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Tissue composition and storage duration affect the usefulness of generic wet-to-dry mass conversion factors in toxicology studies

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ABSTRACT

All ecosystems are exposed to a variety of anthropogenic contaminants. The potential threat posed by these contaminants to organisms has prompted scores of toxicology studies. Contaminant concentrations in wildlife toxicology studies are inconsistently expressed in wet or dry mass units, or even on a lipid-normalized basis, but tissue composition is rarely reported, and the conversion between dry and wet mass units, notably, is often based on assumed empirical moisture contents in tissues. However, diverse factors (e.g., tissue, storage conditions) may affect tissue composition and render comparisons between studies difficult or potentially biased. Here, we used data on the concentration of mercury, a global pollutant, in tissues of red foxes (*Vulpes vulpes*) to quantify the effects of diverse variables on moisture and lipid contents, and their consequences on contaminant concentration in different tissues, when converting between wet and dry mass units (lipid extracted or not). We found that moisture content differed largely between organs, enough to preclude the use of a single conversion factor, and decreased by 1% per year when stored at −80 °C. Although most fox tissues had low lipid concentrations, lipid content affected water content and their extraction affected the wet to dry mass conversion factor. We thus recommend reporting tissue composition (at least water and lipid contents) systematically in toxicology studies of mercury specifically and of contaminants in general, and using tissue/species specific conversion factors to convert between dry and wet mass concentration.

1. Introduction

Anthropogenic industrial development resulted in a massive release of a diversity of inorganic and organic contaminants, such as trace elements, polycyclic aromatic compounds, polychlorinated biphenyls, or organochlorines (Rattner, 2009; Thomas et al., 2021). Many of these anthropogenic contaminants can be transported over considerable distances through long-range atmospheric transportation, and ocean and river currents (e.g., Fisk et al., 2005); they may bioaccumulate in organisms and some may even biomagnify throughout food webs (Ali and Khan, 2019). As a result, many of these contaminants have become ubiquitous in worldwide ecosystems. Chemical contaminants can have major effects on natural selection, or disrupt ecosystem functions, because their effects on wildlife communities through mortality events, altered reproductive or immune functions, or altered individual behavior can be strong (Rohr et al., 2006; Saaristo et al., 2018). Reports on their detrimental effects on both wildlife and human health have proliferated since the beginning of the 20th century (Wiener et al., 2002; Rattner, 2009).

In toxicology studies, contaminant concentrations are inconsistently expressed either per wet, dry or lipid mass units (Espín et al., 2016; Cresson et al., 2017), but most toxicology studies do not report the composition of tested tissues, because systematically determining tissue composition adds resource-consuming steps during the analysis process (e.g., Cresson et al., 2017). Especially, if the main topic of a study does not require accurate tissue composition, those additional steps may be deemed unnecessary. Therefore, researchers have adopted diverse strategies to compare their results to published data. Some convert between contaminant concentration per dry and wet mass using species- and/or organ-specific empirical conversion factors (e.g., Denton et al., 1980; Ma, 1989; Siebert et al., 1999; Kalisinska et al., 2012; Eccles et al., 2017), or a general empirical conversion factor (Pugsley et al., 1985). A common assumption is that mammalian soft tissues contain on average 75% moisture (Skelton, 1927; Dainowski et al., 2015; Treu et al., 2018), although other values are sometimes assumed (e.g., 70%) (Eccles et al., 2017; Ziętara et al., 2019). Others measured conversion factors specific

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to their study but do not provide information on how factors such as tissue composition, lab methods, or sample storage may have influenced those values (e.g., Steimle et al., 1990; Aastrup et al., 2000).

The use of empirical factors may generate errors because multiple factors may affect tissue composition and thus contaminant concentration. Tissue composition differs between organs, species, or may vary spatially in a given habitat (Yang and Miyazaki, 2003; Kojadinovic et al., 2006). Lipid content, specifically, may be an important source of moisture variation since water and lipid proportions in tissues are usually negatively related (Cresson et al., 2017), but lipids could also affect contaminant concentration *per se* since different contaminants have different affinities to lipids (e.g., Hoffman et al., 2002). Chilling and freezing (+4 °C to −80 °C) can affect the chemical composition of animal tissues (e.g., Arannilewa et al., 2006; Gandotra, 2012). Yet, biological samples are commonly collected and then stored frozen for multiple years until being analyzed. Long-term storage would thus likely affect at least the moisture content, and therefore comparing contaminant concentration based on wet massfrom samples spanning over multiple years, without knowledge of the dehydration rate, might lead to erroneous conclusions.

