

# final report

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## The correlation of cadmium levels in sheep liver and kidney

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## Abstract

Liver and kidney samples were collected in NSW and Victorian abattoirs during 2016-18 from 226 adult, homebred sheep and tested for cadmium concentration. Sheep from high rainfall areas have low correlation between cadmium in kidney and liver and are more likely to exceed international MRLs, whereas sheep from medium to low rainfall areas are less likely to exceed the MRLs and have stronger correlation. A further 24 sheep were then selected for multiple sampling of their livers to determine the individual liver variability for cadmium concentration.

When testing livers a single cadmium result yielding a low values of cadmium concentration (<0.5 mg/kg) is a highly reliable measure of the overall liver concentration. It appears there is not a systematic variation between locations within the liver with respect to cadmium residues but there is a random variation that increases with mean liver cadmium concentration.

## Executive summary

Cadmium can occur in high concentrations in liver and kidney tissue and has the potential to cause market access issues in certain residue-sensitive markets. Meat processors have identified the potential for large economic gains from a survey to identify regions where cadmium is concentrated in adult sheep livers. Currently offal from adult sheep is treated as waste due to concerns of cadmium limit violations, which leads to significant production loss within the sheep supply chain.

MLA, to help address this issue, funded a project to look at the correlation of cadmium levels between liver and kidney of adult sheep which was managed by Animal Health Australia. The information was needed so that a large survey based only on liver cadmium could inform on cadmium levels in kidney.

Phase 1 testing for the project involved a total of 226 sheep from high, medium and low rainfall areas of NSW and Victoria being selected from direct homebred lines by a meat inspector following a set work instruction. Both liver and kidney samples from these animals were tested at the same laboratory for cadmium concentration.

Phase 2 testing for this project was to understand the value (i.e. reliability) of single observations made on liver and the extent of within-liver variation in cadmium residues. Repeat sampling of 24 individual, adult sheep livers was undertaken by a meat inspector. Five samples were taken from each liver based on a work instruction and then tested at the same laboratory that performed the phase 1 testing.

The issue with data from the paired samples on liver-kidney cadmium concentration in adult sheep is that there are large variations in measurements on both tissues when higher concentrations of cadmium are present. This abolishes the ability to derive a linear relationship across the entire range of cadmium levels encountered. However, since high cadmium concentrations are strongly associated with high rainfall regions, the problem of variation in proportion to the mean is much less in median rainfall regions and negligible in low rainfall regions. Thus in medium and low rainfall regions levels of cadmium in kidney are an extremely useful guide to the level of cadmium likely to be present in kidneys.

Within individual livers from adult sheep, a single measurement of cadmium that yields a low value (i.e. <0.5 mg/kg) is a highly reliable measure of the overall liver concentration. Fortunately this includes the range of the MRLs for important markets such as EU, China (0.5 mg/kg). For high values of MRL (about 1.25 mg/kg), single measurements on a liver tend to give a less reliable assessment of that liver, although it does not matter what sampling location is used for the single assessment. It appears there is not a systematic variation between locations within the liver with respect to the NRS testing for cadmium residues but there is a random variation that increases with mean liver cadmium concentration.

From this project the cadmium level within liver in medium and low rainfall regions will be low, and is an extremely useful guide for kidneys as well. When testing for cadmium in livers and kidney for markets sensitive residues. Cadmium concentration above 0.5 mg/kg will have poor correlation and increase variation, and an individual test results does not necessary reflect the result of the batch. These data will add to the information eventually to be obtained from the NRS surveys.

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## 1 Background

Cadmium is a heavy metal which concentrates in the livers and kidney of livestock. Maximum residue limits (MRL) for cadmium apply for the shipment of offal to certain countries. Cadmium can occur in high concentrations in liver and kidney tissue and has the potential to cause market access issues in certain residue sensitive markets. Meat processors have identified large economic benefits from a survey to identify regions where cadmium is concentrated in sheep livers. Currently sheep offal is treated as waste due to concerns of cadmium limit violations.

The most recent work in Australia on cadmium in sheep offal was in the late 1990's, which makes the data unsuitable for analysis. Little research has been undertaken since 2002 and MLA has assessed the market risk and opportunities to the industry as sufficient to warrant further work.

The aim of this project is to understand how the risk of exceeding international cadmium maximum residue limits (MRLs) varies according to the geographic origin of the sheep.

## 2 Project objectives

1. To obtain paired kidney and liver samples from adult sheep originating in NSW. Some samples from other eastern states are permitted.
2. To measure cadmium levels in the above samples and to quantify the relationship in concentration of cadmium in kidney and in liver.
3. Tabulate the data by the geographic location of the property of origin to Local Government Area<sup>1</sup> level from which the sample sheep were obtained.
4. Prepare a scientific report suitable for publication in a peer referred journal [to be completed by project epidemiologist]

## 3 Methodology

### 3.1 Phase 1

#### *Correlation of cadmium levels in sheep liver and kidney study*

Animal Health Australia (AHA) was initially to collect approximately 400 paired samples (approximately 800 samples in total) from abattoirs in NSW and Victoria, using existing National Sheep Health Monitoring Project (NSHMP) inspection staff who are employed through a third party labour company. Only adult, homebred sheep had their livers collected and sampled for this study.

There was a mid-point evaluation after 200 paired samples had been collected to ensure an adequate spread of locations from which sheep had been sourced. The remaining samples were then collected from sheep originating from low and medium rainfall areas. Sourcing of sheep during this phase of testing proved difficult due to a lack of suitable sheep entering the abattoirs where the inspectors were located.

#### **Sample collection**

One sheep was selected from direct lines of home bred sheep presented for slaughter. The fifth sheep and every subsequent tenth sheep in each of the selected lines were the animals that were selected for sample collection.

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<sup>1</sup> Property of origin is defined as the property identified by PIC on the NVD accompanying the sheep.

From each sheep selected the following specimens were obtained: a) one kidney sample and b) one liver sample.

Sample collection was via a Work Instruction (WI), which applied across all participating plants and to all samples selected. The WI aligned to existing plant WIs and quality procedures. Samples were to be a minimum of 200gm.

All samples were:

- Individually identified in permanently labelled containers and placed in an Esky on ice until they could be transferred to the chillers prior to dispatch to the laboratory.
- All samples were chilled or frozen after collection.
- Samples were then sent to Symbio Laboratory in Queensland for cadmium testing.
- 

Samples collected were accompanied by the following information recorded on the Symbio sample request form:

- the date, shift and abattoir
- the name of the individual collecting the samples
- individual sheep data including:
  - PIC of origin
  - approximate age
  - gender and breed
  - the tissue type (kidney or liver)

When Samples reached Symbio, they were tested using the Symbio NATA Accredited Method Code ESM02 TOTAL ELEMENTS IN FOOD AND AGRICULTURE SAMPLES (by ICP-MS). Samples are acid digested and analysed using ICP-MS technique. Quality control data is set up by 10 determinations of QC sample. The mean value and standard deviation are calculated. A QC sample is run every batch and the result is checked against the mean value and standard deviation. Accept/Reject criteria for are in place for checks against the QC sample, duplicates, blanks, and spikes which are all run for every batch.

The results of the testing carried out by Symbio Laboratory were then entered into the Endemic Disease Information System (EDIS), managed by AHA.

Samples were to be collected in the financial year 2015-16 but as the supply of sheep from appropriate sources became harder to source, this extended through to 2017-18.

There were 200 sheep initially sampled with kidney and liver cadmium concentrations tested for each. There was then an initial analysis of the data by the project epidemiologist. No further sampling of sheep from high rainfall areas was required at this point. Samples were then collected from a further 26 sheep from low and medium rainfall areas for comparison of kidney and liver cadmium concentrations. Data from this phase of testing is at Appendix 1.

