BASIC GUIDELINES FOR RESIDUE TRIALS

Supervised residue trials serve as the primary source of information for determining residue levels. Specific information on the numbers of trials, time points, animals (male and female if relevant) per time point and tissues required are specified in this standard. The residue risks are considered different for pioneer uses and non-pioneer or generic uses.

Background
The overall objective of the assessment of the toxicological and related data is to determine the acceptable daily intake (ADI) of the active ingredient for humans. The ADI for humans is calculated using the lowest No-Observed-Adverse-Effect-Level (NOAEL) obtained in the most sensitive test and the most susceptible species, usually from the chronic toxicity studies and an appropriate safety factor (SF). This calculation is done by the Department of Health, Directorate: Food Control.

If the active ingredient(s) has or have been evaluated by the Joint Expert Committee on Food Additives (JECFA) and accepted by Codex the ADI and Maximum Residue Limits (MRLs) will be accepted by the South African registration authorities.

Residue studies need only be done on food producing animals. Manufacturers are required to submit tissue residue and depletion rate data on all new veterinary medicines and animal health products, including a method of detection of residues. Residue studies are done in the target species at the recommended dosage with the particular formulation to be marketed. This is done on a non-radio-active labelled product using a fully validated isolation technique. Methods of analysis for measuring residues in food commodities are in most cases available in published manuals or in chemical literature [Food and Drug Administration (FDA) General Guidelines and European Union (EU) Directives]. Appropriate sources of methods for many compounds are available in the Guide to Codex Recommendations Concerning Residues. Treated animals are slaughtered or specimens of edible products (e.g. tissue, milk or eggs) are collected at specific intervals after treatment and the residues in different tissues determined. Residue studies with formulations of veterinary medicines and animal health products done in other countries on the target food animals will be acceptable.

Note: All residue trials must be conducted according to Good Clinical Practice (GCP) where an independent and objective Animal Ethics Committee has granted approval (as for all GCP trials involving animals).

Residue trials

Two trial options are possible:

Option 1. Three or more time points with the specified number of animals at each time point. This option allows a limited extrapolation of data beyond the data time points supplied in the trial.

Option 2. One time point only. Election of a proposed WHP and selection of the specified number of animals for the trial to enable regulatory assessors to be assured that the required residue conformance is met at the assessed WHP. If this is so, then that time point (if it is between the permitted WHP and the next permissible one after it) becomes the assessed WHP. No other extrapolation is permissible in this electable option. For example, if MRL conformance is not met at, e.g. a 5 day time point, then the evaluator will assess a suitable WHP based on a conservative interpretation of the data. It is very unlikely that the (long) default WHP would be offered but each case would be judged on its merits. Trial data presented under Option 2 with fewer than the specified number of animals will also be interpreted conservatively as specified for an unallocated
WHP and waiver situation unless the supplementary documentation in the waiver is sufficient to remediate the data deficiency.

Applicants should note that Option 1 must be followed for MRL determinations, where no internationally acceptable MRLs are available (e.g. CODEX, APVMA, FDA, Japan). NCE’s would also follow Option 1.

All trial design and execution must be conducted in compliance with GLP.

6.1 *Analytical testing done in GLP accredited facilities*

6.1.1 Any analytical processes carried out in GLP accredited facilities and carried out according to GLP do not need to supply full documentation of the procedure. A brief summary is sufficient. However, the actual formulation used, the interval elapsing between manufacture and use, and the storage conditions subsequent to manufacture must be documented.

6.1.2 Any processes carried out in a GLP accredited facility and carried out according to GLP do not need to supply any raw data records associated with the procedure.

6.2 *Analytical testing done in non-GLP accredited facilities*

6.2.1 Where any part of the study is not conducted in a GLP accredited facility the applicant must supply all of the following:
- Full documentation of all physical aspects of the facility;
- Full documentation of other accreditations held by the facility;
- Full documentation of the CV of any auditors employed for the study and the audit schedule, including validation details;
- Full CV of all staff involved in the study;
- All raw data produced within the non-accredited facility pertinent to the study;
- Full documentation of any audits or peer reviews of the facility conducted within 1 year of commencement of the study;
- The foregoing applies to all subcontractors who contributed to any element of the study;
- Documentation showing complete traceability of all relevant physical and observational data generated by the study.

