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APPENDIX F

METABOLISM AND DISTRIBUTION IN DOMESTIC ANIMALS

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1. **Introduction**

Uptake of pesticides by animals, leading to the occurrence of residues in animal products, can occur following ingestion of feed containing a pesticide residue. Consequently residues in products of animal origin (meat, milk, eggs, edible offal) resulting from the ingestion of feed containing pesticide residues need to be evaluated.

Domestic animal metabolism data quantify total residues and characterize the chemical nature of residues which may occur in edible tissues (including milk and eggs) of livestock exposed to pesticides. They are required when pesticide use may lead to significant residues (generally considered to be >0.1 mg/kg total diet) in livestock feed.

A livestock metabolism study should primarily identify the definition and expression of the residue and hence which analytical methods are needed in order both to determine residues in tissues, eggs or milk and whether residues are stored or accumulated. A livestock metabolism study should also elucidate the efficiency of extraction of the various components of the residue so that extraction/residue release procedures can be developed as part of the analytical methods.

For these studies, the position of radiolabelling (preferably 14C) must be chosen so that the label is unlikely to be easily lost by metabolic transformation. For example, ring labelling is recommended for aromatic and other cyclic compounds.

2. **Objectives**

The objectives of these studies are:

- to identify the major components of the total terminal residue in edible animal products;
- to quantify the rate of degradation and excretion of the total residue in certain animal products (milk or eggs) and excreta;
- to indicate the distribution of residues between relevant edible animal products;
- to quantify the major components of the residue and to show the efficiency of extraction procedures for these components;
- to generate data from which a decision on the need for livestock feeding studies (...) can be made;
- to decide on the definition and expression of a residue.

3. **Extent of data required**

Studies must be carried out in lactating ruminants (e.g. cows or goats) and laying poultry (chickens). Where it becomes apparent that metabolic patterns differ significantly in the rat as compared to ruminants, a pig study must be conducted unless the expected intake by pigs is not significant.
For each treatment regime chosen a minimum of one ruminant or three chickens should be treated.

4. **Experimental design and reporting requirements**

4.1 Dose material

Experiments will generally be carried out with radiolabelled chemicals (preferably 14C). It may be appropriate to use parent compound, one or more plant metabolites or in specific cases feed prepared from crops with incurred residues. The position(s) of radiolabelling should be carefully chosen to ensure that maximum useful information is

obtained. Reports should state: radiochemical purity (desirable >95 %), labelling position and specific radioactivity.

4.2 Animals

Reports should state: species and breed, age, body weight and milk or egg yield, if appropriate.

4.3 Site

Animals should be acclimatised prior to dosing. Reports should state: date of study, location and housing conditions.

4.4 Treatment

For chemicals which may occur in animal feed oral administration,
usually feed together with encapsulated radiolabelled pesticide, is appropriate. Dosing should be continued for at least three consecutive days. Dose rates should be at least equivalent to the likely maximum daily exposure. In practice there may be analytical problems if dose rates of less than 10 mg/kg diet are used. If necessary for identification of metabolites administration of higher doses may be useful.

If the primary purpose of the study is to identify the major metabolites so that appropriate analytical methodology can be established for a feeding study, then dosing need only continue for three days.

However, in some cases a study of this type may be used to indicate that transfer of residues is minimal and that the expected dietary exposure will generate residues less than the limit of determination in a feeding study and hence such a study is unnecessary. In such a case dosing until plateau levels are reached in milk or eggs would be necessary (plateau levels usually occur after five days in ruminants, 14 days in poultry).

Reports should state: dose rate, number and timing of treatments and method of dosing.

4.5 Sampling

Animals should be killed within 24 hours after last treatment. The edible tissues of interest are meat, fat, liver, kidney (ruminants and pigs only), milk and eggs. Excreta should be collected so that the overall recovery of radioactivity can be judged and as source for metabolites likely to be present in edible tissues. Milk must be radioassayed whilst fresh (however samples may be frozen for subsequent analysis).

Reports should state: nature, number and size of samples taken. Storage duration and temperature prior to analysis should be stated for both samples and extracts (a general statement will suffice).

4.6 Analysis

It may be necessary to perform studies on the stability of residues during storage. Provided samples are frozen within generally 24 hours after sampling and unless a compound is otherwise known to be volatile or labile, data are not normally required for samples extracted and analyzed within 30 days from sampling (6 months in the case of radiolabelled material).

If, following treatment at the highest likely feeding rate, total radioactive residues in individual edible tissues are <0.01 mg/kg then no further characterisation is required. In some circumstances, for example where there is a particular toxicological concern, it may be necessary to identify terminal metabolites, even when they are present at concentrations lower than 0.01 mg/kg.

If total radioactive residues are > 0.05 mg/kg then the aim should be:

a) to characterise and identify all components of the residue which exceed 0.05mg/kg or 10% of the total radioactive residue,

b) to characterise fractions or individual components of the residue which represent 0.01 - 0.05 mg/kg to the extent that they can be placed in one of the following categories:

1) organosoluble but does not cochromatograph with postulated metabolites and cannot be converted to these compounds by chemical or enzyme hydrolysis;

2) watersoluble and either cannot be converted to organosoluble material by chemical or enzyme hydrolysis or is converted to organosoluble material of nature 1) above; or

3) not extracted by solvents; (including refluxing conditions); water, acid or alkali (including refluxing conditions;) or enzyme digestion.

These trigger values of 0.05 mg/kg or 10% of total radioactive residues are only meant as guidance. In some circumstances, generally governed by toxicological concerns, it may be necessary to identify terminal metabolites, which are present at concentrations lower than 0.05 mg/kg or <10% of total radioactive residues. The trigger values only indicate that it is not generally necessary to identify metabolites which are present at very low and insignificant levels.

Characterization refers to the elucidation of the general nature/characteristics of the radioactive residue short of metabolite identification.

Identification refers to the exact structural determination of components of the total radioactive residue. Typically, this is accomplished by comparing chromatographic behaviour to that of known standards and/or actual spectroscopic analysis (MS, NMR,etc.).

Reports should include: full details of the methods of analysis (including extraction scheme), recoveries of radioactivity in relation to the measured radioactivity in the specific tissue, and sufficient representative chromatograms, spectra and photographs of autoradiograms to illustrate the quality of the data and to indicate the confidence which can be attached to characterizations and identifications.

4.7 Interpretation

- The relevance of results should be discussed in relation to the proposed uses.

- The significance (toxicity, quantity) of metabolites found in edible tissues (including chemically unavailable or non-extractable residues) should be considered.

Generally, if the non-extractable residue is less than 0.05 mg/kg or 25 % of the total radioactive residue and a significant proportion of the total residue has been identified, no further work is required. A significant proportion means 75%; however this may not be possible in all cases. All deviations must be fully justified.

If non-extractable residues exceed 0.05 mg/kg or 25 % of the total radioactive residue, then depending on the absolute level of residue represented by the non-extractable residue and the toxicity of the parent molecule it may be necessary to investigate the biological availability of these residues.

- A proposal for the residue definition should be made.

- The extractability of residues should be discussed in relation to the proposed analytical methodology.

- The need for livestock feeding studies should be discussed.

5. **References**

UK-MAFF (1992):
Data Requirements for Approval under the Control of Pesticides Regulations 1986, Appendix 6.

US-EPA (1982):
Pesticide Assessment Guidelines, Subdivision 0, Residue Chemistry.
EPA-540/9-82-023 (1982) and EPA/738-B-92-001 (1992)