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APPENDIX G

LIVESTOCK FEEDING STUDIES

1 Foreword

Plant protection products may be ingested or absorbed by livestock in three ways:
- following direct application of the product to the animal
- through residues in feedstuffs
- as a result of treatment of their accommodation.

If residues in crops or parts of crops fed to animals are likely, the livestock feeding studies provide the data necessary to establish maximum residue levels for food of animal origin.

Livestock feeding studies provide data on quantitative transfer of residues to meat, milk, eggs and edible offal.

2 Objectives

The objective of these studies is to determine the residue in products of animal origin which will result from residues in feedingstuffs or fodder crops.

3 Extent of data required

Feeding studies are only required:

- when significant residues (0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crop (e. g. trimmings, waste) fed to animals

and

- metabolism studies indicate that significant residues (0.01 mg/kg or above the limit of determination if this would be higher than 0.01 mg/kg) may occur in any edible animal tissue taking into account the residue levels in potential feedingstuffs obtained at the 1x dose rate.

Where appropriate separate feeding studies for lactating ruminants and/or laying poultry should be submitted. Where it appears from the metabolism studies submitted (...) that metabolic pathways differ significantly in the pig as compared to ruminants, a pig feeding study must be conducted unless the expected intake by pigs is not significant.

4 Performance of the trials

4.0 Introduction

If accurate results are to be obtained from feeding studies, it is essential that the trial be set up and performed in a suitable manner in order to keep errors to a minimum. It is also absolutely essential to standardise the techniques and methods used to perform the trial.

4.1 Test substance(s)

The initial active substance is often the relevant part of the residue. In other instances, a metabolite or metabolite mix may also be used in the trial. The test substance(s) should be incorporated into the feed or applied in another suitable form (e.g. in capsules), so as to simulate as far as possible the customary residue concentrations in the feed. In some cases it may be appropriate to feed the animals feedstuffs containing "incurred" residues. However, this may cause problems, particularly when setting dosage rates (see also 4.4), e.g. when "producing" the necessary residue concentration, homogenising the feedstuff and determining the storage stability of the residues.

4.2 Animal species

Feeding trials on ruminants and poultry are usually performed on dairy cattle or goats and laying hens. In special cases, feeding studies on additional species may prove necessary (see 3).

4.3 Number of components

The feeding trial should comprise:
- a control group
- a group treated with the expected residue level (1 x dose) and groups treated with excess doses (3-5 x dose and 10 x dose).

Excess dosage trials demonstrate whether the residues in the animal are proportionate to the dose administered. They also provide additional data if future additional fodder crops are to be treated further with the plant protection product containing the active substance in question.

4.4 Dosage rates

When setting the 1 x dose, a theoretical feed ration must be compiled. It is advisable to compile this ration in such a way that, on the one hand, those feedstuffs which contain the maximum residues are considered, and, on the other, a composition which is balanced and customary for the species of animal in question is obtained. The composition of animal feed varies from one country to another.

The following table indicates maximum incorporation rates for crops in feed of domestic animals. Diets vary enormously; therefore the figures in the table are maximum likely levels but clearly they are not additive. Feeds which can substitute one for another have been grouped together but in practice substitutions are not always simple, for example 2 kg field beans may be replaced by 1 kg of cereal plus 1 kg of rapeseed meal.

The table listed below can be used to construct a worst case diet and to calculate the 1x dose for relevant domestic animals. Only one crop per group should be used to construct a diet consisting of up to 100 %. Calculations should be made in such a way as to present the highest likely intake for the animals concerned. Maximum feed intake is expressed in terms of dry matter.

Table: Maximum feed intakes expressed in percentage terms for certain feedingstuffs frequently used in the nutrition of the four indicator livestock species.

