

Residue Guideline No. 31 – Residues in Poultry Tissues and Eggs

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Introduction

This residue guideline provides information on the conduct and reporting of residue trials with poultry[†]. The use of chemicals and veterinary drugs in the production of poultry may leave residues that need to be addressed in the registration process. The design of appropriate trials must include consideration of the type of poultry farm, the intended function of the birds, the developmental stage of the birds at the time of treatment, and the route of treatment / medication.

Poultry Management and Husbandry Systems: Within Australia, poultry may be farmed using a cage system, a “free range” system or a barn/deep litter system. The management and husbandry practices employed in each system will influence the way chemicals and drugs are administered, and the way any resulting residues are managed.

Broilers, Breeders and Layers: The intended use of the product on either broilers, breeders or layers, is fundamental to the design of residue trials, as it will determine the type of samples necessary for residues analysis ie edible tissues and/or eggs. It is important that current Industry practices are considered. For instance, if birds/eggs that are culled from the breeding programs are to enter the food chain, then treatment of breeder birds will require the generation of both tissue and egg residue data.

Developmental Stage of Treated Poultry: The developmental stage of the birds at the time of treatment (ie day-old chicks, replacement pullets, adult birds and/or fertilised eggs) will also have an impact on the likelihood of residues occurring in food commodities from treated birds/eggs. For instance, fumigation of fertilised eggs is unlikely to result in detectable residues when the hatchlings produced from the treated eggs have grown to market weight (broilers). Similarly, treatment of day-old chicks is unlikely to produce detectable residues in broilers at market weight, or in eggs from layers, owing to the lengthy period of time between treatment and production of edible animal commodities. However, when the time period between treatment and “harvest” of edible commodities is decreased (for example, when growing broiler chickens, replacement pullets approaching the point-of-lay, and adult birds are treated), then there is a higher probability of residues occurring in both eggs and edible tissues, and residues trials would be required.

Route of Treatment/Medication: The mass medication routes (direct veterinary treatments) employed by the poultry industry include:

- medicated drinking water (eg antibiotics, coccidiostats, antiprotozoal agents, anthelmintics);
- feed additives (eg antibiotics, growth promotants, endoparasiticides, digestive aids, electrolytes and dietary supplements);
- injections (eg vaccination of eggs, day-old chicks and birds); and
- other methods, such as eye drops, wing stabs, inhalants, fogging, dusts and sprays.

Consideration of residue aspects of vaccines and the indirect exposure routes (outlined below) falls outside the scope of this guideline.

Indirect exposure of poultry and eggs to pesticides may occur via:

- treatment of walls, litter etc in poultry sheds (further residues advice may be found in *Residue Guideline No. 13 – Animal housing*);
- use of sanitary products by workers to avoid contamination of poultry sheds; and
- feeding of treated feeds to birds eg the inclusion of pesticide-treated grain into the poultry diet (further residues advice may be found in *Residue Guideline No. 1 – Animal transfer studies*).

[†] Poultry are farmed domestic birds; the term primarily refers to domestic fowl, but includes ducks, geese, turkeys and other poultry (such as guinea fowl, pheasant and quail).

Scope of the Guideline

Residues trials are needed when poultry are directly exposed to chemicals and veterinary drugs that may produce residues in edible poultry commodities. The scope of this guideline is to outline the data required for the purpose of establishing Maximum Residue Limits (MRLs) and Withholding Periods (WHPs), without conducting more trials, or sacrificing more birds, than is necessary. This is achieved by ensuring that residue trials address the maximum use-pattern described on the proposed product label, that samples of edible tissues/eggs are collected at appropriate times, and that due care is exercised in relation to the practical aspects associated with the route of medication.

This guideline should be read in conjunction with the APVMA *Vet Requirements Series (Part 4, Metabolism and Kinetics and Part 5A, Residues)* and FAO Guidelines[§] (*Pesticide Residue Trials to Provide Data for the Registration of Pesticides and the Establishment of Maximum Residue Limits*, 1986 and the *FAO Manual on the Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed*, 1997). All trials involving poultry should be conducted in accordance with the *Model Code of Practice for the Welfare of Domestic Poultry*.