6.2.2 Applicants should note that after (date to be determined) applications under 6.2 will not be compliant with the stock remedy policy. Applications under this option must be accompanied by a valid waiver application.

Applicants are reminded that a waiver may not necessarily be granted.

**Note:** Reporting requirements are much less onerous for trials in GLP accredited facilities. Overseas data will be acceptable if trials are performed according to internationally acceptable standards.

6.3 *Residue trials and primary products*

Residue trials should aim at providing, as accurately as possible, a measure of the residues likely to occur in edible portions of animal origin (edible tissues, milk, milk products, eggs). A residue trial may be in the form of obtaining a residue depletion curve (depletion over time) with multiple time points, or residue measurements at one time point.

In particular, dose rates in the trial must not be less than proposed dose rates. If it can be demonstrated that bioavailability is a direct and linear function of dose, then results from higher
dose rates may be extrapolated to (inferred) levels at the proposed dose rate as applied for, for doses not exceeding 10% of the proposed label dosage rate.

6.3.1 Milk
For milk residues the trial data must be generated on, and reported from, individual trial animals generally at the maximum dosage/animal. Where intra-mammary treatments are not applied to all teat canals or quarters then the following shall prevail:
If milk is aggregated at milking then the assigned residue level for any sample so collected will be adjusted pro-rata for the proportion of teat canals treated in the animal.
If teat canals are treated and milk collected and the residues analysed separately then the residue will be calculated as the mean of those separate values.

Any factors such as partial udder treatment and partial herd treatment in a given situation, which could result in a reduction in the milk WHP (with suitable registration conditions), may be alluded to by an assessor and may be taken into account by the Registrar: Act 36 of 1947 at the time the registration is granted.
For residue assessment and WHP purposes, milk will be assessed as pertaining to that bulked product obtained from the test group (i.e. a herd) at a given milking.

6.3.2 Meat, (including fish) and eggs
Analytical data must be reported on the produce from individual animals, eggs as the case may be.

6.4 Design of residue trials

6.4.1 Treatment frequency, dose and timing
The dose and frequency of application, as well as administration route, plus the interval between treatments should be the same as specified on the package insert (PI) and/or label. The dose should be the maximum of any electable dose specified on the PI and/or label.
If the trial conditions differ from those specified on the label or from those currently in farm practice in South Africa, then this should be addressed in the report. All procedures applied to animals prior to application of the formulation must be documented in full (e.g. cleaning, clipping, sterilising).

6.4.2 Field component of residue trials
It is not required that residue trials are conducted on animals suffering from the disease for which the trade name product is (claimed to be) a remedy. The definitive residue depletion study must be conducted on animals suitably certified as free of clinical disease. However, where pharmacodynamics and kinetics of the active ingredient(s) are known or suspected to be affected by disease states for which the veterinary medicine is indicated or by some unrelated disease, then this must be addressed. Any research results obtained for other purposes and which shows any interaction or otherwise between the absorption, distribution, metabolism, and excretion (ADME) of the active substance and the disease state for which it is a remedy or any other disease present in livestock will assist assessors in ascertaining a more accurate risk profile of the residues.

While, in general, the time points selected should cover the rise, plateau and elimination phases of the residue depletion curve for withholding period (WHP) assessment, only the depletion phase is significant for the purpose of setting a withholding time.

Minimum number of data elements at one time point to establish a residue depletion curve.
TABLE: To specify the minimum number of animals that must be included and reported upon at any sampling given time point in a residue depletion trial for meat, edible offal, fat, eggs and milk. The requirements are specified according to the mode of application of the product.

