Portable X-ray fluorescence for identifying lead exposure in wild mammals through hair analysis

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Abstract

Lead toxicity threatens scavenging wildlife globally. To allow inexpensive measurement of long-term lead exposure from archived wildlife tissues, portable X-ray fluorescence (XRF)[1] devices have been validated to measure lead concentrations in bone samples from bird species. In this study, we evaluated the efficacy of portable XRF for hair lead measurement in a specialist mammalian scavenger, the threatened Tasmanian devil (*Sarcophilus harrisii*). We attempted to first validate the method by analyzing relatively large (~1.0 g) hair samples from 39 deceased animals initially with XRF, and then with inductively coupled plasma mass spectrometry (ICP-MS) (validation study). We then measured lead concentrations via XRF only in a further 61 hair samples taken from live devils trapped in a plantation forestry landscape, where devils may scavenge on browsing marsupials that are regularly culled using lead ammunition (plantation study). Portable XRF measures of hair lead levels were positively correlated with ICP-MS values (R^2 = 0.95), although the relationship was weak at low lead concentrations. In the validation study, 95% of sampled Tasmanian devils had relatively low lead levels (≤ 3 mg/kg), but the remaining two animals showed considerably elevated lead levels (> 15 mg/kg), suggesting that harmful long-term lead exposure can occur in this species. In the plantation study, lead levels were comparable to those found in the hair of mammals in other studies, though a higher proportion (11%) of devils showed levels > 3 mg/kg than in the validation study, suggesting devils may have higher exposure to lead in a landscape subjected to regular culling of browsing mammals. Our results suggests that portable XRF is a promising emerging technology, and after some refinement, may allow non-invasive real-time measurement of lead concentrations in the hair of mammals.

Keywords: Tasmanian devil, marsupial, scavenger, ecotoxicology

1. Introduction

[1] XRF: X-ray fluorescence
[2] DFTD: Devil facial tumour disease

Lead is a widely studied heavy metal with many well-known toxic effects. Contemporary research has implicated lead-based ammunition as a major source of anthropogenic environmental lead around the world (Arnemo et al., 2022; Sonne et al., 2023), which is a threat to scavenging wildlife (Plaza and Lambertucci, 2019). Many scavenging species, particularly avian species, have been found with elevated lead exposure levels from lead-based ammunition (Pain et al., 2019), which can cause both lethal and sublethal effects, such as altered behavior (Ecke et al., 2017). Lead exposure in wildlife scavengers is a global issue, but relatively little research has been conducted in Australia, beyond studies of lead exposure in one avian species, the wedge-tailed eagle (*Aquila audax*) (Hampton et al., 2021; Lohr et al., 2020; Pay et al., 2021). This is concerning, given that culling, hunting and commercial harvesting of fauna is ubiquitous in Australia, and relies almost exclusively on lead-based ammunition (Hampton et al., 2022, 2018).

In contrast to avian species, relatively few studies have assessed the effects of lead pollution from ammunition in wild mammalian scavengers (Chiverton et al., 2022). The exception to this trend has been studies of bears (*Ursus* spp.) in the Northern Hemisphere (Boesen et al., 2019; Brown et al., 2023, 2022; Fuchs et al., 2021). One recent study from the Southern Hemisphere demonstrated harmful lead levels in an Australian marsupial species, the Tasmanian devil (*Sarcophilus harrisii*), when fed whole shot carcasses in captivity (Hivert et al., 2018). The Tasmanian devil is the largest marsupial carnivore in the world and a morphologically specialized scavenger (Jones et al., 2003). They are endangered due to devil facial tumor disease (DFTD)[2], a transmissible cancer which has caused population declines of over 80% since 1996 (Cunningham et al., 2021). Due to their endangered status, the species is the focus of intense conservation efforts, including proactive attempts to identify threatening processes that may prevent or delay their recovery, such as threats from anthropogenic activities (Jones et al., 2023, 2007). The aforementioned study of Hivert et al.

(2018) also measured lead exposure in wild devils but restricted their investigations to blood lead concentrations, reflecting only short-term exposure (Pain et al., 2019). Most of these wild devils had blood lead concentrations within the normal range, though 6% showed higher concentrations of lead (0.21 to 0.65 μmol/L).