PART A: General Requirements

1. Residue Definition

Residues of veterinary medicines are defined as pharmacologically active substances (whether active principles, excipients, or degradation products) and their metabolites, which remain in foodstuffs obtained from animals that have been administered the veterinary medicine in question. Before residue trials can be undertaken, a suitable residue definition must be determined. This definition will be based on the findings of metabolism studies and analytical methodology. *Part 4, Metabolism and Kinetics* of the *Vet Requirements Series*, and *Residue Guideline No. 6: Definition of residues for the purpose of setting an MRL* should be consulted for further information.

2. Residue Trial Design

Applications to register drugs for use in/on poultry must contain reports of residue trials that allow the establishment of, or confirmation of compliance with, MRLs in tissue and eggs, as appropriate. All factors that might contribute to the variability of residues in poultry commodities must be considered and taken into account when planning and conducting trials, and a number of specific considerations are discussed in detail in Part B of this guideline.

Good Laboratory Practice (GLP) requirements: All residue trials must be GLP-compliant. Further information on the GLP requirements may be found in the NRA Gazette of March 5, 2002 p36.

Numbers of residue trials: When new MRLs are to be established, residue data from a minimum of **two trials** are required for each commodity (ie 2 trials for tissues and 2 trials for eggs, when relevant), and these trials must be conducted at the maximum treatment regimen for the proposed use-pattern, thereby resulting in the highest tissue/egg residue levels. If a repeat dosage regimen can be reasonably anticipated in practice, even if not specified on the label, then repeat treatments should be applied and residues assessed.

Where the purpose of residue trials is to confirm compliance with existing MRLs for poultry commodities (ie confirmatory trials), residue data from a minimum of **one trial** are required (ie one trial each for tissues and eggs, where relevant). All trials should be conducted under Good Agricultural Practice/Good Practice in the Use of Veterinary Drugs (GAP/GPVD)[†] and GLP, using the final commercial formulation.

[§] <http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPP/Pesticid/Code/Guide.htm>

[†] GAP/GPVD is defined as the currently accepted 'best practice' standard that is applied to the use of veterinary medicines and agricultural compounds in animal husbandry and production. This is reflected in the usage rates on the product label, and is the minimum quantity of chemical or drug that is required to achieve effective pest or disease control while leaving the smallest practicable residue.

Test birds and housing arrangements: The birds used in residue trials should be healthy, and from contemporary, commercial genotypes. No concomitant drug therapy should be used on any bird or pen during the trial(s). Animal housing, feeding and care should follow recommendations for welfare, including vaccination according to industry practices. The housing conditions for experimental birds (ie pen areas, stocking densities) should reflect commercial practices in the geographical location of the study, and take into consideration seasonal variations in temperature and the weight of the birds. Likewise, the number of birds per feeder or waterer should reflect commercial practices. This information should be provided in the final report. A minimum 10-day acclimatisation period is recommended. Birds should be monitored daily throughout the trial to determine any reactions that may adversely affect animal welfare or the validity of trial results.

Numbers of birds: The actual numbers of birds used in each trial will depend on:

- the type of residue trial being undertaken (ie residue decline studies to demonstrate residue depletion over time, or single point trials where residue measurements are taken at one time point); and
- whether pooling tissues from a number of birds (to form composite samples) is necessary to obtain adequate material for analysis (due to the immaturity of the bird, or small organ size).

Typically, residue depletion studies are performed by administering drug to a sufficient number of previously unmedicated birds, to permit the serial sacrifice of groups of birds at intervals after the last treatment. Usually, birds are sacrificed at zero withdrawal[‡], and at three or more later time-points. A minimum of five (5) unique samples of the various tissues (muscle, liver, skin/fat) should be collected at each slaughter time point, to demonstrate the variability in tissue residue concentrations. These samples may be comprised of tissues taken from either a single bird, or pooled from a number of birds, as detailed in the following table:

Growth stage of poultry	Samples collected from a single bird, or pooled from a number of birds			
	†Muscle	Liver	Skin/fat	Eggs
Immature	Single	Composite	Composite	N/A
Mature	Single	Composite	Single	*Composite/Single

† Muscle samples should be comprised of breast and thigh tissue collected from a single bird.

N/A: Not Applicable; *See note in text box.