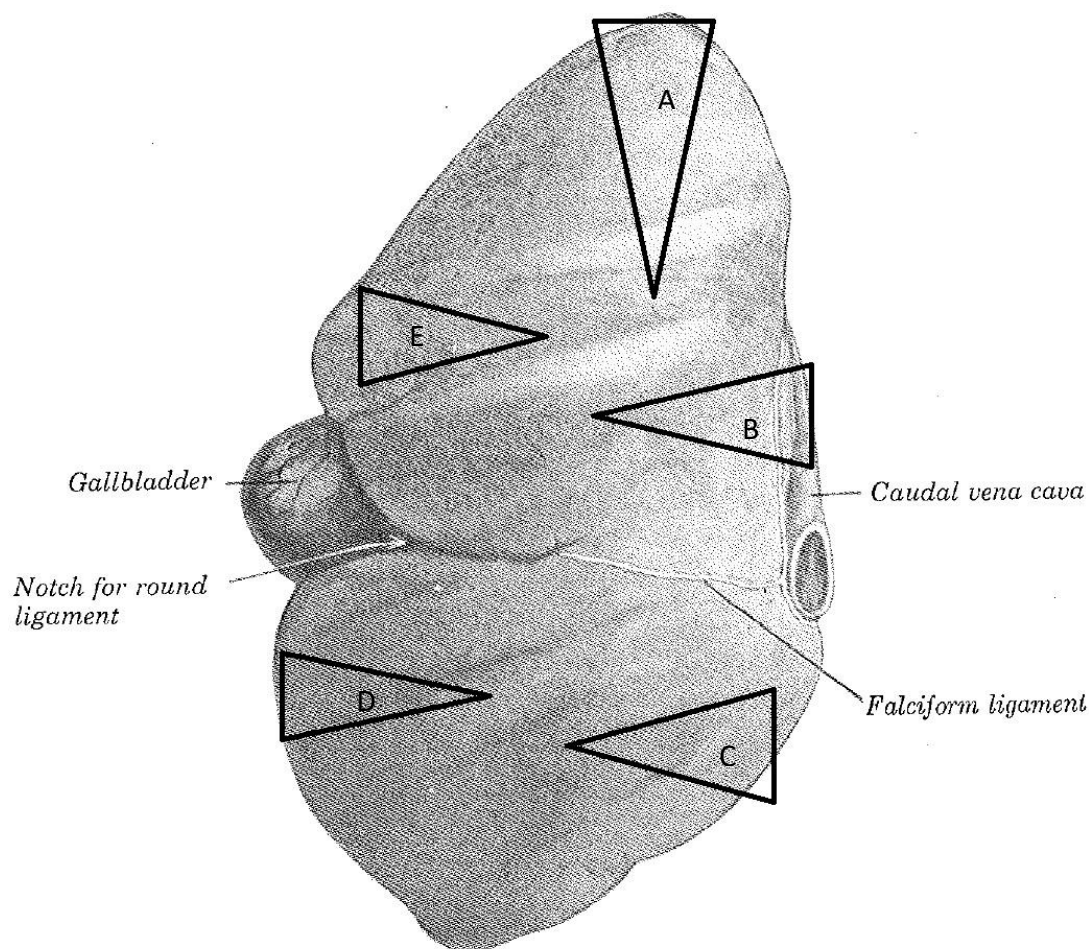
## 3.2 Phase 2

### *Within-liver variation in cadmium levels study*

The purpose of this phase of testing was to understand the value (i.e. reliability) of single observations made on liver and the extent of “within-liver variation” in cadmium residues. Repeat sampling of 24 individual adult livers was undertaken.

A WI was developed for the NSHMP inspector to carry out testing. For this phase only one inspector was used to standardize the testing.

Five wedges were taken from each sampled liver as per Diagram 1. Excisions were large enough to obtain the required mass of liver at each site. All samples were again sent to Symbio Laboratory in Brisbane. Once completed all results were sent to the project epidemiologist for analysis.



**Figure 1.** Diagram of the adult sheep liver showing the convex (smooth) surface normally in contact with the diaphragm in the live animal. Each triangular wedge shape (labeled A-E) shows the approximate position of incisions required to obtain the five liver samples.

A total of 24 sheep livers were collected within Victorian abattoirs by the same inspector in May-June 2018. These livers were sampled as per the WI and then five wedge samples forwarded to Symbio Laboratory for individual testing. Results from this sampling are in appendix 2.

## 4 Results and Discussion

### 4.1 Analysis of Phase 1: correlation of cadmium levels in adult sheep liver and kidney

As described in Section 3.1, the aim of this component was to assess the relationship between cadmium concentration in liver and kidney of adult sheep to inform on the interpretation of survey

now underway where only the concentration of cadmium in liver is assessed. The information required by the industry and regulators relates to both liver and kidney cadmium concentration for assessing their suitability for various domestic and international markets, however, it is very expensive to include both commodities in large surveys and hence only liver is included in the present NRS work.

#### 4.1.1 Methods

Paired samples (meaning one of each type from the same animal) of kidney and liver were obtained from adult sheep (two tooth and older) in Australian abattoirs from March 2016 to August 2017. Samples were forwarded to a laboratory assayed for cadmium concentration. Flocks of origin and local government areas were assigned an “assumed median rainfall” representing the median rainfall for the largest town in that local government area with this data obtained from Bureau of Meteorology ([www.bom.gov.au](http://www.bom.gov.au)). Local government areas were classified into three rainfall regions based on the assumed median annual rainfall as follows: high rainfall – exceeding 500 mm, medium rainfall ranging from 361 mm to 500 mm, and low rainfall – 360 mm and less. The data were analysed descriptively, by simple linear regression and using the Box-Cox transformation to identify if any reasonable regression model could be used to describe the data.

#### 4.1.2 Results

The distribution of flocks from which adult sheep originated and their respective local government areas is given in Table 1. Figure 2 (kidney) and Figure 3 (liver) demonstrate tissue cadmium concentrations grouped according to flock-of-origin so to demonstrate within-flock and between-flock variation. Both plots have the sheep flocks shown on the x-axis ranked in ascending order of the flock-median cadmium concentration (liver or kidney). In both cases the variation of cadmium measured within a flock increases noticeably as the flock median cadmium concentration increases.

Table 2 exhibits how each paired measurement of cadmium in liver and kidney would be interpreted if the EU MRL's were applied. More explicitly, it assesses the value of EU liver status (whether a liver passes or fails the EU MRL for cadmium) as a test for EU kidney status. From Table 2 it can be seen that across all sheep a total of 52 failed the EU kidney MRL for cadmium but only 7 of these also failed the EU liver MRL. Thus EU liver status for cadmium has a sensitivity of 13% (7/52) for detecting kidneys that would fail the EU MRL for cadmium. However, the sensitivity is shown to vary according to rainfall region which is strongly associated with median (flock) cadmium concentration in both kidney and liver.

Figure 4 shows the relationship between individual kidney and individual liver cadmium concentrations with the data broken down according to the rainfall zone (low, medium, high) of the flock of origin. It is also evident in Figure 4 that the poor ability to predict MRL status of kidney using the result for liver (Table 2) is largely a phenomenon of high rainfall areas where cadmium levels are demonstrated to be high. While there is poor correlation of liver and kidney cadmium concentrations at high rainfall location there is reasonable correlation at low rainfall locations such that liver EU MRL status perfectly predicts kidney EU MRL status in sheep from low rainfall flocks (Table 2). Figure 5 gives the data are summarised at the flock level by taking the median values of cadmium concentration in liver and cadmium concentration in kidney as the measures of flock status.



