round piece) muscle	After coarse pre-chopping, macerate in a mincer and then mix carefully.	0.5 kg
Fat	Collect approx. equal quantities of subcutaneous, mesenterial and perirenal fat	After coarse pre-chopping, macerate in a mincer and then mix carefully[1].	0.5 kg
Liver	Collect the entire organ or representative parts thereof, e.g., a cross-section of the lobes	After coarse pre-chopping, macerate in a mincer and then mix carefully.	0.4 kg
Kidney	Sub-sample from both kidneys	Macerate tissue in a mincer and then mix carefully.	0.2 kg
Raw milk[2]	Collect milk from each animal separately		0.5 L[3]

[1] For fat-soluble compounds, samples of perirenal, mesenterial and subcutaneous fat from ruminants should be analysed individually, not as a composite.

[2.] For fat-soluble compounds, residues in the milk fat need to be determined at the end of dosing in addition to the plateau level. The fat should preferably be separated from the milk by physical means, not by chemical solvent extraction, because in solvent extraction residues are extracted from both the aqueous and the lipid phase. As in this way, cream (containing 40 – 60% fat) and not 100% milk fat is obtained; the lipid content of the cream should also be reported. Where a depuration phase is included after the dosing period, samples taken at a minimum of four time-points after the last day of treatment is recommended.

[3] In case an intermediate storage in deep-frozen stage becomes necessary, the sample size of pooled milk samples can be reduced to amounts representing an analytical sample each.

Tissues from different animals should not be combined or pooled at sampling.

B. Poultry

Sample Material[1]	Sampling Method	Analytical Sample Preparation	Weight/unit (homogenised) Laboratory Sample
Meat	Collect approx. equal pieces of leg and breast	Macerate pieces of meat from 3 hens in a mincer and then mix carefully.	0.5 kg
Skin with Fat	Collect all the abdominal fat from at least 3 hens	Chop the fat of 3 hens [2]	0.05 kg
Liver	Collect the entire organ	Chop the livers of 3 hens [2]	0.05 kg
Eggs		Clean shells, break eggs from 3 hens, combine the whites/yolks, discard the shells [3] Limited analysis of yolk and white separately for some chemicals[3,4]	3 units

[1] For dermal uses on poultry, skin should also be analyzed.

[2] The prerequisite for combining of sample material is that at least 3 samples per dose group are available (i.e., at least 9 animals are involved).

[3] Samples can be prepared either before or after transport to the analytical laboratory. The eggs are homogenised by addition of solvent on commencement of analysis.

[4] Analyses of eggs should be conducted on the egg yolk and white combined in one sample, For fat-soluble residues some analysis of the deposition of residues into yolk and white fractions may be conducted to determine how the residue partitions between the egg fractions. The residue levels in yolk and whites may be analysed separately, provided the weights of each are known, so that the residue can be calculated on a whole egg basis for the purpose of MRL setting. Yolk and white would require separation prior to storage of the samples.

C. Pigs/Swine

Sample Material[1]	Sampling Method	Analytical Sample Preparation	Weight/unit (homogenised) Laboratory Sample
Meat	Collect approx. equal pieces of loin, flank or hind-leg (round piece) muscle	After coarse pre-chopping, macerate in a mincer and then mix carefully.	0.5 kg
Fat	Collect approx. equal quantities of subcutaneous, mesenterial and perirenal fat	After coarse pre-chopping, macerate in a mincer and then mix carefully.[2]	0.5 kg
Liver	Collect the entire organ or representative parts thereof	After coarse pre-chopping, macerate in a mincer and then mix carefully.	0.4 kg
Kidney	Sub-sample from both kidneys	Macerate tissue in a mincer and then mix carefully.	0.2 kg
Skin	Collect approx. equal pieces of back, flank and belly	After coarse pre-chopping, macerate in a mincer and then mix carefully	0.5 kg

[1] For dermal uses on swine, skin should also be analyzed.

[2] For fat-soluble compounds, samples of perirenal, mesenterial and subcutaneous fat from ruminants should be analysed individually, not as a composite.

Sample Analysis

65. The analytical method including sample extraction and clean-up procedures 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 LOQ for the animal products will be related to the toxicity of the compound but should generally be in the order of 0.01-0.05 mg/kg or less, if deemed appropriate from a dietary risk assessment perspective.

