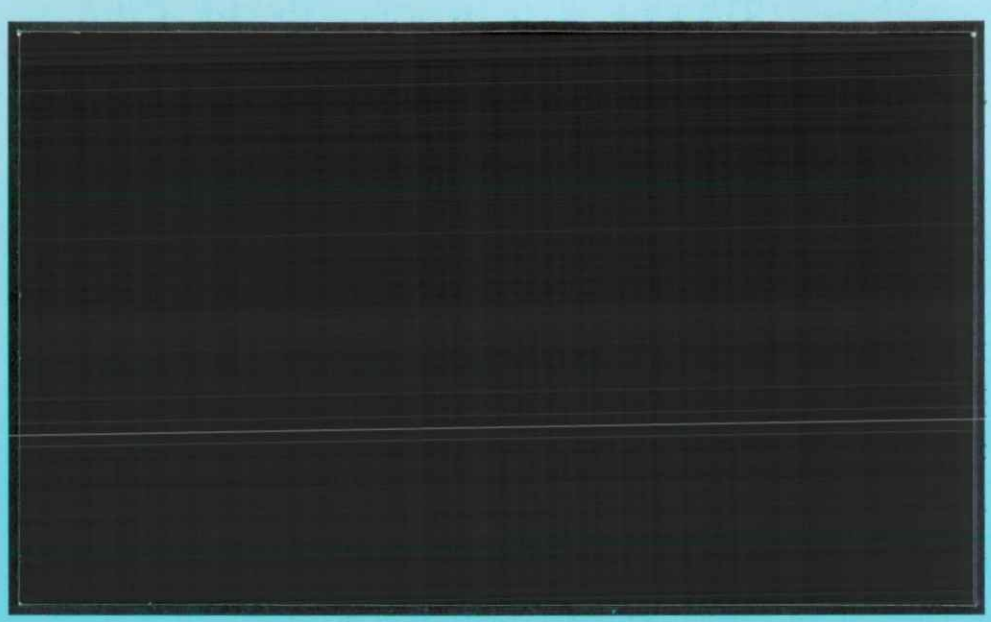

Natural Environment Research Council

ITE has administrative headquarters north and south, and the geographical distribution of its 250 staff in six Research Stations throughout Britain allows efficient use of resources for regional studies and provides an understanding of local ecological and land use characteristics.

ITE NORTH

Edinburgh Research Station
and ITE(N) Directorate
Bush Estate
Penicuik
Midlothian EH26 0QB
Tel: 031 445 4343; Telex 72579
Fax: 031 445 3943

Banchory Research Station
Hill of Brathens
Glassel
Banchory
Kincardineshire AB31 4BY
Tel: 033 02 3434
Fax: 033 02 3303

Merlewood Research Station
Grange-over-Sands
Cumbria LA11 6JU
Tel: 05395 32264; Telex 65102
Fax: 05395 34705

ITE SOUTH

Monks Wood
and ITE(S) Directorate
Abbots Ripton
Huntingdon
Cambs PE17 2LS
Tel: 048 73 381; Telex 32416
Fax: 048 73 467

Bangor Research Unit
University College of North Wales
Deiniol Road
Bangor
Gwynedd LL57 2UP
Tel: 0248 370045; Telex 61224
Fax: 0248 355365

Furzebrook Research Station
Wareham
Dorset BH20 5AS
Tel: 0929 551518
Fax: 0929 551087

The ITE Research Marketing Officers for ITE North and South are based at Banchory and Monks Wood, respectively.

Development of a Tissue Bank for Quality Assurance in Chemical Analysis
Internal Report 3: Rodenticides
(1st and 2nd pilot)

1st Draft 24/4/93

M.J. Craig

1. INTRODUCTION

Chemical analysis plays a fundamental role in many aspects of scientific research. It is imperative that the results of such analysis are reliably accurate and repeatable in order to promote confidence in them. Good quality assurance is therefore essential to support results and without it, it is becoming increasingly difficult to publish data. Consequently, the need to find ways in which to improve both internal and inter-laboratory quality assurance is becoming increasingly more important in chemical analysis. One such way of improvement would be to introduce the use of a tissue bank reference standard, which has the chemical bound to biological material, as part of the routine analytical procedure.