Table 1. Local government areas represented in the data, number of paired ovine liver and kidney samples for each, number of flocks represented, assumed medium rainfall (medrain) and rainfall zone (High > 500mm; 500mm ≥ Medium > 360mm, Low ≤ 360mm).

| Local government area     | Number of flocks | Number of samples | Medium rainfall* | Rainfall zone |
|---------------------------|------------------|-------------------|------------------|---------------|
| Alpine (Vic)              | 1                | 1                 | 1169             | High          |
| Ararat (Vic)              | 1                | 5                 | 615              | High          |
| Balranald-Wentworth (NSW) | 3                | 15                | 323              | Low           |
| Bourke (NSW)              | 1                | 1                 | 352              | Low           |
| Campaspe (Vic)            | 1                | 5                 | 428              | Medium        |
| Cobar (NSW)               | 3                | 8                 | 351              | Low           |
| Delatite (Vic)            | 1                | 2                 | 671              | High          |
| East Gippsland            | 2                | 9                 | 644              | High          |
| Gannawarra (Vic)          | 1                | 3                 | 373              | Medium        |
| Gundagai (NSW)            | 1                | 5                 | 623              | High          |
| Hume_NSW (NSW)            | 15               | 47                | 707              | High          |
| Indigo (Vic)              | 2                | 4                 | 957              | High          |
| Loddon (Vic)              | 2                | 10                | 367              | Medium        |
| Moirra (Vic)              | 1                | 4                 | 442              | Medium        |
| Moyne (Vic)               | 2                | 9                 | 688              | High          |
| Murray (NSW)              | 9                | 26                | 450              | Medium        |
| Riverina (NSW)            | 3                | 15                | 405              | Medium        |
| SA20 (South Australia)    | 1                | 4                 | 633              | High          |
| Strathbogje (Vic)         | 2                | 9                 | 648              | High          |
| Towong (Vic)              | 3                | 9                 | 714              | High          |
| Wagga Wagga (NSW)         | 1                | 5                 | 523              | High          |
| Wangaratta (Vic)          | 3                | 10                | 609              | High          |
| Wellington (NSW)          | 3                | 15                | 606              | High          |
| Yass (NSW)                | 1                | 5                 | 650              | High          |
| <b>Total</b>              | <b>63</b>        | <b>226</b>        |                  |               |

\* Median rainfall per year for largest population centre within each local government area

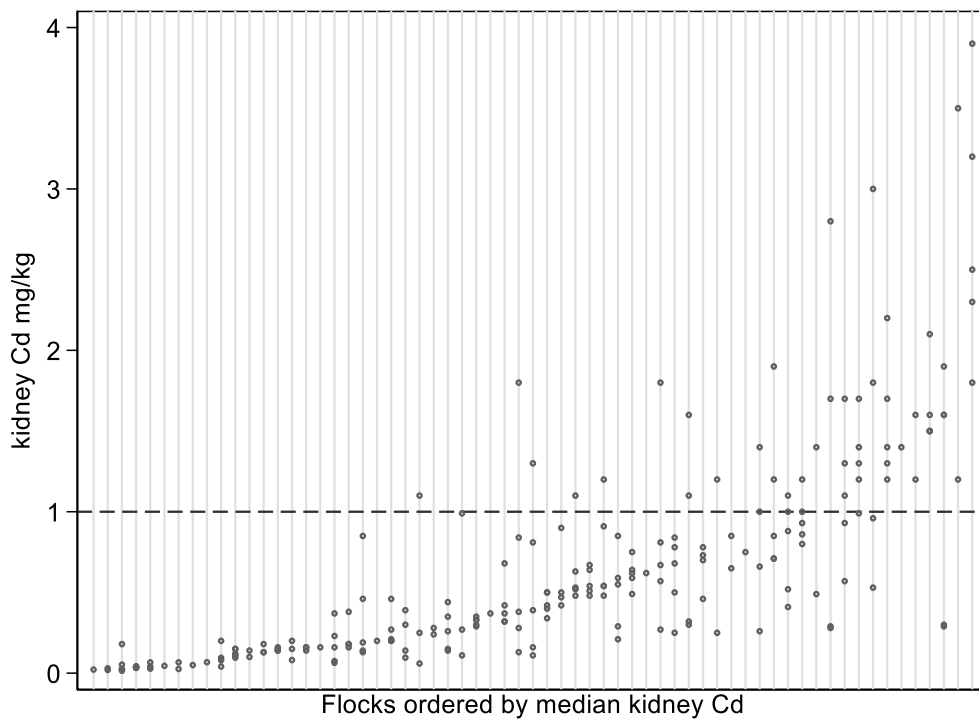


Figure 2. Kidney cadmium concentration - within and between flock variation. Each x-axis position represents a flock, with flocks ordered from lowest to highest value of median kidney Cd level. Each observation within a flock represented by a hollow circle (dashed line represents EU MRL = 1.0 mg/kg).

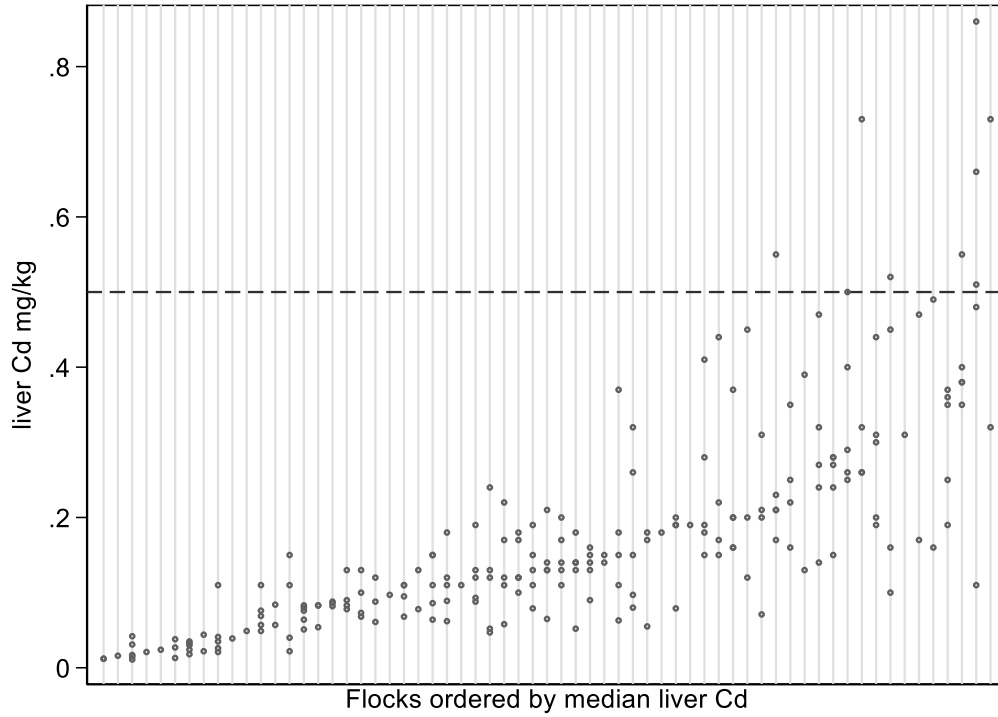


Figure 3. Liver cadmium concentration - within and between flock variation. Each x-axis position represents a flock with flocks ordered from lowest to highest value of median liver Cd level. Each observation within a flock represented by a hollow circle (dashed line represents EU MRL = 0.5 mg/kg).