Milk and eggs

66. Samples should be analyzed for each group on day 0 and at intervals of every 3 to 4 days until residues plateau. Once the plateau is reached, egg and milk samples may be analyzed at weekly intervals (e.g., days 14, 21, 28). Three unique samples of milk and eggs should be analyzed for each dose group at each time point. However, if quantifiable residues are not found in samples from the higher/highest dose group(s), analyses are not necessary for samples from the lower dose group(s).

67. This information can be used for MRL/tolerance setting and for refined dietary risk assessments.

Meat and edible tissues

68. It is advisable to start with analyses of samples from the highest dose group first. If no quantifiable residues of a pesticide are observed in a tissue at the highest dose level, no further analyses of that tissue at lower feeding levels is necessary.

69. The commodities to be analyzed in a feeding study include the following tissues that are used as human food: muscle, fat, liver for poultry and also kidney for ruminants and swine. For dermal uses on poultry or swine, skin should also be analyzed.

70. As noted 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 or pigs, this usually means one sample per animal as three animals 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

Dermal treatment of livestock

71. Sampling and analysis of tissues and milk, as indicated above for feeding studies, equally apply for sample collection and analysis from direct application treatments. It is, however, recommended that the registrant consult with the relevant national authority prior to designing a study.

Storage Stability Data

72. Appropriate storage stability data should be collected on representative livestock commodities. For samples not analysed within 30 days, storage stability data should be generated to provide sufficient evidence that there was no significant degradation of the residue of concern between sampling and analysis.

CONSIDERATIONS FOR DATA REPORTING

Data

73. The following elements should be considered during the design and conduct of the test.

Summary

- Summary of key results: residues transfer to meat, milk, eggs, liver, kidney from oral dosing, dermal application or livestock housing treatment, preferential accumulation in certain organs, highest residues, and occurrence of a plateau in residue concentration in milk or eggs,
- Evaluation of these results,
- Any anomalies of the study, an evaluation of their relevance with reference to the objective.

Objective

A description of the aims of the study in detail including the questions to be dealt with in the study.

Test material

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

- Chemical name (IUPAC);
- Common name (ANSI, BSI, IS0) (if available)
- Chemical Abstracts Service (CAS) name and number.

– The source and purity of each compound should be specified.

– Chemical structure graphics of these compounds are also desired.

– A rationale for feeding compounds other than parent pesticide.

In-life part

– The animal housing should be described. Factors to consider include: Sizes of enclosures, individual versus group housing, food and water containers, temperature, lighting and waste handling.

– Test animals:

(i) A description of the test animals should include: Species, breed, age, weight (including record of weight changes), and general condition and health status. (Table 1 of template).

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

(iii) Body weights and egg/milk production should be reported for the acclimation dosing and withdrawal periods, if relevant.

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

(v) Changes/observations in animal liver and kidney when samples were taken after slaughter should be reported. Attention should be paid to situations when such observations were reported in studies with laboratory animals.

– Feed:

(i) The diet of animals 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.

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

(ii) Feed consumption (dry weight for ruminants) should be reported on an individual or treatment group basis throughout the study. See note for recording on a group basis in paragraph 31.

– Dosing:

(i) The preparation of the dose should be described (mixing with feed or concentrate ration, gelatin capsule, bolus, etc.). The level of the test material in the total diet in parts per million (mg/kg feed)(dry weight basis for ruminants) is needed. The recommended doses are 1X, 3X and 10X, additional doses or deviations should be justified.

(ii) For direct treatment, the nominal concentration and maximum concentration of the treatment should be recorded for each group (mg/kg bodyweight), as well as the concentration for each animal.

(iii) For livestock premise treatments, the concentration of the chemical per area basis or other units should be recorded.

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

(v) A brief description of the method used to analyze fortified 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.

(vi) Details of the method of application or treatment should be reported if the chemical is not administered via an oral route, with a description of the equipment used and duration of application or treatment if relevant, e.g. plunge dip or shower.

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

(viii) The dates of the initial and final doses or applications (or the total length of the dosing period) should be indicated including withdrawal periods where applicable. Dose rates or application concentrations should be reported as mg/kg diet, mg/animal/day and mg/kg bw/day.

(ix) Number of animals per feeding group or treatment group and control group.