Here, we quantified the effects of variations in moisture and lipid contents on total mercury concentration when converting between wet and dry forms (lipid extracted or not) using red fox (*Vulpes vulpes*) tissue samples — its high trophic position and densities for a carnivore species, making it a a good potential sentinel species for monitoring contaminants in the less-studied terrestrial food webs —, as an illustration to quantify the consequences of using empirical factors when converting between wet and dry mass units (lipid extracted or not). We chose mercury because it is a global pollutant that affects ecosystem and human health and is being regulated by the Minamata Convention, a legally binding, international treaty. We also quantified the loss of moisture during long-term storage. Specifically, we quantified 1) composition (lipids and moisture) difference between tissues and 2) deviation from an empirical 75% moisture content, 3) the relationship between moisture and lipid content, 4) the effect of lipid extraction on mercury concentration, and 5) the effect of storage length on moisture content.

2. Methods

2.1. Sample collection and storage

We collected carcasses of 123 red foxes (49 females, 72 males, and 2 foxes of unknown sex; 73% of 103 aged foxes were <1 year old) legally harvested by fur trappers near Churchill, Manitoba, Canada, on western Hudson Bay (58°N, 94°W) from 2012 to 2020. Carcasses were collected by the staff of the Churchill Northern Studies Centre and stored frozen for up to 4 months, until we harvested the tissue samples *in situ*. Each year, we collected a canine tooth (for ageing) and muscle samples from the quadriceps of each fox, avoiding the outer 2–4 mm layer of muscle to exclude any external contamination of the skinned carcasses. In 2018 only, we also collected the entire brain, kidneys, and liver. Samples were brought to the University of Manitoba and archived at −80 °C (muscle) or −20 °C (brain, kidneys, liver). We subsampled livers and brains by taking small pieces from each lobe (after removing 1–2 mm of the tissue surface). We separated renal cortices and medullas since mercury concentration in the cortex and medulla can differ substantially (Dainowski et al., 2015; Warret Rodrigues, 2023), and took small pieces for each renal tissue separately at regular interval along the median craniocaudal axis of the kidney. Analysis of brain, liver and kidney tissues were done in 2019, whereas muscle samples were analyzed in 3 batches in 2019, 2022 and 2023. Therefore, muscle sample storage prior to analysis ranged from 1 to 10 years. Foxes were aged using cementum annuli count at Matson's lab (Manhattan, Montana, USA), note however, that carcasses from 2013 to 2020 have not been aged.

2.2. Determination of tissue composition and levels of mercury

We weighed between 0.087 g and 2.177 g of wet tissue that was freeze-dried for 48 h at −50 °C (collector temperature) using a Labconco FreeZone 2.5 L Benchtop Freeze Dry System freeze dryer. We extracted lipids from the homogenized freeze-dried samples using a Soxhlet apparatus 12 h, with petroleum ether (Ref.: 67,482–540, Anachemia, VWR International, Canada) as solvent (Elliott et al., 2023). We then dried the samples for 72 h in a Fisher Scientific Isotemp® drying oven at 60 °C. Samples were weighed between each step to determine tissue composition.

We measured total mercury concentration (hereafter THg) in the samples at each step (wet, freeze-dried, and dry-lipid-extracted) using the direct mercury analyzer Hydra IIc (Teledyne Leeman Laboratories, Hudson, NH) at the Centre for Earth Observation Science, University of Manitoba. Hydra IIc is an automated spectrophotometer that analyzes mercury concentration directly in solid samples by thermal decomposition (all forms of mercury are pyrolyzed to Hg (0) in the presence of a catalyst), followed by amalgamation (Hg (0) is collected onto a gold trap), and detection by atomic absorption spectroscopy, following U.S. EPA Method 7473 (US U.S. EPA, 1998). This instrument requires no sample digestion. Hydra IIc calibration curves were generated using the solid certified reference materials (CMR) MESS-3 (marine sediment, mercury concentration = 91 ± 9 ng/g), MESS-4 (marine sediment, 90 ± 40 ng/g), and PACS-3 (marine sediment, 2980 ± 360 ng/g). The calibration curve was validated by running several certified reference standards to ensure accuracy of the full range of the curve. Throughout analysis, quality assurance and quality control were maintained by running the CRM MESS-3, MESS-4, TORT-2 (lobster hepatopancreas, 270 ± 60 ng/g), TORT-3 (lobster hepatopancreas, 292 ± 22 ng/g), DORM-4 (fish protein, 412 ± 36 ng/g), DOLT-5 (dogfish liver, 440 ± 180 ng/g ng/g), NIST 2709a (soil, 900 ± 200 ng/g), NCP III-9 S^2 (fish muscle, 933 ± 55.5 ng/g), and PACS-3, two to three times every 14 samples (see Table S2 for details on accepted CRM runs). If a CRM concentration was outside the certified range, we ran up to two more CRMs. If the extra CRM were still outside the certified range, we recalibrated the Hydra IIc.Most samples were replicated 2 to 5 times and we averaged the replicates (average coefficient of variation: $CV_{wet} = 0.08$, $CV_{dry} = 0.06$, $CV_{dry\text{-}lipid\text{-}extracted} = 0.03$; number of samples replicated: $n_{wet} = 165$, $n_{dry} = 126$, $n_{dry\text{-}lipid\text{-}extracted} = 38$). However, ~20% of our samples were presented without replicate data due to low sample amount (mostly dry and dry-lipid-extracted) or replicates being discarded after a quality control check. Based on measured mercury content (ng/g) in wet and freeze-dried samples we calculated an empirical dry and wet mass mercury content respectively, using a 75% moisture content (e.g., Eccles et al., 2017; Treu et al., 2018) to illustrate the impact of assuming approximate empirical tissue composition. We quantified the difference between measured and empirical mercury contents in all tissues, but did not perform an inferential statistical test to characterize these differences, because the importance of a particular difference depends on each study goal and context, and providing a p-value in that context may be misleading.