The low population size of Tasmanian devils and their reliance on scavenging makes the potential impact of lead contamination from eating culled carcasses particularly concerning, especially to local devil populations in modified landscapes where culling of browsing mammals is common (Hutchinson et al., unpublished results). For instance, in Tasmanian timber plantations (henceforth 'plantations'), culling of the marsupial browsers Bennett's wallaby (*Notamacropus rufogriseus*), rufous-bellied pademelon (*Thylogale billardierii*) and brushtail possum (*Trichosurus vulpecula*), which constitute primary prey for devils (Andersen et al., 2017), is regularly conducted using lead-based ammunition prior to planting in coupes to protect seedlings (FPA, 2017).

To our knowledge, all past studies of lead exposure in scavenging mammals have utilized invasive sampling via collection of blood (Boesen et al., 2019; Brown et al., 2023; Fuchs et al., 2021; Hivert et al., 2018; Rogers et al., 2012), teeth (Brown et al., 2022) or liver (Kelly et al., 2021). Recent reviews have highlighted the value of non-invasive sampling of heavy metal exposure in wild mammals (Jota Baptista et al., 2022). One non-invasive approach to investigating heavy metal exposure in mammals is through hair analysis, a technique used by some recent studies on non-scavenging mammal species such as fruit bats (*Pteropus* spp.) (Sánchez et al., 2022), Malay civets (*Viverra tangalunga*) (Evans et al., 2022) and roe deer (*Capreolus capreolus*) (Draghi et al., 2023). Collecting hair samples from wild animals can be done remotely using hair snares (Azad et al., 2019) or through minimal handling of trapped wild animals. Invasive sampling studies typically require *ex situ* analysis via

inductively coupled plasma mass spectrometry (ICP-MS) in a laboratory to analyze lead concentrations, which is expensive and time-consuming (Hampton et al., 2021).

X-ray fluorescence (XRF) is another option for analyzing lead exposure levels in hair. The XRF device analyzes the elemental content of samples through the fluorescence produced by X-rays from a low energy x-ray tube (50 kV). XRF has long been used in human medicine (Specht et al., 2019a) and has a wide range of uses in wildlife research, such as examining elemental characteristics of tissues such as antlers (Singh et al., 2022), tracking monotreme species in the illicit wildlife trade (Brandis et al., 2018), and identifying trafficked animal parts such as ivory (McLellan et al., 2022). Portable XRF units have recently become commercially available. As they are handheld and battery-powered, these units are relatively inexpensive, quick and practical for use in the field, with most functions taking only three minutes to measure samples.

Advantages of this method over ICP-MS include lower cost and non-destructive analysis of samples (Hampton et al., 2021). Portable XRF has been validated for measurement of lead in bones of several avian species, showing strong correlations with ICP-MS values, such as wedge-tailed eagles (Hampton et al., 2021), California condors (*Gymnogyps californianus*) (Specht et al., 2018b), Pacific black duck (*Anas superciliosa*) (Nzabanita et al., 2023), and common loons (*Gavia immer*) (Specht et al., 2019b). However, portable XRF has been validated for measurement of lead in the hair of few mammalian species other than humans (Buddhachat et al., 2016).

Here, we investigated hair lead concentrations in wild Tasmanian devils in two stages. The first stage was a validation study (comparing portable XRF to traditional ICP-MS methods) and the second stage was a study of lead exposure in a forestry plantation landscape. Our hypotheses were: 1) portable XRF measures of hair lead levels in Tasmanian devils are similar enough to ICP-MS results that they can be used as a proxy, and 2) portable XRF can

rapidly identify mammals with markedly elevated lead exposure levels through non-destructive and non-invasive analysis of hair samples.

2. Materials and methods

2.1 Validation study

Hair from Tasmanian devils (Fig. 1) (n = 39) was collected from archived collections held in the Tasmanian Museum and Art Gallery (TMAG) and the University of Tasmania (UTAS). All samples were collected between April 2017 and February 2022 from across Tasmania but concentrated on urban areas surrounding Hobart (Hutchinson et al., unpublished results). Approximately 1 g of hair was shaved from whole frozen devil carcasses. Hair samples were analyzed via both XRF and ICP-MS analysis, with the same sample used for each method to minimize the potential for confounding effects of variation in lead concentrations between hair from different parts of the body (Specht et al., 2019a).