		% Dry Matter (DM)	Chicken	Dairy Cattle	Beef Cattle	Pig
Body weight Daily Maximum Feed (Dry Matter) DM			1.9 kg 120 g	550 kg 20 kg	350 kg 15 kg	75 kg 3 kg
Maximum Percentage			% DM	% DM	% DM	% DM
Group	Crop/ Commodity					
I	Green Forage (include. Hay)					
	Grasses	20	-	100	100	-
	Alfalfa/Clover	20	-	40	40	15
	Forage Rape	14	-	-	35	15
	Kale/Cabbage	14	5	35	35	15
	Sugar Beet leaves and tops	16	-	30	30	25
	Silage (Clover, Grasses (...)	20	-	100	100	15
	Fruit Pomace (Apples, Citrus)	23	-	10	30	-
	Hay	85	-	100	100	15
II	Grains					
	Grains except Maize	86	70	40	80	80
	Maize	86	70	30	30	40
	Bran (Wheat and Rye)	89	15	20	20	20
III	Straws (cereals)	86	_	20	50	-
IV	Pulses	86	30	20	20	40
V	Root and Tubers					
	(e.g. Potatoes,	15	20	30	60	60
	Swede/Turnip/	10	20	30	60	60
	Sugar and Fodder Beet	20	20	30	60	60
VI	Oil Seed (Meal, Cake) (eg Soya bean, Peanuts, Rape seed, Sunflower seed, Linseed	86	10	30	30	20

To illustrate the use of this table examples are given below:

Residues in studies were found to be:

sugar beet leaves	5 mg/kg
straw	10 mg/kg
small cereal grains	0.5 mg/kg
potatoes	2 mg/kg
oilseed rape cake	1 mg/kg

Calculation for a dairy cow weighing 550 kg and consuming 20 kg dry matter/day.

Sugar beet leaves form 30 % of the diet so the intake in terms of dry matter will be 6 kg/animal/day (30 % of 20 kg).

Adjusting the figure for 16 % dry matter in sugar beet leaves gives an intake of 37.5 kg of fresh material/animal/day (6/16 x 100).

Fresh material contains 5 mg/kg residues therefore the intake of residues from sugar beet leaves is 187.5 mg/animal/day (37.5 x 5).

This calculation is carried out for the other commodities being consumed and the following table can be constructed:

Group crop	Percent of diet as crop	Intake of dry matter from crop (kg/animal/day)	% dry matter	Intake of fresh material (kg/animal/day)	residue in crop (mg /kg)	Residue intake by dairy cattle (mg/animal/day)
I Sugar beet leaves	30	6	16	37.5	5	187.5
II Small cereal grains	40	8	86	9.3	0.5	4.7
III Straw	20	4	86	4.65	10	46.5
V Potatoes	30	6	15	40	2	80
VI Oilseed rape cake	30	6	86	7.0	1	7.0

From this table the intakes which will lead to the highest intake by the animal of concern can be selected (in this example a dairy cow). In doing this not more than one crop should be selected from any one group and the diet should not consist of more than 100 %.

In the example shown above the largest contribution of residue comes from

Crop	mg/animal/day	Percent of diet
sugar beet leaves	187.5	30
potatoes	80	30
straw	46.5	20
oilseed rape cake	4.7	20
sum	318.7	100

The intakes are added together to give an intake of
318.7 mg/animals/day which is equivalent to 0.58 mg/kg bw/day (318.7/550).

This calculation can be carried out for the appropriate animals and is the basis for the 1x dose. For plant protection products with other use pattern a calculation can be carried out in the same way to compare the potential intake by livestock animals with the intake used in the livestock feeding studies to give an indication of the likely residues in products of animal origin.

4.5 Application

To apply the required dose, part of the animal feed (usually the feed concentrate ration) may be mixed with the test substance, the latter being dissolved in a suitable solvent (e.g. maize oil). The required quantity of treated feed concentrate is then mixed with the animal feed and fed to the individual animals.

In the case of laying hens, it may be preferable to apply the total feed quantity to the required active substance content and to allow the animals to feed freely. In these cases the feed intake must be monitored in order to calculate the residues consumed.

Other types of application, such as capsules or adding to drinking water, may also be suitable. The application method selected must be justified.

Suitable precautions should be taken to ensure that as far as possible the animals do not become contaminated externally with the test substance (e.g. via drinking water).

It is recommended that the individual dosage groups are not treated on the same day, since this may cause capacity problems on slaughtering.

The animals should be treated until such time as a residue plateau for each dose appears in the milk or eggs. This usually occurs within 28 days. Treatment should, however, last for at least 28 days, even if the plateau is reached at an earlier stage.

Before commencing the trial it should be ensured by means of analysis that the content and stability of the test substance in the feedstuff, water or capsules will remain constant over the feed period. Otherwise frequent mixing will be necessary. When administering the test substance with the feed, uniform distribution must also be ensured.