2.3. Statistical analyses

We performed data exploration and all statistical analyses using the packages car v.3.0.7 (Fox and Weisberg 2019), nlme v.3.1.147 (Pinheiro et al. 2020), lme4 v.1.1.23 (Bates et al. 2015), lmerTest v.3.1.2 (Kuznetsova et al. 2017), glmmTMB v.1.1.7 (Brooks et al. 2017), esc v.0.5.0 (Lüdecke 2019), DHARMa v.0.4.6 (Hartig 2022), multcomp v.1.4–13 (Horntorn et al. 2008), effsize v.0.8.1 (Torchiano 2020), Hmisc v.4.4–0 (Harrell 2020), RVAIdememoire v.0.9–75 (Hervé 2020), dplyr v.0.8.5 (Wickham et al., 2020A), tidyr v.1.0.2 (Wickham et al., 2020B), fitdistrplus v.1.1.1 (Delignette-Muller and Dutang 2015), rstatix v.0.5.0 (Kassambara, 2020), and base R v.4.0.0, 4.1.1 and 4.2.3 in the R software (R Core Team 2020). Graphs were produced using the ggpubr

v.0.3.0 and ggplot2 v.3.3.0 package. We checked data sets for outliers, deviation from normality, and homoscedasticity by inspecting the residual vs fitted values plots and the QQ plot of residuals (Zuur et al. 2009, 2010), and dealt with potential issues as we built and validated models using the guidelines in Zuur et al. (2010) and Zuur and Ieno (2016). Points with a Cook's distance >0.7 were considered outliers with high leverage and reviewed individually. We excluded all outliers with high leverage because they likely arose from instrument-related issues or abnormal sample degradation (Mcdonald 2002; Zuur and Ieno 2016). Our results are reported as mean ± SE unless otherwise indicated.

We tested if sex and age influenced water or lipid content by running two GLMMs (family beta, link logit), controlling for fox ID as a random effect because some samples were run multiple times at different years (i.e., we have a total of 137 data points coming from 123 different foxes). In addition to the variables of interest (i.e., sex and age), we controlled for the effect of storage length and lipid proportion on proportion of water and allowed for the variance in water proportion to vary over storage time, and for the effect of year of collection on proportion of lipids. The residuals of these two models showed no pattern, and no outlier with high leverage. We couldn't directly compare models with and without age and sex using AICc due to missing data without losing information and statistical power (e.g., Symonds and Moussalli, 2011). Because sex and age did not affect tissue composition, we dropped these variables from further analyses, to take advantage of our full data set.

To determine if moisture content differed among organs, we fitted a generalized least squares model and allowed the variance to differ between organs using the varIdent function of the nlme package (Zuur et al., 2009) and a posthoc Tukey pairwise comparison. We used a series of one-sample two-sided t-tests with a 99% confidence interval ($\alpha = 0.01$) to assess if the moisture content of each organ deviated from 75%. We reported effect sizes as Hedges' *g* (Hedges, 1981). We calculated the percent error associated with converting from dry to wet mass assuming an empirical value of 75% (and thus a conversion factor of 4) as:

$$\%\text{Error} = \frac{\left|\left([Hg]d_{measured}/4\right) - [Hg]w_{measured}\right|}{[Hg]wmeasured} \times 100$$

where $[Hg]d_{measured}$ is the mercury concentration per tissue-mass (dry) and $[Hg]w_{measured}$ the mercury concentration per tissue-mass (wet), and repeated the procedure to simulate a conversion from wet to dry mass We tested if these percent errors between observed and calculated mercury concentrations were affected by water or lipid content using a gamma GLM (link = log) for each conversion direction. Subsetting muscle, we tested if water proportion affected the percent error between observed and calculated mercury concentrations for both conversion directions.

