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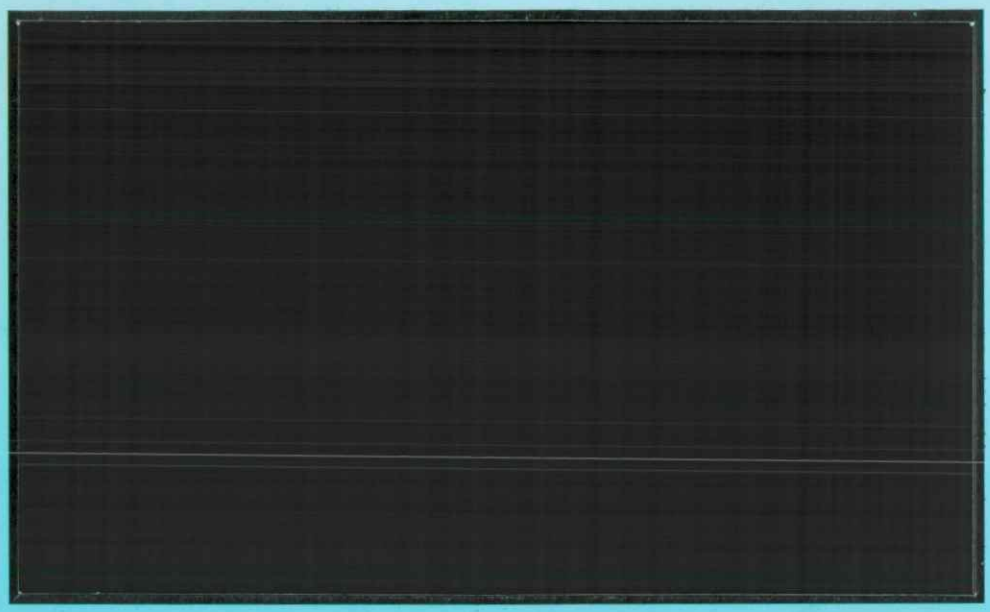

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Development of a Tissue Bank for Quality Assurance in Chemical Analysis
Internal Report 2: Polychlorinated biphenyls (2nd pilot)

1st Draft 23/4/93.

1. INTRODUCTION

Chemical analysis plays a fundamental role in many aspects of scientific research. It is imperative that the results of such analysis are reliable, accurate and repeatable. Good Quality Assurance (QA) is therefore essential to support results and promote confidence in them. Consequently, the need to find ways in which to improve both internal and inter-laboratory QA 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 2nd pilot study was to continue the work started in the first pilot (appendix 1) on the development of a tissue bank consisting of freeze dried avian liver and blood containing biologically incorporated polychlorinated biphenyls (PCB's). Progress towards the development of similar tissue banks for organochlorines, metals and rodenticides is reported elsewhere (Craig 1993a and Craig 1993c).

1.1. Selection of reference chemicals.

The PCB arochlor mixtures used in this study were 1248, 1254 and 1260. These mixtures were chosen since they contain relatively high proportions of the congeners 8, 52, 118, 130 and 180. Residues of these congeners have been shown to appear in tissues of wild animals and birds 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 individually marked young cockerels (one Miran and two Welsumers) housed together in an outside aviary measuring 2m x 2m x 3.5m. The Miran cockerel was dosed with arochlor 1248, one Welsumer cockerel was dosed with arochlor 1254 and the remaining Welsumer cockerel was dosed with arochlor 1260.

The birds were fed *ad libitum* with a commercial chick starter diet from hatching up to the age of ten weeks and a compound mix of chick starter crumbs, wheat and split maize thereafter. Water was provided *ad libitum*. All three birds were weighed at the start of dosing and when sacrificed. All birds were observed closely throughout the dosing period for possible symptoms of toxicity; reduction in body weight, changes in behaviour, reduction in food consumption (not quantified) and general loss of condition.

2.2. Dosing regime

A suitable exposure level for each of the arochlors was chosen based on previous studies found in bird toxicology literature in which birds were orally dosed with arochlors of known congener confirmation. The dosing regime (Table 1) was expected to result in sufficient accumulation of congener residues in the liver but not to induce clinical symptoms of toxicity.

Table 1. 2nd pilot dosing regime for PCB's

Arochlor	Vehicle	Mean dose rate (mg/bird/day)[1]	Estimated mean intake (mg/kg BW/day)[2]	Duration (Days)
1248	Vegetable oil	114.5	30.3	22
1254	Vegetable oil	78.4	30.1	22
1260	Vegetable oil	88.6	30.3	22

[1] Due to the increase in body weight of birds between the time of dosing capsule preparation and the *start* of dosing, extra capsules were administered to the birds (spread over the dosing period) to obtain the original intended intake rate. Mean dose/bird/day over the 22 day period is therefore stated.