For edible tissues, the total number of treated birds in a trial may be calculated as $5 \times$ the number of birds per composite sample (where necessary) \times the number of sampling time points. At least one group of untreated control birds must be included in each residue trial.

For egg trials with laying hens, the number of treated birds may be calculated as $5 \times$ the number of birds required to produce the composite egg sample (see below). Clearly, the same groups of birds can be used for successive samplings of eggs. Eggs from birds within a dosage group may be pooled, if necessary, to form composite samples so that adequate sample weight is available for analysis and retained samples. Five unique samples of eggs should be analysed at each time point. Where composite egg samples are required, five (5) groups of laying birds should be treated (each group should contain sufficient birds for at least 3 eggs/group/day). All eggs from each group should be bulked daily, and the numbers and weights of eggs/group/day should be recorded. Where composite samples are not employed, provision should be made for possible loss of birds/eggs during the trial, and for the fact that not all birds lay an egg every day.

*Analysis of residues in composite egg samples does not provide a true measure of the variability that occurs in individual eggs ie the residue result from a composite sample represents the average of the residue levels occurring in the individual eggs that make up the composite sample. Residue variability is an important factor that needs to be considered when establishing appropriate MRLs for residues in eggs, and when considering the acute dietary intake of residues in eggs from treated birds. The JMPR^f (2000) determined that the short-term (acute) dietary exposure calculation for poultry tissues/eggs should not incorporate a variability factor, but should utilise the maximum residue found in an individual egg. Therefore, where

[‡] For tissues of poultry, zero withdrawal is considered to be within six hours of the last treatment.

^f JMPR is the Joint FAO/WHO Meeting on Pesticide Residues, and can be located at <http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/cap2.pdf>.

residues in composite egg samples are above the Limit of Quantitation (LOQ), it is important that a measure of spread of residue is obtained. One way of addressing this issue would be to individually analyse for residues in all eggs collected at one sampling time point. Composite samples could be analysed at all sampling times except at one sampling time point (preferably when maximum residues are expected), where all eggs are analysed individually. Alternatively, eggs from birds dosed at the highest anticipated rate may be analysed on an individual basis.

Treatment regimen: The dose should be the highest intended treatment rate, and should model exposure received by target animals. Treatment regimens can be broadly classed as:

- single point treatments, where a drug is given once to an animal for a specific therapeutic effect. In this case, a single dose of drug is the appropriate exposure regimen during residue trials. However, if a repeat dosage regimen can be reasonably anticipated in practice, even if not specified on the label, then repeat treatments should be assessed;
- short-term treatments, where animals are dosed for a number of days, on either one or more occasions/treatment cycles. In this case, test birds should be dosed for the maximum period permitted on the proposed label. Where the re-treatment interval is short, or the drug/chemical has a propensity to remain in edible tissues, residue trials may need to incorporate multiple treatment periods at the shortest proposed re-treatment interval; and
- long-term treatments, where animals are treated for prolonged periods eg medicated feed and/or water treatments, and where a nil withholding period is desired. In this case, birds must be treated at the maximum proposed rate for a period that is sufficient to enable the residue concentrations in edible commodities (tissues and eggs) to reach a steady-state equilibrium (ie demonstrate that residue levels have reached a plateau).

Sampling times: The sampling times employed throughout a residue trial will depend on the type of residue data required. In general, residue decline information is required when a short withholding period is proposed. For instance, treatment of broiler chickens would require the generation of residue decline data in edible tissues, to demonstrate that residue levels are below the proposed MRL at the proposed WHP. Similarly, treatment of laying hens would require the generation of data to show that residues in eggs are always less than the proposed MRL, since nil egg WHPs are preferred when whole sheds of laying birds are treated. Note that, in the case of breeder birds (where excess eggs – exceeding hatchery requirements – may be made available for human consumption) the APVMA will consider the establishment of longer egg WHPs, such that eggs are discarded, or re-directed through avenues that do not involve human consumption.