<table>
<thead>
<tr>
<th>Model of application</th>
<th>Wild Ruminants/Buck</th>
<th>Ruminants</th>
<th>Pigs</th>
<th>Birds including chickens</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meat and edible tissues</td>
<td>Milk</td>
<td>Meat and edible tissues</td>
<td>Meat/Eggs</td>
<td>5</td>
</tr>
<tr>
<td>Oral Systemic</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Oral Non Systemic</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Topical Systemic</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Topical Non Systemic</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Parenteral Preparations</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Intrauterine Preparations Intramammary Lactating Animal Preparations Intramammary Dry Cow Preparations</td>
<td>3</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bioequivalence trial results normally used to demonstrate comparative bioavailability (e.g. by comparative measurements of plasma samples) between a reference and a test product may or may not prove bioequivalency. In cases where bioequivalence is said to be proven, the WHP of the reference product is applied directly to the test product. This may not necessarily be the case, depending on depletion curve dynamics of the test product. To obtain the required degree of conformance for residues, the depletion curve must be less than or exactly similar to the depletion curve (at any time point) of the reference product.
6.5 Samples and sampling

6.5.1 Sampling procedures
The procedure for taking samples for residue analysis must be fully documented with particular attention to the practical avoidance of contamination of samples. Failure to comply fully with this provision may result in inclusion of outlying (high and possibly arising from contamination) data points unnecessarily in the evaluated data set. This may result in the imposition of an unnecessarily conservative WHP.

6.5.2 Sample storage
The storage of the samples must be fully documented from the time of removal from the animal to receipt within the laboratory, up to and including analysis, and then for storage until the study is completed. The sample packaging must be shown to be free from components that interfere with the residue analysis.
All samples should be taken in duplicate (the second as a reserve sample).
All samples must be kept very cold, approximately 2-4°C with sufficient ice packs in a sealed cooler box, whilst transporting (preferably <12 hours), or kept well refrigerated until transported to laboratory. For transportation time that will take more than 12-24 hours, a refrigerated vehicle is preferable.
Samples taken in non-GLP accredited facilities must supply all raw data sheets, sampling protocols, and freezer and transport logs.

6.5.3 Sample types
Tissues are taken on a wet mass basis.
For residue studies:
- Meat is muscle (voluntary, e.g. not heart) obtained from any of the major muscles; the correct anatomical name is required (e.g. latissimus dorsi) from a specified part of the animal. Muscle must be trimmed of fat,
- Fat is omental or renal fat,
- Kidney is homogenised whole kidney with fat trimmed,
- Liver is any part of the liver,
- Offal is any edible part of the gastrointestinal tract,
- Eggs are homogenised whole eggs without shell and
- Fish meat is without skin/scales.
It is not required to report residues on kidneys of fish or poultry.
A minimum sample of 100 g of any one tissue must be taken from which the required subsample must be taken (excluding eggs).

**Extreme care must be taken not to cross-contaminate samples.**

A minimum of 100 ml of milk must be taken from each animal for which the required subsample must be taken.

In the case of trade name products for application into or on the udder, then the milk must be identified as either originating from either, specified quarter(s) of the treated udder or, combined with milk from untreated quarters from the same animal.

6.6 **Residue data from other countries**

Registration data in support of a withholding period for a veterinary medicine does not necessarily have to be generated from trials conducted in South Africa except in the case of topically applied parasiticides on sheep (where wool residue data must be provided for exportation). One South African trial on wool sheep/Angora goats is required for each of the claimed use patterns, e.g. off-shears and or long-wool use.

7. **METHODS OF RESIDUE ANALYSIS**

**Analysis in GLP accredited facilities**

Any analytical processes carried out in GLP accredited facilities and carried out according to GLP do not need to supply full documentation of the method. A brief summary is sufficient.

Any analytical processes carried out in a GLP accredited facility and carried out according to GLP do not need to supply any raw data records associated with the sample analyses.

Any analytical processes carried out in a GLP accredited facility and carried out according to GLP do not need to supply any raw data associated with the method of validation. It is sufficient to tabulate the performance specifications obtained during the validation.

Registrations under this option must provide:
- copies of the analytical laboratory’s accreditation status,
- any audit reports and
- any deviations and amendments to the study plan.

A document detailing the study participants, their role and experience must be supplied.