– Milk and egg sampling:

(i) The collection of milk and eggs should be described with any deviations from normal practice explained. Any compositing or pooling of samples should be noted, although milk from animals within a dosage group should not be pooled.

(ii) For feed through formulations, collection of urine, feces (or cage wash) should be reported.

Post- slaughter sampling

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

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

Sample handling and storage stability.

(i) 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. Time interval between sampling and storage

4. Storage temperature;

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

6. Mode of shipping, if applicable.

(ii) Where samples are not analysed within 30 days, evidence should be presented showing that the storage did not affect the results of the study, i.e. demonstrate or reference adequate storage stability.

Extraction, clean-up, determination, evaluation.

A description of the method used to prepare and measure the samples; identification of the residue levels in tissues, milk and eggs and the methods used to assess the results.

Analysis of samples.

(i) A detailed description of the analytical method employed (including method validation data, recovery and method sensitivity) to measure residues 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. Preparation and handling of the sample throughout the method should be described in detail. Note that methods for metabolites may also be needed.

(ii) 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.

(iii) Identify instrumentation, equipment and reagents used and the operating conditions of the instrumentation. If the extraction/clean-up procedure is complex, a flow diagram should be submitted.

(iv) 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 fortification level.

(v) 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.

(vi) Analytical responses of standards (calibration curves), copies of representative chromatograms should be supplied for control, fortified, and treated samples of each matrix (milk, eggs, each edible tissue, etc.) along with at least one sample calculation of residue levels and percent recoveries using the raw data. Examples of calibration curves of analytical standards should also be provided.

Results and discussion.

This section of the report discusses and evaluates the reported measurements in the light of the questions outlined in the objective section. The relevance of results should be discussed in relation to the proposed uses of the plant protection product.

(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 residue of concern 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 needed for poultry). The individual values should be listed for all samples (not merely averages or ranges). It should be clearly indicated whether residue values have been corrected for recoveries. If the residue of concern consists of several compounds, the residues of each should be reported, as far as analytically achievable.

(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 and, if so, when did residues plateau in milk and eggs? Do they preferentially accumulate in certain tissues? In the case of direct treatment, discussion should also include an appropriate slaughter interval or withdrawal period for the target tissue after treatment. Are the results consistent with the livestock metabolism studies?

Conclusion.

A conclusion should be reached as to whether residues of the pesticide transfer from feed items, direct application or livestock housing treatment to meat, milk and eggs. If so, the extent of transfer should be discussed. The results can be summarized in a table (the preferable format) showing either the ranges or maximum residues in type each of sample for each feeding level.

Study Report

74. The study report should contain the following information:

- description of the test animals and their body weights, daily feed consumptions, egg/milk production, and any health problems.

- preparation and administration of the test dose, including dose concentration or concentration of test material applied, storage conditions of the dose, analysis of spiked feeds (if applicable), method of application or administration, frequency and times of dosing or treatment and withdrawal if applicable, and number of animals per dose level group.

- description of the sampling, compositing, pooling, and storage of milk and egg samples, or for feed-through formulations urine, faeces and cage wash.

- description of slaughter and sampling, including the interval from last dose, or application, or withdrawal of dose/treatment to slaughter, tissues harvested (weights, combining from multiple animals).

- description of sample handling and storage from sample collection until analysis.

- description of the extraction and clean-up of samples.

- discussion of the analytical method(s) used to determine the residues, including the validation of the method and its sensitivity with concurrent recovery samples.

- presentation the results of the measurements, including individual recovery values for fortified meat, milk, poultry, and eggs, storage stability of the residues with time in the various animal matrices, and concentrations of the residue of concern in each tissue, milk, and eggs at each feeding level and during the withdrawal period if applicable, and at various time intervals for milk and eggs.

- discussion of the results including whether a transfer of pesticide residue to milk, eggs, fat, muscle, liver, and/or kidney occurs, when the residues plateau in milk and eggs and, for dosing or direct treatment, whether residues deplete after withdrawal, and a comparison to the results of the livestock metabolism studies.

- conclusions on the extent of transfer of the residue of concern to fat, muscle, kidney, liver, milk, and eggs at each of the feeding levels.

LITERATURE

The following documents provide additional guidance on conducting livestock feeding studies.