In contrast, when there is an extensive time lapse between treatment and “harvest” of the edible commodities, residue decline data are generally not required. For example, residue decline data are not required after medication of day-old chicks, as there is 5 to 8 weeks before the treated chicks have reached market size as broilers. Similarly, residue decline profiles would not be required when replacement layer pullets of less than 12 weeks of age are treated, as dosing of birds prior to sexual maturity (~12 weeks) is unlikely to result in significant residues in the immature ova, and any residues deposited in immature ova are substantially diluted as the ova mature. However, treatment of pre-lay replacement pullets after sexual maturity (>12 weeks of age) will require the provision of confirmatory residue data in eggs, where residue levels are determined in the first eggs collected for human consumption. Finally, treatment of pullets at the point-of-lay (ie ~17 weeks), or adult layers, would require the production of residue decline data in eggs³. In some circumstances, the depletion of residues to the Limit of Quantitation (LOQ) should be conducted, to facilitate trade with countries that have residue tolerances that differ from those established in Australia (see NRA Gazette February 5, 2002 p 39).

Data from the residue trials must be reported according to *Residue Guideline No. 11: Reporting of residue trials*, along with other data as required by this guideline.

³ In cases where there are adequate residues decline data available for tissues from treated birds, the APVMA may be able to extrapolate an egg WHP from these data. In order to facilitate this approach, the residue decline profiles in both muscle and fat/skin need to have been monitored down to the Limit of Quantitation (LOQ). Where applicable, Registrants are encouraged to discuss this approach with the APVMA Chemistry and Residues Program prior to the commencement of any residues trials.

3. Sampling

Edible tissues: With poultry trials, the normal tissues collected for residues analysis are lean muscle (composite of breast and thigh muscle), liver, abdominal fat, and skin with adhering fat. If metabolism studies show the chemical/drug has a predisposition for another edible tissue, that tissue should also be sampled. For example, neomycin is known to accumulate in kidneys, so they would need to be sampled for a neomycin product. Where metabolism or other residue studies show that residues do not occur in one of the above tissues, that tissue can be omitted from future work. Stability of samples during storage should comply with Residue Guideline No. 8: *Stability of residues during storage*.

Eggs: As mentioned earlier, it is the policy in Australia (and in overseas countries such as the USA) that egg WHPs be nil for whole shed treatments of layers producing eggs for human consumption. The transfer of veterinary drugs into eggs is a function of both drug pharmacokinetics and the physiology of egg production. Large variations are observed in the relative ratios of residues in egg yolk and albumen with different drugs, and this variation is largely dependent on the relative lipid-solubility and water-solubility of the drug. Drugs that tend to be lipid soluble are found in much higher concentration in egg yolk than in albumen. In contrast, those that tend to be water soluble are found in higher concentrations in albumen.

The albumen fraction of eggs is produced and excreted within 24 hours of laying. Therefore, drug residues in albumen are expected to result in more standard residue profiles, with highest residues observed soon after treatment. In contrast, the turnover time for yolk is in the order of 68 days. There is evidence to demonstrate that residues transfer into pre-ovulatory yolks, but do not appear to transfer back out and are sequestered until the developing yolks are ovulated (Donoghue, 2001; Donoghue *et al.*, 1996). Thus, even chemicals with an extremely short half life may be stored and released in laid eggs for days or even weeks after the drug has been depleting from the rest of the bird. Furthermore, residues in yolk may continue to increase (even after treatment has ceased) as drugs that are stored in body fat are released back into the blood and the residues in other poultry tissues start to decline. The exception to this situation is where eggs are treated directly (eg on-farm fumigation); in this case, residue levels are expected to be highest soon after treatment.

The timing of egg collection for residues analysis needs to factor in the phenomena discussed above, thereby ensuring that collection times coincide with the highest residue levels. Analysis of eggs should be conducted on the egg yolk and white combined in one sample (without shell). However, some analysis of the deposition of residues into yolk and albumen fractions should be conducted, to determine how the drug/chemical partitions between the egg fractions. The residue levels in yolks 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, as MRLs will be expressed on a whole-egg basis.

4. Analytical Method

The analytical method must address the residue definition, and must be capable of determining residue levels in all relevant tissues and poultry commodities. Residue Guideline No. 19: *Residue Analytical method* and Guideline No. 26: *Veterinary drug residue analytical methods* should be consulted for further information. The analytical methodology used should be suitable for use as a regulatory or enforcement procedure, and the analytical phase of all residue trials must be GLP-compliant (see NRA Gazette of March 5, 2002 p36).