4.6 Number of animals and accommodation

In the case of dairy cattle, a dosage group (test component) should include at least three animals and, in the case of poultry (laying hens), at least 9. In total the animals involved should produce a constant average quantity of milk or eggs before treatment commences. If it is desirable (e.g. in the case of substances with a tendency to accumulate) to determine the reduction in residues following completion of the treatment, additional animals must be treated.

During the trial the animals involved in the study should be housed in a suitable manner: dairy cattle in barns and laying hens in cages.

5 **Sampling**

5.0 Introduction

There are two types of sampling: sampling during the feeding period and sampling after slaughter.

When extracting and handling the key animal materials particular attention should be paid to the avoidance of contamination. If correct laboratory samples are to be obtained, scrupulous compliance with all details is essential.

5.1 Definition

The 'animal sample' is the sample produced and obtained in accordance with the study plan, instructions for use or other directions. It consists of all the material obtained from the animal in each case.

The 'laboratory sample' is the animal sample prepared for analysis in accordance with the study plan, instructions for use or other directions. It is obtained from the animal sample (cleaned as appropriate) either
- by taking representative quantities (e.g. raw milk, meat, fat);

- by using the complete animal sample (e.g. cattle liver); or

- by combining several animal samples (e.g. the meat of laying hens).

The material is then chopped and carefully mixed.

The 'analytical sample' is that part of the laboratory sample which is used for analytical examination in accordance with the analysis regulations or the working instructions. It is obtainded by removing an aliquot quantity from the laboratory sample.

5.2 General

Specially trained personnel complying with the statutory requirements (e.g. animal protection) and able to recognise and interpret the significance of the test are essential for sampling and, in particular, for slaughtering.

5.3 Handling samples and preventing contamination

Any damage to or spoiling of the samples which could affect residue levels must be avoided.

It should be ensured that sampling equipment and containers or bags used for transportation and storage are clean and non-contaminating. New, suitably sized and adequately robust containers and bags should therefore be used. The material should not adversely affect analysis or adsorb residues.

Samples should be taken first from the control group and then from the treated animals, starting from the group with the lowest dose and rising to the maximum dose. When taking samples of milk, separate milking equipment should be used for the control group and the dosage groups.

Avoid contaminating samples by touching them with hands or clothes which have been in contact with the test substance.

Udders should be thoroughly cleaned before milking, to prevent contamination resulting from contact with excrement.

Any excrement adhering to eggs should also be removed.

Before commencing treatment, samples of milk or eggs should be taken from all animals in order to determine starting values. Samples should also be taken on at least two days per week during the feeding period to identify the formation of plateaus. Milk samples should be collected from each animal separately.

The animals must be slaughtered within 24 hours of administering the final dose, with the exception of any animals which are to undergo further observation.

When slaughtering the animals it should be ensured that tissue samples are not contaminated by blood, urine, faeces or other body fluids.

After slaughtering, the respective laboratory tissue samples are obtained from the animal samples. The method is described in Appendices I A and B.

6 **Report**

6.0 Introduction

A report on feeding studies should include all relevant data in a suitable format.

The report for an entire feeding study could for example be subdivided into the following sections:
- Summary
- Objective
- In-life part
- Sample preparation
- Extraction, clean-up, determination, evaluation
- Results and discussion.

6.1 Summary

This summarises the key results, the evaluation of these results and any anomalies of the study, with reference to the objective.

6.2 Objective

The objective section of the report again describes the aims of the study in detail and formulates the questions to be dealt with in the study.

6.3 In-life part

This section of the report summarises the key points documented in the log book (e.g. type, number and weight of animals used and any unusual behaviour or health effects noted). Dose rates must be reported as mg/kg diet, mg/animal/day and mg/kg bw/day. Duration of treatment and method of dosing must be reported.

Reference should be made to the critical points of the animal trial component, and special techniques and events should be described.

6.4 Sample preparation

This section should be used to describe sampling techniques including nature, number and size of samples taken and, where appropriate, intermediate storage, as well as the production of the laboratory or analysis samples and the storage and dispatch thereof.

6.5 Extraction, clean-up, determination, evaluation

This essentially describes the method used to prepare and measure the samples.

This section of the report contains the measuring results and the methods used to assess the measurements.

6.6 Results and discussion

This section of the report dicusses 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.

Critical appraisal of study and results.

In particular the following points must be addressed:
- a residue at or about the limit of determination in control samples
- adverse effects on egg production, milk yield and health of the livestock
- the relationship between the dose rate used in the study and the highest likely residue levels in feed
- proposal for MRL and/or limit of determination with reasoning.