(1) OECD Guidance Document: Overview for Residue Chemistry Studies (2006)

(2) OECD Guidance Document on the Definition of Residue (2006)

(3) European Community (2003). The Rules Governing Medicinal Products in the European Community, Volume 8; Notice to Applicants and Note for Guidance: Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin, June 2003.

(4) Food and Agriculture Organisation of the United Nations (1986). Guidelines on Pesticide Residue Trials to Provide Data for the Registration of pesticides and the Establishment of Maximum Residue Limits, Food and Agriculture Organisation of the United Nations, Rome.

(5) Food and Agriculture Organisation of the United Nations (2002) Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed, Rome.

(6) European Community (1992). Conduct of Pharmacokinetic Studies in Animals, September 1992, Directive 81/852/EEC; 7AE3a.

(7) European Community (1993). Conduct of Bioequivalence Studies in Animals, May 1993, Directive 81/852/EEC; 7AE4a.

(8) Craigmill, A.L and Cortright, K.A. (2002). Interspecies Considerations in the Evaluation of Human Food Safety for Veterinary Drugs. AAPS PharmSci. 4(4), article 34.

(9) The European Agency for the Evaluation of Medicinal Products (2002). Note for Guidance For the Determination of Withdrawal Periods For Milk, Committee for Veterinary Medicinal Products, Evaluation of Medicines for Veterinary Use. EMEA/CVMP/473/98-Final.

(10) The European Agency for the Evaluation of Medicinal Products (2002). Note for Guidance: Approach Towards Harmonisation of Withdrawal Periods, January 1997. Committee for Veterinary Medicinal Products, Evaluation of Medicines for Veterinary Use. EMEA/CVMP/036/95/ Final.

(11) United States Environmental Protection Agency (1996). OPPTS Test Guidelines, Series 860: Residue Chemistry Test Guidelines.. EPA Report 712-C-96-182, Washington, D.C..
http://www.epa.gov/pesticides/science/guidelines.htm

(12) Canada Pest Management Regulatory Agency (1998). Dir98-02
Regulatory Directive, Residue Chemistry Guidelines. Section 8 Meat/Milk/Poultry/Eggs.

(13) Food and Agriculture Organization of the United Nations/World Health Organization (1991). Evaluation of Certain Veterinary Drug Residues in Food, WHO Technical Report Series 815. 38th Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva.

(14) Food and Agriculture Organization of the United Nations/World Health Organization (1995). Evaluation of Certain Veterinary Drug Residues in Food, WHO Technical Report Series 851. 42nd Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva.

(15) United States Food and Drug Administration, Center for Veterinary Medicine. Guideline 3. I. Guideline For Metabolism Studies And For Selection Of Residues For Toxicological Testing.
http://www.fda.gov/cvm/

(16) United States Food and Drug Administration, Center for Veterinary Medicine. Guideline 3. VI. Guideline For Establishing A Withdrawal Period.
www.fda.gov/cvm/guidance/guideline3pt6.html

(17) Food and Agriculture Organisation of the United Nations (1990). Guidelines on Producing Pesticide Residues Data from Supervised Trials; Part 4 Metabolism Studies and Supervised Residue Trials in Animals, Rome.

(18) Australian Pesticides and Veterinary Medicines Authority Veterinary Requirements Series, Part 5A, Residue Guidelines, Guideline No. 27 Ectoparasiticide Residues in Sheep Tissues.
http://www.apvma.gov.au/

(19) Australian Pesticides and Veterinary Medicines Authority. Veterinary Requirements Series, Part 5A, Residue Guidelines, Guideline No. 31 Residues in Poultry Tissues and Eggs.
http://www.apvma.gov.au/guidelines/guidln31.pdf

(20) Australian Pesticides and Veterinary Medicines Authority. Veterinary Requirements Series, Part 5A, Residue Guidelines, Guideline No. 23 Data Requirements for Animal Tissue Residue Trials. http://www.apvma.gov.au/guidelines/guidln23.shtml

(21) European Community (2003). Guidance document Part C Livestock feeding studies (Unpublished draft 23-November 2003). Guidelines for the generation of data concerning residues as provided in Annex II part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market: Appendix G - Livestock feeding studies, (Doc. 7031/VI/95), 22 July 1996. http://europa.eu.int/comm/food/plant/protection/resources/publications_en.htm