The measurement of residues in tissue bank samples during routine chemical analysis of biological samples serves several purposes. Firstly, it will allow the detection of inaccuracies during the analysis caused either, by poor laboratory technique in preparation of calibration standards, or by machine failure. Secondly, it will show any *variation with time* in both extraction and detection efficiencies, thereby giving confidence to time series data. Third, it will permit laboratory inter-calibration for identification and quantification. Finally, a tissue bank may also be used to measure repeatability within in any one analysis series by running repeat tissue standards. The use of a tissue bank for these purposes, rather than solution standards or topically spiked material, is desirable as solutions can evaporate, absorb and degrade. Also, slight changes in the structure and properties of residues caused by biological incorporation into tissue will not be reflected by either solution standards or topical spiking.

The aim of the present study was to develop a tissue bank consisting of freeze dried avian liver and blood containing biologically incorporated rodenticides. Progress towards the development of similar tissue banks for metals, organochlorines and polychlorinated biphenyls (PCB's) is reported elsewhere (Craig 1993a and Craig 1993b).

1.1. Selection of reference chemicals.

The second generation rodenticides brodifacoum, difenacoum, flocoumafen and bromadiolone were used in this study. Residues of these chemicals have been shown to appear frequently in tissues of wild animals and birds, (as a result of secondary poisoning in many cases) and as such, are often the subject of routine analysis at many research centres. It was therefore considered appropriate that these chemicals be selected as reference standards for the purpose of quality assurance.

2. MATERIALS AND METHODS

2.1. Experimental birds

The experiment was conducted using three adult female Japanese quail housed individually in metal cages with raised wire floors. The cages were located in an environmentally controlled room with an ambient temperature of 20-25°C. A commercial chick starter diet and water were supplied *ad libitum* throughout the experiment. Two birds were dosed with rodenticides and one bird was left untreated (control). All three birds were weighed daily throughout the experiment. All birds were observed closely throughout the dosing period for possible symptoms of toxicity; bleeding, reduction in body weight, changes in behaviour, reduction in food consumption (not quantified) and general loss of condition.

2.2. Dosing regime

A literature survey was carried out of previous studies on bird toxicology (appendix 1). A suitable exposure level for each of the rodenticides was chosen based on this survey taking into account the fact that several rodenticides would be administered concurrently as a single oral dose, unlike those in many of the published studies. The dosing regime (Table 1) was expected to result in sufficient accumulation of rodenticide residues in the liver but not to induce clinical symptoms of toxicity.

Table 1. Pilot dosing regime for rodenticides

Rodenticide	Vehicle	Dose Rate (mg/bird/day)	Estimated intake (mg/kg BW/day)[1]	Duration (days)
Flocoumafen	Milled sucrose	0.15	0.66	2
Brodifacoum	Milled sucrose	0.10	0.44	2
Difenacoum	Milled sucrose	0.10	0.44	2
Bromadiolone	Milled sucrose	0.15	0.66	2

2.3. Preparation of dosing compound

A dosing compound of 0.37 mg g^{-1} bromadiolone (technical grade; Lipha, Lyon, France), 0.36 mg g^{-1} flocoumafen (96.4% pure; Sorex Ltd., Widnes, Cheshire), 0.24 mg g^{-1} difenacoum (98% pure; Sorex Ltd., Widnes, Cheshire) and 0.24 mg g^{-1} brodifacoum (97.4% pure; Sorex Ltd., Widnes, Cheshire) was prepared as described below:

[1] Estimated intake based on mean weight of birds at start of dosing (0.226 kg). The dose administered was kept constant through out the dosing period irrespective of any change in weight of the birds.

A coloured indicator, used to assess the homogeneity of the rodenticide dosing compound, was first prepared by adding approximately 15 drops of food colouring to 10 g of finely ground castor sugar. The colouring was added 3-4 drops at a time and the sugar dried in an oven at 80 °C between additions. After drying, the mixture was ground thoroughly with a pestle and mortar until a uniform colour was obtained.