Table 2. Data from cross-tabulation of paired liver and kidney cadmium concentrations interpreted as pass or fail using the European Union MRL for cadmium in liver (0.5mg/kg) and for cadmium in kidney (1.0mg/kg). Estimates of sensitivity refer to liver cadmium EU failure being used as a test for kidney cadmium EU failure.

| Rainfall zone    | Kid pass<br>Liv pass | Kid pass<br>Liv fail | Kid fail<br>Liv pass | Kid fail<br>Liv fail | Sensitivity*<br>(95% ci)      | Specificity*<br>(95% ci)        |
|------------------|----------------------|----------------------|----------------------|----------------------|-------------------------------|---------------------------------|
| All<br>(n=226)   | 173                  | 1                    | 45                   | 7                    | 7/52 = 0.13<br>(0.056-0.258)  | 173/174 = 0.99<br>(0.968-0.999) |
| High<br>(n=139)  | 89                   | 1                    | 42                   | 7                    | 7/49 = 0.14<br>(0.059-0.272)  | 89/90 = 0.99<br>(0.940-0.999)   |
| Medium<br>(n=63) | 60                   | 0                    | 3                    | 0                    | 0/3 = 0<br>(0-0.708)          | 60/60 = 1<br>(0.940-1)          |
| Low<br>(n=24)    | 24                   | 0                    | 0                    | 0                    | NA due nil<br>kidney failures | 24/24 = 1<br>(0.878-1)          |

\*Here "sensitivity" is the proportion of sheep with unacceptable kidneys that also have unacceptable liver (by EU cadmium MRL's). A low sensitivity indicates that a high proportion of kidney failures are NOT detected by assessing liver failures (high false negative or failure-to-detect problem). "Specificity" is the proportion of sheep with acceptable kidneys that also have acceptable livers (by EU cadmium MRL's). A high specificity indicates a high proportion of acceptable kidneys would be classified as such based on the result for liver (low false positive problem).

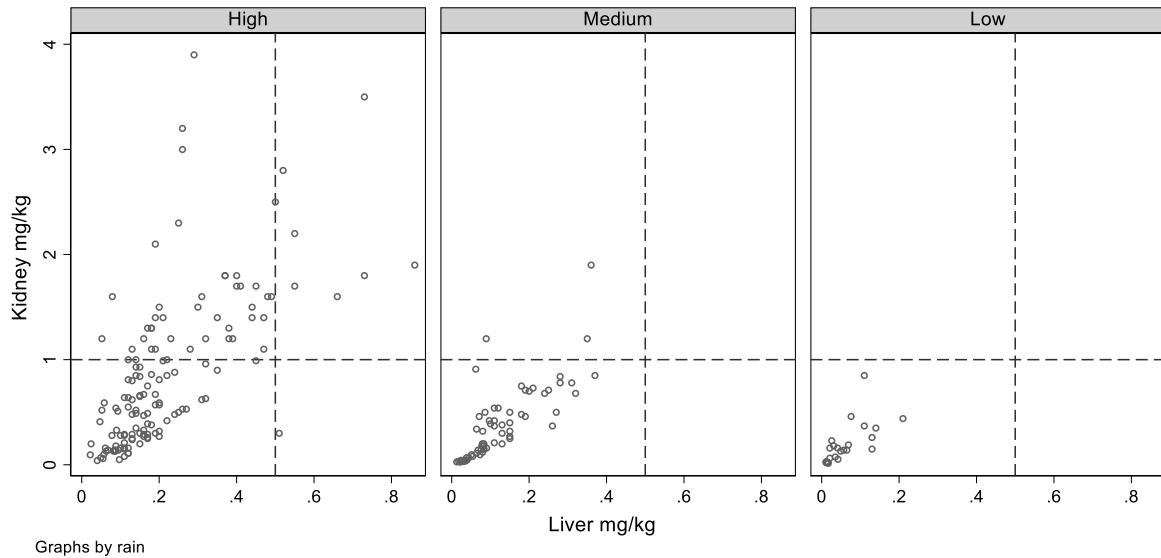


Figure 4. Scatter plots of cadmium concentrations (mg/kg) in paired samples of kidney and liver from adult sheep at slaughter showing observations for sheep from high rainfall flocks (n=139), medium rainfall flocks (n=63) and low rainfall flocks (n=24), reference lines represent the EU MRL for liver (vertical) and kidney (horizontal).

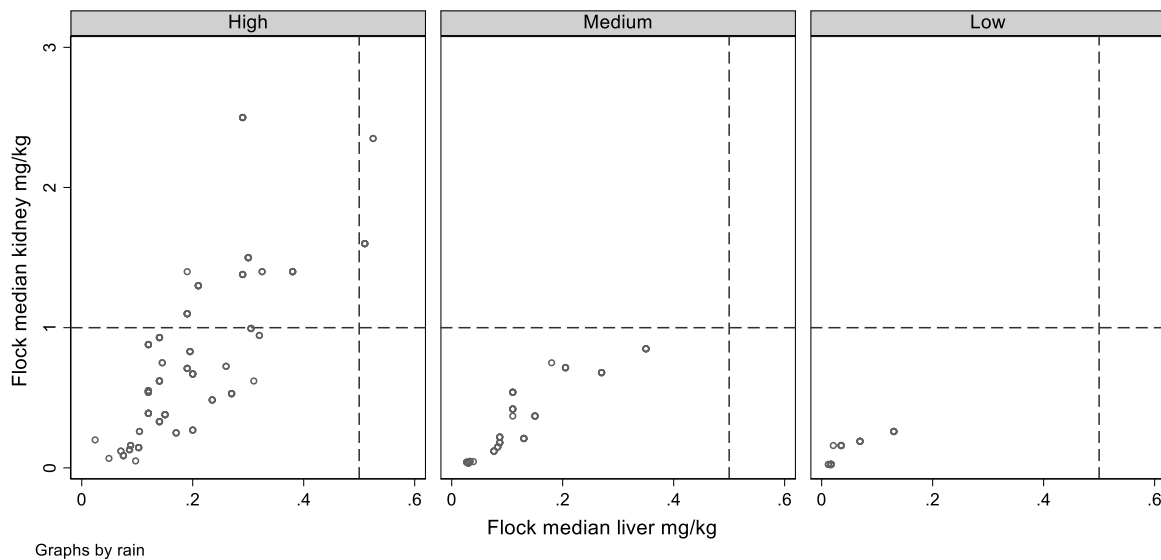


Figure 5. Scatter plots of flock-median cadmium concentrations of kidney vs liver in samples taken from adult sheep at slaughter showing median observations for high rainfall flocks (n=39), medium rainfall flocks (n=17) and low rainfall flocks (n=7) reference lines represent the EU MRL for liver (vertical) and kidney (horizontal). This is the same data as the previous Figure 4 but summarised at the flock level.

The purpose of fitting regression models was to understand whether the relationship between kidney cadmium concentration and liver cadmium concentration could be expressed as a simple equation. The output from ordinary least squares regression revealed a poor fit of the model to the data. The residuals were not normally distributed and exhibited the typical pattern encountered when variation increases as a function of observation means. The adjusted r-squared for the least squares linear regression model was 0.52 (low to moderate). The Box-Cox transformation procedure was then applied – it is a process that attempts to find an optimum power transformation of the dependent variable that increases the fit of data to a linear least-squares model. The optimum power transformation was kidney cadmium concentration raised to the power of 0.2807, however, this did not greatly improve the fit of the data, yielded a model with adjusted r-squared of 0.51 and residuals not substantially improved from the model without any data transformation.

### **4.1.3 Discussion**

The fundamental issue with the data from paired samples on liver-kidney cadmium concentration in adult sheep is that there are large variations in measurements made on both tissues when higher concentrations of cadmium are present. This effectively destroys the ability to derive a linear or curvilinear relationship across the entire range of values of cadmium levels. For this reason there is not a single model that can be fit to the data that would meaningfully assist the interpretation of future survey data based only on liver measurements. However, because high cadmium concentrations are strongly associated with high rainfall regions, the above noted problem of variation proportional to the mean is much less in median rainfall regions and negligible in low rainfall regions. Thus in medium and low rainfall regions levels of cadmium in kidney are an extremely useful guide to the level of cadmium likely to be present in kidneys.