7. **References**

"P. Beutel, H. Frehse, P. Hertl, B. Krebs, H. Morgenthaler, H.-J Scheuermann und G. Timme (1992):
Rückstandsversuche - Teil IV: Fütterungsstudien mit landwirtschaftlichen Nutzlieren, Industrieverband Agrar e.V. (IVA), 2. Auflage 1992

P. Beutel, H. Frehse, P. Hertl, B. Krebs, H. Morgenthaler , H.-J. Scheuermann and G. Timme (1992):
Residue Studies - Part IV: Feeding studies with farm animals, Industrieverband Agrar e.V. (IVA); 1st impression 1992"

Codex Committee on Pesticide Residues (CCPR) (1986):
Guidelines on Supervised Studies to Provide Data on the Nature and Amount of Pesticide Residues in Human Foods of Animal Origin. ALINORM 87/24, Appendix IV, Annex I.

Environmental Protection Agency (1982):
EPA Guidelines-Subdivision O: Residue Chemistry, § 171-4.

Environmental Protection Agency (1994)
Follow-up Guidance for:
- Updated livestock Feeds tagles
- Aspirated Grain Fractions (Grain Dust): A Tolerance Perspective
- Calculating Livestock Dietary Exposre
- Number and Location of Domestic Crop Field Trials
EPA 738-K-94-001, June 1994

Environmental Protection Agency (1995)
Residue Chemistry Guidelines, 860 Series, OPPTS 860.1480: Meat/Milk/Poultry/Eggs

Federal Biological Research Centre for Agriculture and Forestry, Federal Republic of Germany (1990):
Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln, Teil I, 1-2, Anleitung zum Antrag auf Zulassung von Pflanzenschutzmitteln (Guidelines for official testing of pesticides, Part I, 1-2, How to apply for registration of a pesticide).

Amtliche Sammlung von Untersuchungsverfahrern nach § 35 LMBG (1988):
Verfahren nach Weibull. Methode L 01.00-20. (Official collection of test methods in accordance with § 35 LMBG [German Food Regulations] (1988): Weibull method. Method L 01.00-20, May 1988.

Deutsche Forschungsgemeinschaft (German Research Association):
Rückstandsanalytik von Pflanzenschutzmitteln. Methodensammlung der Arbeitsgruppe "Analytik". 1. bis 11.
(Residues analysis of pesticides. Method compilation of the "Analysis" working group, 1 to 11. 1989 issue, Chapters XI and XI-A, in conjunction with Chapter V, 4-7. VCH-Verlagsgesellschaft mbH, Weinheim, Germany.

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

Appendix 1 : Sampling, sample preparation and sample sizes

A. Dairy cattle

Sample material	Sampling method	Sample preparation	Weight/unit (homogenised) Laboratory sample
Meat	Collect approx, equal pieces of loin, flank or hind-leg (round piece) and diaphragm muscle	After coarse pre-chopping macerate in a mincer and then mix carefully	1 kg
Fat	Collect approx, equal quantities of sub-cutaneous, mesenterial and renal fat	After coarse pre-chopping macerate in a mincer and then mix carefully	1 kg
Liver	Collect the entire organ or representative parts thereof	After coarse pre-chopping macerate in a mincer and then mix carefully	1 unit or 4 x min. 50 g
Kidneys	Collect the entire organ or representative parts thereof	If possible remove urine residues. Macerate tissue in a mincer and then mix carefully	1 unit or 4 x min. 50 g
Raw milk	Collect milk from each animal separately	Homogenize each sample of milk	1 l[1)]

[1)] In case an intermediate storage in deep-frozen stage becomes necessary, milk should only be deep-frozen in amounts representing an analytical sample each.

Appendix 1 : Sampling, sample preparation and sample sizes

B. Laying hens

Sample material	Sampling method	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 [1)]	300 g
Fat	Collect all the abdominal fat	Chop the fat of 3 hens, where appropriate combined[1)]	30 g
Liver	Collect the entire organ	Chop the livers of 3 hens, where appropriate combined[1)]	3 units
Eggs		Clean shells; Break eggs from 3 hens; Combine the whites/yokes; Discard the shells[2)]	3 units

[1)] The pre-requisite for combining sample materials is that there are at least 3 samples per dose group available (i.e. at least 9 animals are involved)

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

Stomach, kidneys and heart need only be analysed in the event of specific problems (e.g. results from metabolic studies).