2.2 Forestry plantation landscape study

Relatively small (~0.1 g) hair samples were collected from 61 live wild devils living within Surrey Hills, a forestry plantation landscape in northwest Tasmania (-41.420S, 145.814E). Surrey Hills is an intensively managed forestry landscape dominated by *Eucalyptus nitens* plantations (Jones et al., 2023). Culling, using lead-based ammunition, is regularly undertaken at the site prior to planting in coupes to protect seedlings from browsing by three marsupial species: Bennett's wallaby, rufous-bellied pademelon, and brushtail possum. All three species are common prey of devils (Andersen et al., 2017), and there is evidence that culled browsers are scavenged by devils (Le Mar and McArthur, 2000). Between February 2021 and March 2022, we captured devils in custom-made 80 cm by 30 cm PVC pipe traps, shaved a 1 cm^2 patch of fur from their right shoulders, and recorded the

age (from tooth wear) and sex of the animal. These samples were analyzed using XRF only, as they were not large enough for ICP-MS methods.

2.3 XRF measurements

For XRF analysis of hair samples, we used similar methods as in past studies of lead in birds (Hampton et al., 2021; Specht et al., 2018b) and humans (Specht et al., 2018a). We used the Niton XL3t GOLDD+ (Thermo Fisher Scientific, Omaha, USA) portable XRF device, the same unit as used as in previous related studies (Bhatia et al., 2021; Hampton et al., 2021; Specht et al., 2018a). The device has a thermoelectric-cooled silicon drift detector (25 mm^2 area, 1 mm thickness). We used the device's customization option to optimize X-ray tube settings and filtration: 50 kV, 40 μA, and a filter combination of silver and iron (Hampton et al., 2021; Specht et al., 2019b, 2018b). We used a 3-minute (180 second) read-time for each sample.

The XRF unit was placed in a stand with hair samples placed on top of it, to ensure that samples were as close as possible to the beam detector (Hampton et al., 2021; Specht et al., 2018b). To analyze the measurements, we used the standard procedure from past studies (Hampton et al., 2021; Specht et al., 2018a), using peak fitting through MATLAB to identify the net counts of the lead peak and the corresponding Compton scattering peak from the silver X-ray tube anode. We used the silver Compton scattering peak to normalize the differences in geometry and mass among samples of different sizes (Hampton et al., 2021; Specht et al., 2018a).

2.4 ICP-MS measurements

The same hair samples (validation study samples only) were then sent to a commercial laboratory at Edith Cowan University Analytical Facility (Joondalup, WA, Australia) to

determine their lead concentrations via inductively coupled mass spectrometry (ICP-MS). Hair samples were freeze-dried and then underwent acid digestion. The hair samples were homogenized and processed as per Pay et al. (2021): specifically, a 0.4 g aliquot was transferred into ICP-grade polytetrafluoroethylene vessels which contained 5 mL of 68% trace analysis-grade nitric acid (Primar Plus™; Thermo Fisher Scientific, Omaha, USA), 0.5 mL of 34–37% trace metal-grade hydrochloric acid (Fisher Scientific), and 3 mL of 30% hydrogen peroxide (Emsure ISO®; Merck, Rahway, USA). The samples were then digested for 15 minutes in a microwave digestion system at 150°C. Samples were subsequently diluted with reverse osmosis deionized water (Milli Q®; Merck) to a total volume of 50 mL before being transferred to polypropylene tubes. For ICP–MS analysis, an iCAP™ Q (Thermo Fisher Scientific) unit was coupled to a CETAC™ ASX 520 AutoSampler (Thermo Fisher Scientific). Qtegra™ (Thermo Fisher Scientific, Omaha, USA) software was used for data acquisition and element quantitation.

A standard curve was created with concentration ranges of iCAP™ Q element standards (Thermo Fisher Scientific) and ICP-MS 68A solutions (High-Purity Standards, North Charleston, USA) to calibrate the instruments prior to analysis. We used Bone Ash Standard Reference Material 1400 (National Institute of Standards and Technology, Gaithersburg, USA) as a positive control Certified Reference Material (CRM). The CRM was digested twice, and two ICP-MS readings were made on each digestion. CRM ICP–MS readings had an average accuracy of 100.2%. We re-analyzed every fifth or sixth sample to create a duplicate read, with an average relative standard deviation (RSD) of 2.2%. We also conducted duplicate blind sample digestions on five randomly selected samples (average RSD = 2.0%). The lead concentrations were measured in mg/kg of dry weight (equivalent to ppm). Limit of detection (LOD) was 0.0015 mg/kg and limit of quantification (LOQ) was 0.005 mg/kg.