## **4.2 Analysis of Phase 2: within liver variation of cadmium**

As described in 3.4, from the livers of 24 adult sheep at slaughter, five wedge-shaped biopsies were obtained from standardised anatomical locations. Liver samples were submitted for estimation of cadmium concentration by mass spectrometry. The purpose of this component was to describe the variability of cadmium measurements within livers to understand the value of a single measurement in describing the overall status of the liver.

### **4.2.1 Methods**

Descriptive tables and plots were formed to investigate the variation in cadmium levels (mg/kg) at different locations in the liver and the relationship between mean liver cadmium concentration and variation in concentration at different sites within liver. Where necessary cadmium concentrations were log transformation for plotting. Natural log transformation was also used to stabilise variance in cadmium mg/kg data prior to using the transformed data as an outcome variable in a mixed model where there was a single fixed effect consisting of sample location within liver (A, B, C, D or E as shown in Figure 1), and a single random effect of sheep (treated as subject). Tukey's test of multiple comparisons was performed to understand significance of difference in the mean cadmium concentrations at different liver sites measured on the log-scale.

#### 4.2.2 Results

Summary statistics for the cadmium concentration measurements obtained in this assessment of repeatability of measurement are given in Table 3. A plot showing variation of cadmium levels within and between livers is shown in Figure 6. Figure 6 suggests a relationship exists between the mean liver value of cadmium and the amount of variance within the liver (amongst sampling sites). To explore this apparent “variance in proportion to the mean effect”, Figure 7 was used to represent the same data as Figure 6 but with a re-arrangement along the x axis so that sheep id’s are ranked in ascending level of mean liver cadmium concentration. The relationship is further confirmed in Figure 8, by formally plotting the mean cadmium concentration against standard deviation for each liver. Figure 9 shows the beneficial impact of log transformation in stabilising the variance of data prior to application of regression.

The results for the mixed-effects regression model fitted to explain variation in cadmium concentration between sampling locations is given in Table 4. Prior to regression the concentration data were log-transformed and inferences made on the log scale owing to the above mentioned issue with variance. Plots of standardised residuals (not shown) revealed that the underlying assumptions for this analysis were satisfied. Table 5 shows the results for the multiple comparison of effect of sampling location. Care should be taken interpreting P values for the regression as the differences between means (either observed in Table 3 or predicted as coefficients on the log scale in Table 4) are small and not biologically meaningful. Overall, the results indicate sampling site within the liver having little practical impact on the level of cadmium result returned.

Table 3. Selected descriptive statistics for cadmium concentration (mg/kg) in the liver of adult sheep when samples were obtained from five anatomical sites (A, B, C, D and E) from each of 24 livers.

| Site       | n   | mean | sd   | min  | 10th<br>pctl | 90th<br>pctl | max  |
|------------|-----|------|------|------|--------------|--------------|------|
| <b>A</b>   | 24  | 0.29 | 0.26 | 0.02 | 0.03         | 0.62         | 1.10 |
| <b>B</b>   | 24  | 0.27 | 0.23 | 0.02 | 0.03         | 0.60         | 0.93 |
| <b>C</b>   | 24  | 0.28 | 0.24 | 0.02 | 0.03         | 0.60         | 0.95 |
| <b>D</b>   | 24  | 0.26 | 0.22 | 0.02 | 0.03         | 0.54         | 0.85 |
| <b>E</b>   | 24  | 0.28 | 0.23 | 0.02 | 0.04         | 0.60         | 0.87 |
| <b>ALL</b> | 120 | 0.28 | 0.23 | 0.02 | 0.03         | 0.60         | 1.10 |

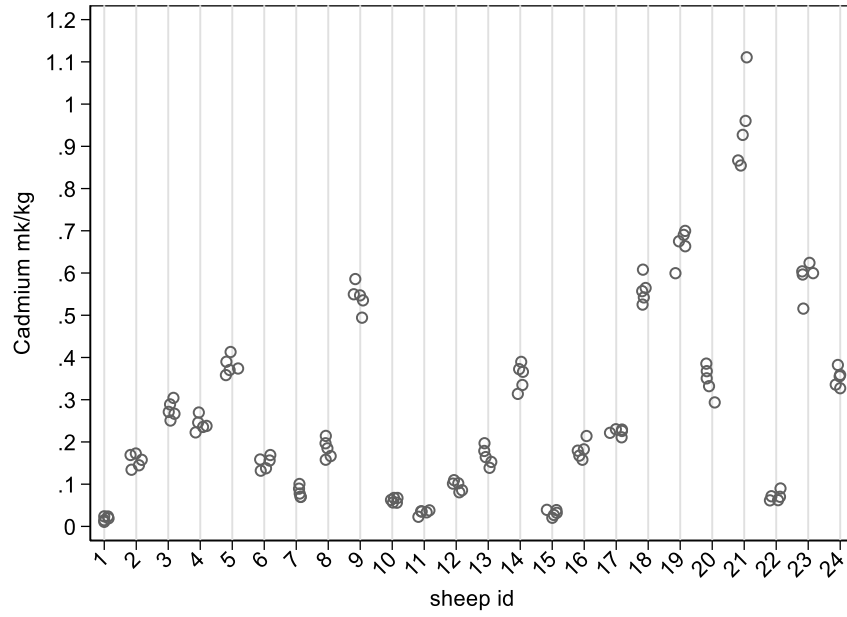


Figure 6. Sheep identifying number versus liver concentration of cadmium (mg/kg) where five samples from each liver were assayed.

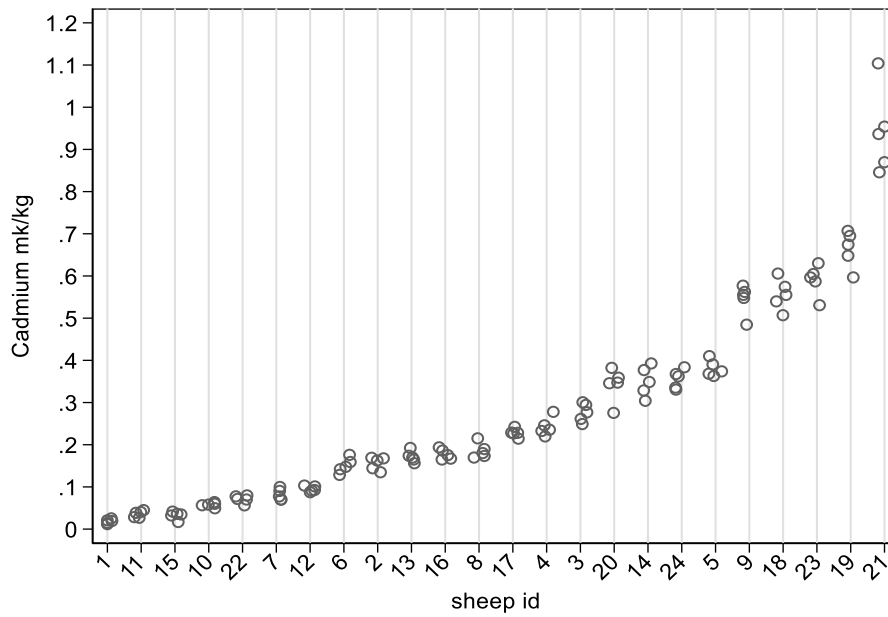


Figure 7. Sheep identifying number versus liver concentration of cadmium (mg/kg) where five samples from each liver were assayed. This is the same data as for Figure 1, however, sheep id are ranked in ascending order of median cadmium concentration.