[2] Intake rate was calculated using the body weight of each of the three birds at the start of the dosing period. The dose administered was kept constant through out the dosing period irrespective of any change in weight of the birds.

2.3. Preparation of dosing solutions.

Dosing solutions of 350 mg ml^{-1} arochlor 1248, 250 mg ml^{-1} arochlor 1254 and 250 mg ml^{-1} arochlor 1260 (Monsanto, USA) were formulated in vegetable oil as described below.

The required quantities of arochlor (5.250 g arochlor 1248, 3.750 g arochlor 1254 and 3.750 g arochlor 1260) were weighed into separate pre-weighed, graduated glass vials. Each solution was then made up to 15 ml with the addition of vegetable oil and placed on a heated stirrer at low heat until the arochlor had dissolved and dispersed evenly through the oil.

Gelatine capsules (Size 2; Farillon Ltd., Romford, Essex) were then each filled with 0.3 ml of this solution using a Gilson pipette and stored in an air tight container until required for dosing.[3]

2.4. Dosing procedure

The dosing solution 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 and freeze drying

Chickens were sacrificed by cervical dislocation followed by immediate decapitation using an amputation knife. 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) for five minutes at half speed. Eight 2 g sub samples were taken from each liver homogenate and four 2 g sub samples were taken from each blood homogenate, placed in 100 ml glass beakers and frozen at -70°C. Four of the frozen liver sub samples and two of the frozen blood sub samples from each bird were then freeze dried before analysis in a freeze drier (Model no. EF03; Edwards High Vacuum Ltd., Crawley, Sussex), with a system vacuum of 760 mm mercury.

2.6. Analysis of residues

INSERT CHEMISTS PROTOCOL FOR PCB ANALYSIS HERE.

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[3] Mean (±SE) density of dosing solution was calculated so that it could be weighed into capsules for greater accuracy of delivery.

3. RESULTS

3.1. Bird health

Birds showed no symptoms of toxicity and were alive and appeared healthy at the end of the dosing period.

3.2. PCB residue analysis

Awaiting results. (22/4/93)

4. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Providing the initial analysis of the PCB contaminated liver and blood homogenates reveals congener residue levels above the minimum target level of 1 µg/g DW, full scale production of a tissue bank can be initiated using the current dosing regime. If, however, the levels of residues are insufficient, or the congeners of interest are not present then a third pilot study may be necessary with revised dosing rates and possibly alternative arochlors.

Ideally, the residue levels should be sufficiently high to permit dilution of the contaminated liver with uncontaminated liver. Dilution in this way will not only allow the final residue levels in the tissue bank to be manipulated, but will also increase the bulk of the tissue bank, thereby extending its longevity. A cautious approach to the dilution is suggested to avoid over diluting the contaminated liver to such a degree that the residue levels in the final tissue bank fall below the minimum target. It is recommended that a 1:1 dilution be carried out in the first instance and that the level of residues in this dilution be checked. This process can then be repeated in two or more stages until the desired residue level with suitable confidence limits is achieved. Sub-samples of approximately 1 g should then be dispensed into appropriate *pre-weighed* glass beakers. The exact mass of the container plus sample should then be carefully recorded for reference and all the samples placed in the freezer. Once this is done, the analytical chemist can then simply select a tissue bank sample from the freezer and run it as a routine part of an analysis series. A comparison between the results obtained, and those expected from the tissue bank sample will quickly reveal any analytical error.

A record of the residue levels detected in each tissue bank sample analysed should be kept for reference. By plotting out these successive residue results against time, trends of any long term variation in residue levels, perhaps due to time-degradation of chemicals, may be identified and future results interpreted accordingly.

CITATIONS

1. Craig, M.J. (1993a) Development of a tissue bank of reference standard for quality assurance in chemical analysis. Internal report 1: Organochlorines and Metals.

2. Craig, M.J. (1993c) Development of a tissue bank of reference standard for quality assurance in chemical analysis. Internal report 3: Rodenticides.

APPENDIX 1.

Development of a Tissue Bank for Quality Assurance in Chemical Analysis: Polychlorinated biphenyls. 1st pilot report.

1st draft. 22/4/93.

1. MATERIALS AND METHODS

1.1. Experimental birds

The experiment was conducted using three individually marked young Welsumer cockerels housed together in an outside aviary measuring 2m x 2m x 3.5m. Two of the birds were dosed with PCB congeners and the remaining bird was left untreated to serve as a control. The birds were fed *ad libitum* with a commercial chick starter diet from hatching up to the age of ten weeks and a compound mix of chick starter crumbs, wheat and split maize thereafter. Water was provided *ad libitum*. All birds were weighed at thirteen, seventeen, nineteen and twenty weeks of age. At the start of dosing the birds were eighteen weeks of age. All birds were observed closely throughout the dosing period for possible symptoms of toxicity: reduction in body weight, changes in behaviour, reduction in food consumption (not quantified) and general loss of condition.