2.5 Statistical analysis

We identified the uncertainty values for XRF measurements as per past studies using the same equipment (Specht et al., 2019b). We calculated the uncertainty (σ) of each measurement using the equation:

Equation 1:

$$\sigma = \frac{c \times \sqrt{\frac{BKG}{t}}}{NET}$$

where *c* is concentration, *BKG* is the background counts as estimated by our fitting, *t* is measurement time, and *NET* is net lead counts from a Gaussian function (Zhang et al., 2021). We retained the negative values for lead in hair because they represent a point estimate of hair lead concentration incorporating uncertainty (Kim et al., 1995; Specht et al., 2016).

Linear regression was used to estimate the relationship between ICP-MS-derived and XRF-derived lead concentrations for sampled tested via both methods (n = 39) with the following equation:

Equation 2:

$$ICP_i = \beta_0 + \beta_1(XRF_i) + \epsilon_i$$

where β_0 is the intercept, β_1 is the slope of the relationship, and ϵ_i is the residual error. The predictive competence of the regression model was evaluated with 10-fold cross-validation repeated three times. Modelling was conducted using the caret package (Kuhn, 2022) in the R statistical environment (RStudio Team, 2023).

For each estimation method, we calculated the proportion of samples classified into ‘high’ or ‘low’ levels of lead exposure. Such classification can be problematic for hair lead concentrations (Fernández et al., 2021), because there is no standard classification for harmful lead concentrations in hair, as there is for blood, liver and bone (Pain et al., 2019).

As such, we defined 'low' lead concentration as ≤ 3 mg/kg where most of our validation data was concentrated, and 'high' lead concentrations as > 3 mg/kg. We used the same classification for both XRF values and predicted ICP-MS values and for the plantation landscape samples. Additionally, we constructed linear models with the predictors of sex and age (juvenile < 1 year, subadult 1–2 years, adult > 2 years) to look for any influence of these parameters on predicted ICP-MS lead levels in devils, using Akaike's Information Criterion (AIC) values to select the best fitting model (Burnham and Anderson, 2002).

3. Results

3.1 Validation of XRF for devil hair samples

We found a positive relationship between lead concentrations in the hair of Tasmanian devils estimated using portable XRF and ICP-MS (n = 39) (Fig. 2). The regression model showed a reasonable correlation between portable XRF and ICP-MS values, explaining 95% of the variability in the data over the range of values observed. The theoretical ICP-MS estimate of hair lead corresponding with an XRF estimate of 0 mg/kg was -0.564 mg/kg and the expected ICP-MS lead concentration increased by 0.947 units (SE = 0.034) for every unit increase estimated by XRF ($R^2 = 0.95$, $t_{37} = 27.825$, $p < 0.001$; RMSE = 1.4). ICP-MS estimates could therefore be predicted from portable XRF estimates across the range of sample data (n = 61) using the equation:

Equation 3:

$$ICP_i = \beta_0 + \beta_1 XRF_i + \epsilon_i$$

where $\beta_0 = -0.564$ and $\beta_1 = 0.947$.

The two highest values exerted a high degree of influence over the form of the regression. We therefore ran a second model excluding these values. This model showed limited,

although still significant, correlation between XRF and ICP-MS values, explaining only 13% of the variability in the data ($R^2 = 0.13$, $t_{37} = 2.323$, $p = 0.03$).

Most of the samples (94.87% as measured via either method) showed lead concentrations of < 3 mg/kg (Table 1) and the relationship between XRF and ICP-MS was weak at these lower values. However, the two points with the highest lead levels (> 15 mg/kg) measured consistently high with both XRF and ICP-MS (Fig. 2). XRF estimates tended to be higher than ICP-MS: the mean lead concentration was 2.33 mg/kg when measured via XRF versus 1.65 mg/kg when measured via ICP-MS. However, the frequency of 'high' lead exposure (> 3 mg/kg) was identical (5.13%) for both methods (Table 1). Given the dissimilarity between XRF and ICP-MS values, we feel that it is important to transform XRF values into ICP-MS equivalents prior to analysis, using equation 3.

3.2 Forestry plantation landscape study

Table 2 shows portable XRF hair lead measurements and uncertainties from devils in the plantation landscape. These data were converted to ICP-MS values using equation 3. Even using this transformed data, 11% of devils displayed 'high' (> 3 mg/kg) lead levels, substantially higher than the proportion of devils displaying 'high' levels in the validation analysis (5%, Table 1). The best fitting model predicting lead levels in devil hair in this landscape was the null model, indicating no influence of age or sex on lead levels in devils (Table 3).