A stock compound of 14.69 mg g^{-1} bromadiolone, 14.49 mg g^{-1} flocoumafen, 9.66 mg g^{-1} difenacoum and 9.66 mg g^{-1} brodifacoum was then formulated by mixing 152 mg, 150 mg, 100 mg and 100 mg of bromadiolone, flocoumafen, difenacoum and brodifacoum respectively with 9.35 g finely ground castor sugar and 500 mg coloured indicator sugar using a pestle and mortar. This compound was then transferred to a conical flask and placed on a rotating mixer for four hours until thoroughly mixed. Two glass agitators were placed in the flask to assist mixing. The dosing compound was then prepared by mixing 100 mg of the rodenticide stock with 3.70 g finely ground castor sugar and 200 mg coloured indicator sugar using a pestle and mortar. This mixture was transferred to a conical flask and placed on a rotating mixer for four hours until thoroughly mixed. Two glass agitators were placed in the flask to assist mixing.

The dosing compound was then put into gelatine capsules. Capsules were filled by first running them through the dosing compound grasped with a pair of forceps. The powder was then tamped down tightly with a glass rod and the capsule filled again. This was repeated until no further powder could be pushed into the capsule. Filling capsules in this way resulted in a mean (±SE) dose per capsule of 414 ± 1.165 mg (n=10). Five sub samples were taken from the stock compound and analysed to check the concentration.

2.4. Dosing procedure

The dosing compound was administered to the birds in a gelatine capsule once daily. To assist their administration, the oesophagus was first lubricated with 0.5 ml of water directed into the pharyngeal cavity using a Gilson pipette and the capsules were briefly moistened with water immediately before being given to the bird. Capsules were placed by hand at the back of the pharyngeal cavity of the birds, the beak held closed and the throat massaged to help the capsule down.

2.5. Sample preparation

Quail were sacrificed by decapitation using a sharp pair of scissors. Blood was collected from the neck in a beaker, and the livers were removed and rinsed with distilled water. Individual livers and blood were homogenised separately using an electric blender (Kinematica, Luzern, Switzerland) at half speed for five minutes. Two 1 g sub samples were taken from each of the liver and blood homogenates for analysis.

2.6. Analysis of residues

Residue levels were determined using high power liquid chromatography (HPLC).

3. RESULTS

3.1. Bird health

Body tremors and bleeding from the feet were observed in both of the treated birds after only two doses. The birds were therefore put down and the study terminated.

3.2. Rodenticide tissue residue analysis

Awaiting results (24/4/93)

3.3 Analysis of stock compound

The mean rodenticide concentrations of the rodenticide stock compound analysed are displayed along side the intended concentrations in Table 2. With the exception of bromadilone, the actual concentration of chemical is slightly lower than the intended level.

Table 2. Concentration of stock compound

Rodenticide	Mean (±SE) rodenticide concentration of stock compound (mg/g)	Target concentration of rodenticide stock compound (mg/g)
Bromadiolone	25.1± 3.8 (n=5)	14.69
Difenacoum	8.5 ± 1.3 (n=5)	9.66
Flocoumafen	8.9 ± 1.3 (n=5)	14.49
Brodifacoum	7.9 ± 1.2 (n=5)	9.66

4.0 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Due to the development of chronic symptoms of toxicity in the quail which were treated with rodenticides, it was recommended that a second rodenticide pilot study should be carried out with much reduced dose rates and a cautious approach.

A second pilot study was subsequently carried out in which the rodenticide dose rates were halved. Symptoms of toxicity were observed in one of the birds after twelve days of dosing and in the remaining bird after eighteen days. The pilot was therefore terminated and tissue samples prepared for analysis. In addition to liver and blood, eggs laid by the birds during the dosing period were also analysed for rodenticides. (Awaiting results).

APPENDIX 1: Literature survey of bird toxicology studies

The following tables display information relating to rodenticide dosing obtained from a literature survey of previous toxicology studies on various bird species.