5. MRL and WHP Proposals

The Applicant must propose Maximum Residue Limits (MRLs) for all relevant tissues and eggs, as appropriate. The Applicant must also propose slaughter Withholding Periods (WHPs), and in this respect Applicants should consult Residue Guideline No. 10: *Withholding periods* for further information.

PART B: Route of Treatment/Medication - Specific Considerations

The method of administration (oral, parenteral, topical), the formulation type, and extent of activity of a product will influence the protocol design for residue trials. The following paragraphs detail some factors that need to be considered for specific routes of treatment/medication.

Medicated drinking water: Administration via the drinking water is often preferable because domestic poultry will drink when they will not eat. However, fluid intake by the birds may vary due to the weather, to the ease of access or hygiene of drinking water dispensers (“drinkers”), or to the unpalatability of the medicated water. Consequently, the quantity of medicated water consumed by birds during residue trials must be reported, to enable determination of the actual dosage received (mg active constituent per kg bodyweight). Trial protocols should endeavour to address the worst-case residue scenario ie maximum consumption/dose of medication.

Consideration should also be given to the composition of the watering system/containers, and the quality of the actual water. Galvanised metal may result in chelation of the drug with metal ions. Other materials may lead to adsorption of the drug onto the container surface. Chelation of the drug may also occur when “hard” water is used to prepare the medication, and the use of chlorinated or otherwise sanitised water may destroy the medication. Each of these factors may result in a reduction of the intended dose delivered to the birds. Therefore, when the drug is to be administered in the water, samples of the medicated water should be collected and analysed to confirm the drug concentration.

Feed additives: Feed additives, incorporated into pellets, crumble or mash, are commonly used in the mass medication of poultry, particularly when the physicochemical properties of the drug make it insoluble in water (ie unsuitable for in-water treatment). Variations in feed consumption are associated with hot or cold weather; housing changes; breed, type, strain and age of bird; body weight; rate of lay; energy and fibre content of feed, and particle size of feed ingredients. These factors may alter the level of feed consumption by 10-20 %, thereby altering the effective dose rate (in the feed) by an equivalent percentage. Therefore, it is important that the quantity of medicated feed consumed by birds during residue trials be reported, to enable determination of the actual dosage received (mg active constituent per kg bodyweight). Trial protocols should endeavour to address the worst-case residue scenario ie maximum consumption/dose of medication.

It should also be noted that absorption of the drug by treated birds may be unpredictable because of binding to feed ingredients. Furthermore, the milling process may affect the stability of the drug(s), as pelleted feeds may be subjected to temperatures of 75-95 °C, which may cause breakdown of the active constituents. Therefore, it is important that residue studies be conducted using the predominant feed form (pellet, crumble or mash), and that samples of the medicated feed are analysed for their drug content before and after milling/processing.

Injection: Intramuscular injections are typically given into the thigh or neck muscle of day-old chicks. At the time of preparation of this guideline, there are no injectable products registered by the APVMA for use in adult poultry. However, injection into the thigh or breast muscle in adult birds is a potential route of administration. Registration of such a use pattern would require consideration of the levels of injection-site residues (refer to the proposed Injectable Veterinary Products guideline). Stakeholders intending to pursue registration of injectable use patterns in adult poultry are urged to discuss their trial protocols with the APVMA, prior to the commencement of any residue trials.

Other routes: Advice on the registration of products that are delivered to poultry by routes other than those described above will be provided on a case-by-case basis. Stakeholders are urged to discuss their trial protocols with the APVMA, prior to the commencement of any residue trials.

Relevant Residue Guidelines Cited

Residue Guideline No. 1: *Animal transfer studies*

Residue Guideline No. 6: *Definition of residues for the purpose of setting an MRL.*

Residue Guideline No. 8: *Stability of residues during storage.*

Residue Guideline No. 10: *Withholding periods.*

Residue Guideline No. 11: *Reporting of residue trials.*

Residue Guideline No. 13: *Animal housing*

Residue Guideline No. 19: *Residue analytical method.*

Residue Guideline No. 26: *Veterinary drug residue analytical methods.*

References:

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The Australian Egg Corporation Limited website: <http://www.aecl.org/>

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