4. Discussion

We present the first investigation of portable XRF for measurement of lead levels in hair in a non-human species, and the first report of lead exposure in a wild-living specialized mammalian scavenger as measured via hair lead levels. Using regression analysis, we were

able to characterize the relationship between XRF and ICPS-MS values at a coarse scale over a wide range of data. However, the relationship was highly variable at lower values, so XRF is not useful for precise predictions of low lead exposure levels in devil hair. We suggest that portable XRF could be used as an initial screening method for mammalian hair to identify individuals with the largest burden of lead exposure versus a typical exposure profile.

Lead levels in devil hair, in both the validation study (mean 1.65 ± 4.61 SD) and the plantation landscape study (mean 1.05 ± 1.02 mg/kg), were comparable to other published research on lead exposure in mammals (both wild and domestic). The levels we found were lower than those found in the hair of domestic dogs (*Canis familiaris*) used for hunting in Argentina (mean 2.37 ± 6.21 mg/kg) (Fernández et al., 2021), similar to those in hair from fruit bats in eastern Australia (medians for three species: 1.26/1.64/2.26 mg/kg) (Sánchez et al., 2022) and roe deer in Italy (mean 1.39 ± 1.63 mg/kg) (Draghi et al., 2023), and higher than those found in hair from Malay civets sampled in Malaysian Borneo (mean 0.52 ± 1.08 mg/kg) (Evans et al., 2022). Similar to several of these studies (Draghi et al., 2023; Evans et al., 2022; Sánchez et al., 2022), there was no evidence of variations in lead levels in devil hair among sexes and age classes.

Without a standard classification of harmful lead levels in hair, even apparently low levels of lead in devil hair may be cause for concern. Two devils, both in the validation study, showed atypically high hair lead levels: 15.77 and 25.34 mg/kg, when no other devils in either sample group had > 4.5 mg/kg (none > 2 mg/kg in the validation study). The source of these unusually high levels is unknown. The generally low hair lead levels are consistent with other studies showing low lead levels in the blood (Hivert et al., 2018) and liver (Hutchinson et al., unpublished results) of wild devils. Grey wolves (*Canis lupus*), cougars (*Puma concolor*) and American black bears (*Ursus americanus*) in the greater Yellowstone ecosystem likewise showed relatively low lead levels in blood and tissue samples, indicating

that some mammalian scavengers in some areas may not be impacted by lead exposure even where hunting using lead-based ammunition occurs (Rogers et al., 2012). Brown bears (*Ursus arctos*) did show elevated lead levels in this landscape, but lead levels did not increase during the hunting season (Rogers et al., 2012). In contrast, brown bears sampled in northern Europe have shown markedly elevated lead levels in blood and milk (Boesen et al., 2019), and lead levels in the teeth of Canadian black bears increase markedly during hunting seasons (Brown et al., 2022). It is clear that further research is required into risk factors for harmful lead exposure in free ranging mammalian species (Chiverton et al., 2022).

The proportion of devils displaying 'high' lead levels (> 3 mg/kg) was more than twice as high in the plantation landscape (11%) than in the validation study (5%), the latter of which used samples from throughout Tasmania but concentrated in urban areas near Hobart (Hutchinson et al., unpublished results). This is a high enough discrepancy to suggest a real difference between these values even when accounting for limited accuracy of the XRF analysis. This result indicates support for the hypothesis that devils living in modified landscapes subjected to regular culling may be at elevated risk of lead contamination compared to devil populations in unmodified habitats (or at least those from urban areas with minimal shooting activity), as has been found in some other mammalian non-specialist scavengers in areas where hunting occurs (Brown et al., 2022; Kelly et al., 2021). Notably, our study results came from only two sample populations with different locations and time periods, so the discrepancy could be due to other factors that we could not incorporate into the analysis. Further investigation to confirm causes is needed. Regardless, these results highlight the importance of investigating lead levels in local scavenger populations in areas with intensive hunting or culling operations, as the level of risk may not be identified by studies of broader populations.

5. Conclusions

XRF provides a cost effective and field-friendly method of analyzing lead levels in mammal hair. Though the relationship was variable, we found sufficient correlation between lead levels in devil hair derived via XRF and ICP-MS to distinguish between high and low lead concentrations. While ICP-MS remains the best approach to quantifying low lead exposure levels in non-human mammalian hair with a high degree of accuracy, XRF is a viable non-invasive alternative in situations where ICP-MS is impractical, such as measurements in the field and where non-destructive sampling is preferred, as with archived museum specimens. At the least, our preliminary data suggest the method has promise as a screening test to distinguish animals with marked lead exposure from those animals with background lead levels. Our data suggest that investigating devil lead exposure levels in landscapes where culling is regularly conducted would be worthwhile to identify risks arising from use of lead-based ammunition.