RODENTICIDE: Brodifacoum

Compound	Organism	Age	Dosing Method	Exposure	Duration	Residue(ppm)		Comments	Reference
						Liver	Egg		
Brodifacoum	Barn owl	1st year	Fed dosed mice	46.08 µg total (= 0.150-0.182 mg/kg BW)	1 day	0.63-1.25		Four of the six birds died 6-17 days after treatment. All showed signs of internal bleeding.	Newton, I., Wyllie, I., & Freestone, P. (1990) Rodenticides in British barn owls. Environ. Pollut., ?:101-117.
Brodifacoum	Barn owl	1st year	Fed dosed mice	92.16 µg total	3 days	#	#	The remaining two birds of the six treated were treated again 77-79 days after the first treatment and survived.	Newton, I., Wyllie, I., & Freestone, P. (1990) Rodenticides in British barn owls. Environ. Pollut., ?:101-117.
Brodifacoum	Barn owl	1st year	Fed dosed mice	184.32 µg total	6 days	#	#	The remaining two birds of the six treated were treated again 75 days after the second treatment and survived but showed prolonged bleeding from feet or mouth for 30 days post treatment.	Newton, I., Wyllie, I., & Freestone, P. (1990) Rodenticides in British barn owls. Environ. Pollut., ?:101-117.
Brodifacoum	Chicken	#	Oral	4.5	#	#	#	LD_{50}	Hayes, W.J & Lawes, E.R, Handbook of pesticide toxicology, Academic Press, New York, 1991. Vol 3: p 1299.
Brodifacoum	Mallard Duck	#	Oral	2.0	#	#	#	LD_{50}	Hayes, W.J & Lawes, E.R, Handbook of pesticide toxicology, Academic Press, New York, 1991. Vol 3: p 1299.

RODENTICIDE: Flocoumafen

Compound	Organism	Age	Dosing Method	Exposure	Duration	Residue(ppm)		Comments	Reference
						Liver	Egg		
Flocoumafen	Chicken	#	Oral dose	>100 mg/kg	#	#	#	Acute oral LD_{50}	Worthing & Hance, The Pesticide Manual, British Crop Protection Council, 1991, Ninth edition, p 399.
Flocoumafen	Japanese Quail	#	Oral dose	>300 mg/kg	#	#	#	Acute oral LD_{50}	Worthing & Hance, The Pesticide Manual, British Crop Protection Council, 1991, Ninth edition, p 399.
Flocoumafen	Japanese Quail	#	In food	37 mg/kg diet	5 day	#	#	LC_{50}	Worthing & Hance, The Pesticide Manual, British Crop Protection Council, 1991, Ninth edition, p 399.
Flocoumafen	Mallard Duck	#	In food	1.7 mg/kg diet	5 day	#	#	LC_{50}	Worthing & Hance, The Pesticide Manual, British Crop Protection Council, 1991, Ninth edition, p 399.

RODENTICIDE: Difenacoum

Compound	Organism	Age	Dosing Method	Exposure	Duration	Residue(ppm)		Comments	Reference
						Liver	Egg		
Difenacoum	Barn owl	1st year	Fed dosed mice	30.51 µg total	1 day	#	#	All six birds treated survived.	Newton, I., Wyllie, I., & Freestone, P. (1990) Rodenticides in British barn owls. Environ. Pollut., ?:101-117.
Difenacoum	Barn owl	1st year	Fed dosed mice	61.02 µg total	3 days	#	#	Birds were treated 11-14 days after first treatment and all survived.	Newton, I., Wyllie, I., & Freestone, P. (1990) Rodenticides in British barn owls. Environ. Pollut., ?:101-117.
Difenacoum	Barn owl	1st year	Fed dosed mice	50.85-101.71 µg total	6 days	#	#	Birds were treated 33-35 days after second treatment and all survived. No external bleeding was observed in any of the above treatments.	Newton, I., Wyllie, I., & Freestone, P. (1990) Rodenticides in British barn owls. Environ. Pollut., ?:101-117.
Difenacoum	Chicken	#	Oral	50 mg/kg	#	#	#	LD_{50}	Tomes data base.