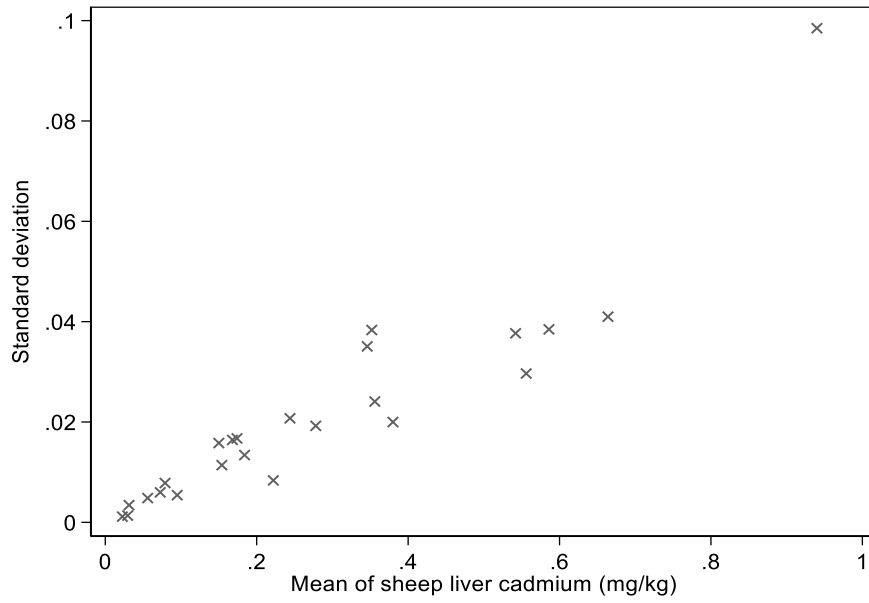


Figure 8. Plot of the mean of five measurements made on cadmium concentration in individual adult sheep liver (mg/kg) versus standard deviation.

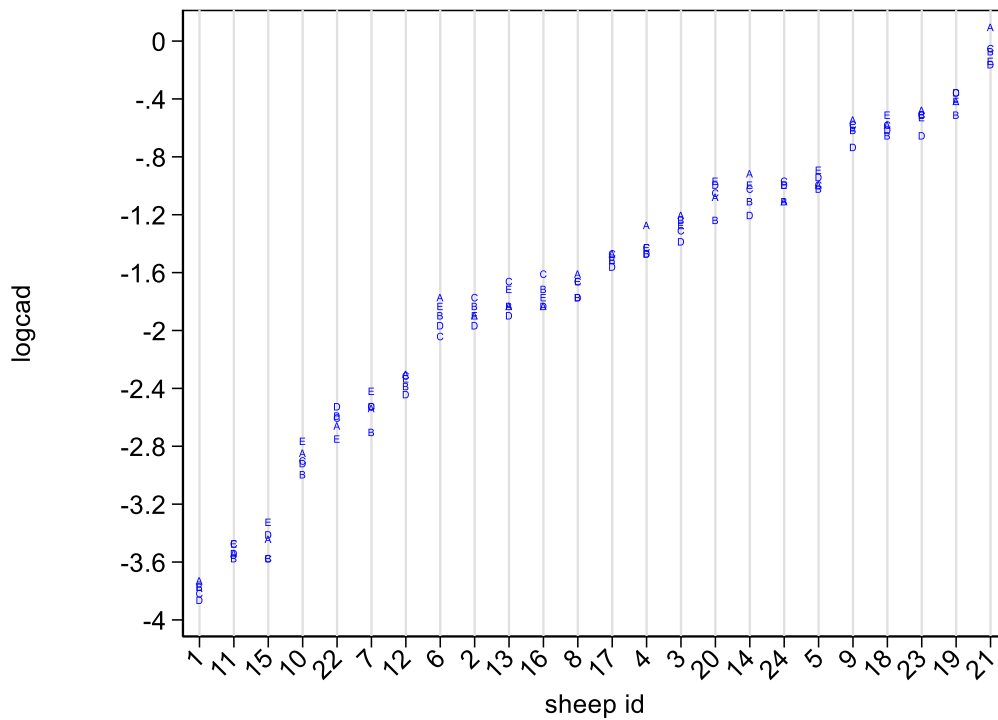


Figure 9. Log of cadmium concentration in sheep liver showing variation within livers at each of five sampling sites (A, B, C, D and E). Sheep livers are ordered in ascending value of median liver cadmium.

Table 4. Co-efficients from a mixed model regressing log of cadmium concentration in liver versus site of sampling within liver (A, B, C, D, E) with subject (sheep id) treated as a random effect.

| Site | Coef.     | Std. Err. | z     | P> z  | [95% Conf. Interval] |           |
|------|-----------|-----------|-------|-------|----------------------|-----------|
| B    | -.0724814 | .0204718  | -3.54 | 0.000 | -.1126054            | -.0323574 |
| C    | -.0124464 | .0204718  | -0.61 | 0.543 | -.0525704            | .0276776  |
| D    | -.0735058 | .0204718  | -3.59 | 0.000 | -.1136298            | -.0333819 |
| E    | .0001942  | .0204718  | 0.01  | 0.992 | -.0399298            | .0403182  |
| cons | -1.665447 | .2065805  | -8.06 | 0.000 | -2.070338            | -1.260557 |

Table 5. Pairwise multiple comparisons (contrasts) of log transformed cadmium mg/kg results for five locations of sampling within ovine livers.

| Sites compared | Contrast  | Std. Err. | Tukey<br>t | P> t  | Tukey<br>[95% Conf. Interval] |          |
|----------------|-----------|-----------|------------|-------|-------------------------------|----------|
| B vs A         | -.024125  | .0683647  | -0.35      | 0.997 | -.2136001                     | .1653501 |
| C vs A         | -.0067917 | .0683647  | -0.10      | 1.000 | -.1962668                     | .1826834 |
| D vs A         | -.027     | .0683647  | -0.39      | 0.995 | -.2164751                     | .1624751 |
| E vs A         | -.0087083 | .0683647  | -0.13      | 1.000 | -.1981834                     | .1807668 |
| C vs B         | .0173333  | .0683647  | 0.25       | 0.999 | -.1721418                     | .2068084 |
| D vs B         | -.002875  | .0683647  | -0.04      | 1.000 | -.1923501                     | .1866001 |
| E vs B         | .0154167  | .0683647  | 0.23       | 0.999 | -.1740584                     | .2048918 |
| D vs C         | -.0202083 | .0683647  | -0.30      | 0.998 | -.2096834                     | .1692668 |
| E vs C         | -.0019167 | .0683647  | -0.03      | 1.000 | -.1913918                     | .1875584 |
| E vs D         | .0182917  | .0683647  | 0.27       | 0.999 | -.1711834                     | .2077668 |

### 4.2.3 Discussion

Based on this analysis, a single measurement on a liver yielding a low values of cadmium concentration (<0.5 mg/kg) is a highly reliable measure of the overall liver concentration. Fortunately this includes the range of the MRLS for important markets such as EU, China (0.5 mg/kg).

As the mean value of Cd concentration for a sheep liver increases so does the variation in the measurements made from that liver. This is much the same relationship as defined in Phase 1 for flocks and regions, i.e. flocks with a high median cadmium concentration in liver or kidney have a lot of variation amongst the samples assessed. Thus, there is much more measurement uncertainty about higher values of Cd than lower values. Moreover, for high values of MRL (e.g. Australian MRL for cadmium in liver =1.25 mg/kg ), single measurements on a liver tend to give a less reliable assessment of that liver, although it does not matter what sampling location is used for the single assessment. This is in part the reason why there is noted variation in the level of cadmium between and within geographic regions and within animals from the same line of sheep or property, noted in Phase 1 of this report.

In this work, there was no meaningful difference in the level of cadmium obtained from different sites of the liver. While the observed means and predicted means from the mixed model show some variation within liver and some statistical significance (but not in multiple comparisons) these differences are too small to be of practical relevance. In conclusion it appears there is not a



systematic variation between locations within the liver with respect to cadmium residues but there is a random variation that increases with mean liver cadmium concentration.