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Fig. 1. A wild Tasmanian devil (*Sarcophilus harrisii*). Photo: Evie Jones.

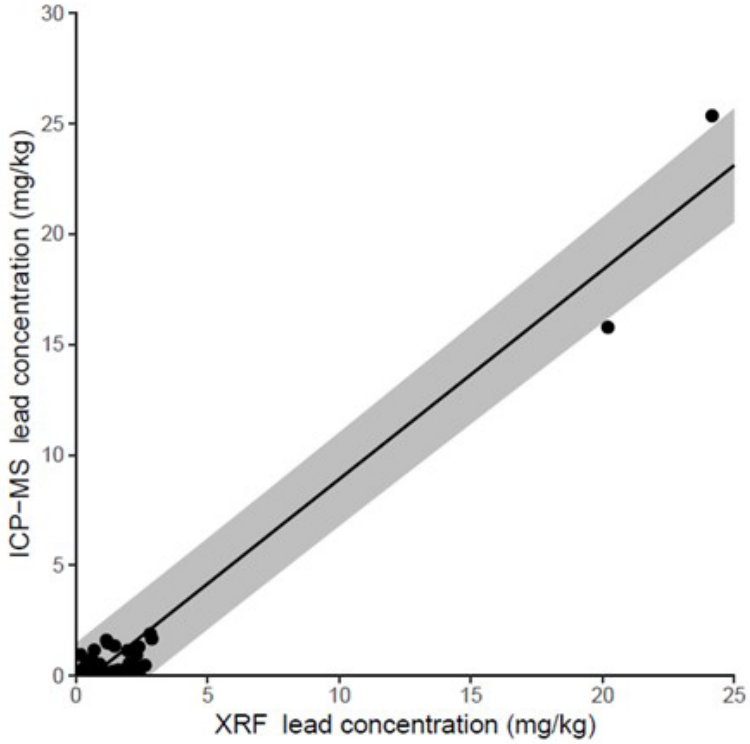

Fig. 2. Correlation between portable XRF and ICP-MS lead concentration measurements in Tasmanian devil (*Sarcophilus harrisii*) hair, including all observed values. Shaded polygon represents the 95% prediction interval.

Table 1. Lead concentration in hair samples from 39 Tasmanian devils (*Sarcophilus harrisii*), estimated using inductively-coupled mass spectrometry (ICP-MS) and portable X-ray fluorescence (XRF). Estimates are partitioned into low (≤ 3 mg/kg) and high (> 3 mg/kg) exposure categories.

	Mean	**Min**	**Max**	**SD**	**% ≤ 3 mg/kg**	**% > 3 mg/kg**
ICP-MS (mg/kg)	1.65	0.12	25.34	4.61	94.87	5.13
Portable XRF (mg/kg)	2.33	0.08	25.16	4.76	94.87	5.13
Portable XRF uncertainty (mg/kg)	0.58	0.20	1.97	0.39	NA	NA

Table 2. Lead concentrations in hair derived from portable XRF and predicted ICP-MS levels from regression analysis for live Tasmanian devils (*Sarcophilus harrisii*), sampled during a trapping program in a forestry plantation landscape (n = 61).

	Mean	**Min**	**Max**	**SD**	**% ≤ 3 mg/kg**	**% > 3 mg/kg**
Predicted ICP-MS (mg/kg)	1.04	-1.88	5.47	1.45	88.52	11.47
Portable XRF (mg/kg)	1.69	-1.39	6.37	1.53	81.97	18.03
Portable XRF uncertainty (mg/kg)	0.85	0.25	2.02	0.45	NA	NA

Table 3. Model ranking testing the importance of Tasmanian devil (*Sarcophilus harrisii*) age and sex in predicting lead levels in devil hair in a forestry plantation landscape. df = degrees of freedom, AIC = Akaike information criterion, ΔAIC = Akaike information criterion difference, AICwt = Akaike information criterion weight.

Model	**df**	**AIC**	**ΔAIC**	**AICwt**
Null	2	221.5	0	0.47
Sex	3	222.8	1.24	0.25
Age	4	223.5	1.96	0.18
Age + sex	5	224.7	3.19	0.10