1.2. Dosing regime and preparation of dosing solution

The PCB congener dosing regime (Table 1) was chosen based on previous studies found in bird toxicology literature in which birds were orally dosed with arochlors of known congener confirmation.

A PCB dosing solution of 0.17 mg ml^{-1} congener 8, 0.17 mg ml^{-1} congener 52, 0.18 mg ml^{-1} congener 118, 0.10 mg ml^{-1} congener 138 and 300 mg ml^{-1} congener 180 (Greyhound Chromatography and Allied Chemicals, Birkenhead, Merseyside) was prepared as described below:

Stock solutions of 5 mg ml^{-1}, 2 mg ml^{-1}, 1 mg ml^{-1}, 1 mg ml^{-1} and 1 mg ml^{-1} of PCB congeners 8, 52, 118, 138 and 180 respectively were formulated in hexane in separate 5 ml graduated screw top bottles. These stock solutions were maintained at 5 ml volume by the addition of extra hexane as necessary. The dosing solution was then prepared by first combining 0.50 ml, 1.25 ml, 2.75 ml, 1.50 ml and 4.50 ml of the respective stock solutions in a 15 ml graduated vial. The solution was then allowed to evaporate down to approximately 2-3 ml before making up to 15 ml volume with olive oil. The solution was left, un-stoppered, in a fume cupboard to allow evaporation of any excess hexane and maintained at 15 ml volume by the addition of olive oil as necessary until no further change in volume occurred.

Forty four gelatine capsules were then each filled with 300 µl of this solution using a Gilson pipette and stored in an air tight container until required for dosing.

Table 1. 1st Pilot dosing regime for polychlorinated biphenyls

PCB Congener	Vehicle	Dose Rate (mg/bird/day)	Estimated intake (mg/kg BW/day)[4]	Duration (days)
No. 8	Olive oil	0.05	0.03	22
No. 52	Olive oil	0.05	0.03	22
No. 118	Olive oil	0.06	0.03	22
No. 138	Olive oil	0.03	0.02	22
No. 180	Olive oil	0.09	0.05	22

1.3. Dosing procedure

The dosing solution 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.

1.4. Sample preparation and freeze drying

Chickens were sacrificed by dislocation of the neck followed by immediate decapitation using an amputation knife. 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) for five minutes at half speed. Eight 2 g sub samples were taken from each liver homogenate and four 2 g sub samples were taken from each blood homogenate and frozen at -70°C. Four of the frozen liver sub samples and two of the frozen blood sub samples from each bird were then freeze dried before analysis in a freeze drier (Model no. EF03; Edwards High Vacuum Ltd., Crawley, Sussex), with a system vacuum of 760 mm mercury.

[4] Intake rate (mg/kg BW/day) was calculated using the mean body weight of the two birds at the start of the dosing period (1.9 kg). The dose administered was kept constant throughout the dosing period irrespective of any change in weight of the birds.

2.0 RESULTS

2.1. Bird health

Birds showed no symptoms of toxicity and were alive and appeared healthy at the end of the dosing period.

2.2. Residue analysis

Results of the PCB congener analysis are displayed in Tables 2 and 3. Blood and liver levels of all of the congeners were below the minimum target residue level of 1 µg/g DW and therefore too low for the potential production of either, a tissue bank or a blood bank.

Table 2. Mean liver PCB residues expressed as µg/g dry weight.[5]

	Congener 8	Congener 52	Congener 118	Congener 138	Congener 180
Fresh test sample (n=8)	0.00	0.05	0.21	0.17	0.32
Freeze dried test sample (n=8)	0.00	0.19	0.19	0.18	0.38
Fresh control sample (n=4)	0.00	0.00	0.00	0.00	0.01
Freeze dried control sample (n=4)	0.00	0.09	0.00	0.00	0.00

Table 3. Mean blood PCB residues expressed as µg/g dry weight.

	Congener 8	Congener 52	Congener 118	Congener 138	Congener 180
Fresh test sample (n=4)	0.00	0.00	0.00	0.00	0.05
Freeze dried test sample (n=4)	0.00	0.00	0.00	0.00	0.04
Fresh control sample (n=2)	0.00	0.00	0.00	0.00	0.00
Freeze dried control sample (n=2)	0.00	0.00	0.00	0.00	0.00

[5] µg/g DW figures for fresh material calculated using mean % water content of freeze dried samples.

3. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

As the levels of the PCB residues in the liver and blood were lower than anticipated, and too low for the large scale production of a tissue bank, it was decided that a second pilot should be initiated using increased dose rates (see pilot 2 report). In doing so, it was important not to raise the dose rates to such a level that would induce symptoms of toxicity in the birds. It quickly became apparent however that the increased dose rates required to attain sufficient liver residues would not be feasible due to the prohibitive cost of the congeners. It was therefore recommended that an arochlor mix should be used instead and that the implications of this should be investigated in the second pilot study.