There are some practical ramifications from the findings reported here. Firstly, variations in sampling location within livers that might occur within a study, between studies or in the process of regulating the commercial supply of liver appear to be irrelevant to the outcome. Nevertheless, as median concentration of cadmium rises in a liver so do the variations in the measurement of concentration. The latter potentially gives rise to false negative results whereby a liver with an average measurement above the MRL is assessed as below the MRL, and conversely false positive measurements. Fortuitously, the (arguably) most important MRL is that of the EU (which is also adopted by a range of other countries in Europe and Asia) and this MRL for cadmium in liver is set sufficiently low for the variability to have a minor impact on false positive and negative errors.

## **5 Conclusions/recommendations**

It can be concluded from this project the cadmium level within liver in medium and low rainfall regions will be low, and is an extremely useful guide for kidneys as well. Effectively, when liver passes the EU MRL standard for cadmium concentration then kidney also passes.

The recommendation from the project is when testing for cadmium in livers and kidney for markets sensitive residues, Cadmium concentration above 0.5mg/kg will have poor correlation and increase variation, and an individual test results does not necessary reflect the result of the batch.

## 6 Appendix

### 6.1 Phase 1 raw data

| Sample number | Kidney (mg/kg) | Liver (mg/kg) | Sample number | Kidney (mg/kg) | Liver (mg/kg) | Sample number | Kidney (mg/kg) | Liver (mg/kg) |
|---------------|----------------|---------------|---------------|----------------|---------------|---------------|----------------|---------------|
| 1             | 0.13           | 0.063         | 41            | 1.3            | 0.17          | 81            | 0.99           | 0.45          |
| 2             | 0.28           | 0.11          | 42            | 1.2            | 0.23          | 82            | 0.27           | 0.2           |
| 3             | 0.84           | 0.15          | 43            | 1.7            | 0.55          | 83            | 0.11           | 0.12          |
| 4             | 0.38           | 0.18          | 44            | 1.6            | 0.48          | 84            | 0.022          | 0.016         |
| 5             | 1.8            | 0.37          | 45            | 0.3            | 0.51          | 85            | 0.041          | 0.027         |
| 6             | 0.05           | 0.097         | 46            | 1.9            | 0.86          | 86            | 0.028          | 0.013         |
| 7             | 0.25           | 0.13          | 47            | 1.6            | 0.66          | 87            | 0.067          | 0.038         |
| 8             | 1.2            | 0.39          | 48            | 0.29           | 0.11          | 88            | 0.42           | 0.22          |
| 9             | 0.3            | 0.13          | 49            | 1.4            | 0.47          | 89            | 0.5            | 0.25          |
| 10            | 0.39           | 0.1           | 50            | 0.49           | 0.17          | 90            | 0.27           | 0.16          |
| 11            | 0.81           | 0.12          | 51            | 0.095          | 0.022         | 91            | 1.8            | 0.37          |
| 12            | 0.16           | 0.1           | 52            | 0.2            | 0.15          | 92            | 0.57           | 0.2           |
| 13            | 0.11           | 0.12          | 53            | 0.041          | 0.04          | 93            | 0.81           | 0.2           |
| 14            | 1.3            | 0.18          | 54            | 0.08           | 0.11          | 94            | 0.67           | 0.16          |
| 15            | 0.39           | 0.17          | 55            | 0.29           | 0.16          | 95            | 0.85           | 0.22          |
| 16            | 0.85           | 0.14          | 56            | 0.28           | 0.1           | 96            | 0.59           | 0.058         |
| 17            | 0.25           | 0.15          | 57            | 2.8            | 0.52          | 97            | 0.21           | 0.11          |
| 18            | 0.78           | 0.28          | 58            | 1.7            | 0.45          | 98            | 0.29           | 0.17          |
| 19            | 0.84           | 0.28          | 59            | 0.2            | 0.024         | 99            | 0.55           | 0.12          |
| 20            | 0.5            | 0.27          | 60            | 0.081          | 0.054         | 100           | 0.068          | 0.049         |
| 21            | 0.68           | 0.24          | 61            | 1.6            | 0.31          | 101           | 1              | 0.22          |
| 22            | 1.2            | 0.32          | 62            | 1.5            | 0.44          | 102           | 0.66           | 0.15          |
| 23            | 3.5            | 0.73          | 63            | 1.5            | 0.3           | 103           | 0.26           | 0.17          |
| 24            | 1.6            | 0.49          | 64            | 1.5            | 0.2           | 104           | 0.32           | 0.2           |
| 25            | 1.2            | 0.16          | 65            | 2.1            | 0.19          | 105           | 0.3            | 0.19          |
| 26            | 0.53           | 0.27          | 66            | 0.1            | 0.057         | 106           | 1.6            | 0.079         |
| 27            | 0.48           | 0.24          | 67            | 0.14           | 0.084         | 107           | 1.1            | 0.19          |
| 28            | 1.1            | 0.47          | 68            | 0.16           | 0.021         | 108           | 1.4            | 0.35          |
| 29            | 0.52           | 0.14          | 69            | 0.18           | 0.088         | 109           | 2.2            | 0.55          |
| 30            | 0.63           | 0.32          | 70            | 0.13           | 0.082         | 110           | 1.2            | 0.38          |
| 31            | 1.4            | 0.19          | 71            | 0.13           | 0.086         | 111           | 0.18           | 0.078         |
| 32            | 0.9            | 0.35          | 72            | 0.15           | 0.13          | 112           | 1.3            | 0.38          |
| 33            | 0.47           | 0.16          | 73            | 0.14           | 0.065         | 113           | 1.7            | 0.4           |
| 34            | 0.16           | 0.061         | 74            | 0.26           | 0.13          | 114           | 0.16           | 0.11          |
| 35            | 0.16           | 0.12          | 75            | 0.35           | 0.14          | 115           | 0.14           | 0.068         |
| 36            | 0.14           | 0.088         | 76            | 0.44           | 0.21          | 116           | 0.14           | 0.095         |
| 37            | 0.28           | 0.078         | 77            | 0.25           | 0.17          | 117           | 0.15           | 0.11          |
| 38            | 0.24           | 0.13          | 78            | 0.06           | 0.055         | 118           | 0.49           | 0.14          |
| 39            | 0.99           | 0.21          | 79            | 1.1            | 0.18          | 119           | 0.62           | 0.13          |
| 40            | 1.4            | 0.21          | 80            | 0.65           | 0.15          | 120           | 0.64           | 0.11          |

| Sample number | Kidney (mg/kg) | Liver (mg/kg) | Sample number | Kidney (mg/kg) | Liver (mg/kg) | Sample number | Kidney (mg/kg) | Liver (mg/kg) |
|---------------|----------------|---------------|---------------|----------------|---------------|---------------|----------------|---------------|
| 121           | 0.59           | 0.2           | 161           | 0.5            | 0.15          | 201           | 0.02           | 0.012         |
| 122           | 0.18           | 0.083         | 162           | 1.3            | 0.18          | 202           | 0.03           | 0.012         |
| 123           | 0.75           | 0.17          | 163           | 1.7            | 0.41          | 203           | 0.045          | 0.039         |
| 124           | 3.9            | 0.29          | 164           | 0.57           | 0.19          | 204           | 0.7            | 0.2           |
| 125           | 2.3            | 0.25          | 165           | 0.93           | 0.15          | 205           | 0.73           | 0.21          |
| 126           | 3.2            | 0.26          | 166           | 1.1            | 0.28          | 206           | 0.46           | 0.071         |
| 127           | 2.5            | 0.5           | 167           | 0.043          | 0.024         | 207           | 0.78           | 0.31          |
| 128           | 1.8            | 0.4           | 168           | 0.037          | 0.035         | 208           | 0.42           | 0.097         |
| 129           | 0.33           | 0.09          | 169           | 0.033          | 0.018         | 209           | 0.32           | 0.15          |
| 130           | 0.33           | 0.16          | 170           | 0.033          | 0.03          | 210           | 0.37           | 0.26          |
| 131           | 0.3            | 0.15          | 171           | 0.15           | 0.083         | 211           | 0.68           | 0.32          |
| 132           | 0.29           | 0.13          | 172           | 0.035          | 0.033         | 212           | 0.32           | 0.08          |
| 133           | 0.38           | 0.13          | 173           | 0.67           | 0.19          | 213           | 0.62           | 0.31          |
| 134           | 0.35           | 0.14          | 174           | 0.51           | 0.093         | 214           | 0.75           | 0.18          |
| 135           | 1.2            | 0.052         | 175           | 0.64           | 0.12          | 215           | 0.37           | 0.11          |
| 136           | 1              | 0.14          | 176           | 0.48           | 0.13          | 216           | 0.14           | 0.068         |
| 137           | 0.93           | 0.14          | 177           | 0.54           | 0.088         | 217           | 0.096          | 0.073         |
| 138           | 0.8            | 0.13          | 178           | 0.21           | 0.11          | 218           | 0.15           | 0.083         |
| 139           | 0.86           | 0.18          | 179           | 0.27           | 0.15          | 219           | 0.2            | 0.083         |
| 140           | 0.52           | 0.052         | 180           | 0.2            | 0.079         | 220           | 0.067          | 0.044         |
| 141           | 1.1            | 0.13          | 181           | 0.2            | 0.13          | 221           | 0.026          | 0.022         |
| 142           | 1              | 0.12          | 182           | 0.096          | 0.051         | 222           | 0.026          | 0.011         |
| 143           | 0.88           | 0.24          | 183           | 0.46           | 0.19          | 223           | 0.053          | 0.042         |
| 144           | 0.16           | 0.09          | 184           | 0.14           | 0.057         | 224           | 0.022          | 0.015         |
| 145           | 0.41           | 0.047         | 185           | 0.85           | 0.11          | 225           | 0.015          | 0.017         |
| 146           | 0.71           | 0.25          | 186           | 0.19           | 0.069         | 226           | 0.18           | 0.031         |
| 147           | 1.2            | 0.35          | 187           | 0.13           | 0.049         |               |                |               |
| 148           | 0.85           | 0.37          | 188           | 0.46           | 0.076         |               |                |               |
| 149           | 0.71           | 0.19          | 189           | 0.23           | 0.026         |               |                |               |
| 150           | 1.9            | 0.36          | 190           | 0.064          | 0.021         |               |                |               |
| 151           | 0.91           | 0.062         | 191           | 0.37           | 0.11          |               |                |               |
| 152           | 1.2            | 0.089         | 192           | 0.16           | 0.041         |               |                |               |
| 153           | 0.54           | 0.12          | 193           | 0.12           | 0.08          |               |                |               |
| 154           | 0.54           | 0.11          | 194           | 0.075          | 0.035         |               |                |               |
| 155           | 1.8            | 0.73          | 195           | 0.11           | 0.064         |               |                |               |
| 156           | 0.48           | 0.18          | 196           | 0.15           | 0.076         |               |                |               |
| 157           | 0.53           | 0.26          | 197           | 0.42           | 0.11          |               |                |               |
| 158           | 3              | 0.26          | 198           | 0.4            | 0.15          |               |                |               |
| 159           | 0.96           | 0.32          | 199           | 0.34           | 0.064         |               |                |               |
| 160           | 1.4            | 0.44          | 200           | 0.5            | 0.086         |               |                |               |

## 6.2 Phase 2 raw data

| Sample Description | Cadmium results (mg/kg) | Sample Description | Cadmium results (mg/kg) | Sample Description | Cadmium results (mg/kg) |
|--------------------|-------------------------|--------------------|-------------------------|--------------------|-------------------------|
| 026L A             | 0.024                   | 043L A             | 0.56                    | 035L A             | 0.058                   |
| 026L B             | 0.023                   | 043L B             | 0.52                    | 035L B             | 0.05                    |
| 026L C             | 0.022                   | 043L C             | 0.56                    | 035L C             | 0.055                   |
| 026L D             | 0.021                   | 043L D             | 0.54                    | 035L D             | 0.054                   |
| 026L E             | 0.023                   | 043L E             | 0.6                     | 035L E             | 0.063                   |
| 027L A             | 0.15                    | 042L A             | 0.23                    | 034L A             | 0.58                    |
| 027L B             | 0.16                    | 042L B             | 0.22                    | 034L B             | 0.54                    |
| 027L C             | 0.17                    | 042L C             | 0.23                    | 034L C             | 0.56                    |
| 027L D             | 0.14                    | 042L D             | 0.21                    | 034L D             | 0.48                    |
| 027L E             | 0.15                    | 042L E             | 0.22                    | 034L E             | 0.55                    |
| 028L A             | 0.3                     | 041L A             | 0.16                    | 033L A             | 0.2                     |
| 028L B             | 0.29                    | 041L B             | 0.18                    | 033L B             | 0.17                    |
| 028L C             | 0.27                    | 041L C             | 0.2                     | 033L C             | 0.19                    |
| 028L D             | 0.25                    | 041L D             | 0.16                    | 033L D             | 0.17                    |
| 028L E             | 0.28                    | 041L E             | 0.17                    | 033L E             | 0.19                    |
| 029L A             | 0.28                    | 040L A             | 0.032                   | 050L A             | 0.33                    |
| 029L B             | 0.23                    | 040L B             | 0.028                   | 050L B             | 0.33                    |
| 029L C             | 0.24                    | 040L C             | 0.028                   | 050L C             | 0.38                    |
| 029L D             | 0.23                    | 040L D             | 0.033                   | 050L D             | 0.37                    |
| 029L E             | 0.24                    | 040L E             | 0.036                   | 050L E             | 0.37                    |
| 030L A             | 0.37                    | 039L A             | 0.4                     | 049L A             | 0.62                    |
| 030L B             | 0.36                    | 039L B             | 0.33                    | 049L B             | 0.6                     |
| 030L C             | 0.37                    | 039L C             | 0.36                    | 049L C             | 0.6                     |
| 030L D             | 0.39                    | 039L D             | 0.3                     | 049L D             | 0.52                    |
| 030L E             | 0.41                    | 039L E             | 0.37                    | 049L E             | 0.59                    |
| 031L A             | 0.17                    | 038L A             | 0.16                    | 048L A             | 0.07                    |
| 031L B             | 0.15                    | 038L B             | 0.16                    | 048L B             | 0.075                   |
| 031L C             | 0.13                    | 038L C             | 0.19                    | 048L C             | 0.074                   |
| 031L D             | 0.14                    | 038L D             | 0.15                    | 048L D             | 0.08                    |
| 031L E             | 0.16                    | 038L E             | 0.18                    | 048L E             | 0.064                   |
| 032L A             | 0.079                   | 037L A             | 0.1                     | 047L A             | 1.1                     |
| 032L B             | 0.067                   | 037L B             | 0.092                   | 047L B             | 0.93                    |
| 032L C             | 0.08                    | 037L C             | 0.099                   | 047L C             | 0.95                    |
| 032L D             | 0.08                    | 037L D             | 0.087                   | 047L D             | 0.85                    |
| 032L E             | 0.089                   | 037L E             | 0.097                   | 047L E             | 0.87                    |
| 044L A             | 0.66                    | 036L A             | 0.029                   | 046L A             | 0.34                    |
| 044L B             | 0.6                     | 036L B             | 0.028                   | 046L B             | 0.29                    |
| 044L C             | 0.7                     | 036L C             | 0.031                   | 046L C             | 0.35                    |
| 044L D             | 0.7                     | 036L D             | 0.029                   | 046L D             | 0.37                    |
| 044L E             | 0.66                    | 036L E             | 0.031                   | 046L E             | 0.38                    |