We determined the strength of the correlation between lipid and moisture contents using a non-parametric Spearman's rank correlation test for each organ. We then examined how much of the moisture content was explained by lipid content and assessed the effect of lipid extraction on the dry to wet mass conversion factor using two GLMMs (family Gaussian, link identity). Since we were only interested in the general fixed effect of lipid proportion on the moisture proportion and of lipid extraction on the conversion factor, we ran our GLMM using "organ" and fox ID as crossed random effects (each fox ID appeared in all or multiple organs). We allowed the intercept to vary in the crossed random effects (fox ID and organ), but not the slope. We determined if the lipid content differed between organs by fitting a linear mixed model controlling for fox ID and a post-hoc Tukey pairwise comparison.

Finally, we assessed the possible effects of long-term storage (up to 10 years) on the water content of muscle samples by running a GLMM (family beta, link logit), controlling for fox ID as a random effect because some samples were run multiple times at different years (i.e., we have a total of 137 data points coming from 123 different foxes), and allowing for the variance in water proportion to vary over time. We also added lipid proportion because of its large partial effect on water proportion (e.g., Zuur and Ieno, 2016). We also ran a beta GLMM to test if storage length affected the proportion of lipids, controlling for fox ID and including year to account for the effect of yearly resource fluctuation on fox diet and thus on their body fat. We report no issues with our model validation, our residuals showing no pattern, and no outlier having high leverage.

3. Results

The average percent moisture of tissues ranged from 70.8 ± 0.6% to 78.3 ± 0.8%, with renal cortex and medulla showing the most extreme values (Fig. 1 and Table 1). The moisture level of brain and muscle were the closest to the empirical 75%, yet moisture content in all tissues diverged from that empirical 75% (Fig. 1 and Table S1). Moisture content also differed between brain or renal medulla *versus* liver, muscle and renal cortex (Table 2), with medium to large effect sizes (Hedges, 1981) ranging from $d_{muscle} = -0.49$ to $d_{renal\ cortex} = 1.25$, leading to mean conversion factors ranging from 2.9 to 4.9 (see Table 1). The percent errors associated with the use of a 75% empirical factor for muscle and brain were low (−1.98% and 4.09% respectively) but was >20% for all other tissues (Fig. 2). Measured mean THg per tissue and mean THg converted between wet and dry mass (both directions) with an empirical factor of 4 are provided in Table 3. Applying an empirical conversion factor of 4 (assuming 75% moisture content in all tissues) inflated or decreased by 8%–50% the converted estimate of mercury level compared to the measured one (Table 3). THg in dry-lipid-extracted brain samples (a lipid-rich tissue) was 72% higher compared to THg converted from wet to dry mass, while for other tissues the difference between measured THg in freeze-dried lipid-extracted samples and wet to dry mass converted THg ranged from 1% to 38% (Table 3). Lipid content affected the percent error when converting from dry to wet ($t = 2.30$, $df = 142$, $p = 0.023$), but water proportion did not ($t = 0.12$, $df = 142$, $p = 0.91$). When converting from wet to dry, the reverse was true, water proportion did affect the percent error ($t = 2.99$, $df = 142$, $p = 0.003$) while lipid content did not ($t = 1.82$, $df = 142$, $p = 0.071$). Water content of muscle stored for up to 10 years did affect the percent error whether converting from wet to dry ($t = 2.03$, $df = 96$, $p = 0.046$) or from dry to wet ($t = 3.02$, $df = 96$, $p = 0.003$).

The strength of the correlation between moisture and lipid content, however, varied greatly across organs (Fig. 3), from no correlation in the renal cortex ($r_s = -0.03$, $n = 32$) to moderately correlated in the brain ($r_s = -0.46$, $n = 29$). Most organs had similarly low lipid contents, ranging from 2.18 ± 0.40% to 2.92 ± 0.18%, except the brain for which lipid content was higher 7.97 ± 0.49% (GLMM (brain as reference level): $t = [-9.25, -10.04]$, $df = 101$, $p < 0.001$, $n = 160$; Table 2). Lipid content significantly affected moisture content after controlling for fox ID and tissue type (GLMM: $t = -5.84$, $df = 157.74$, $p < 0.001$, $n = 160$). Lipid extraction significantly changed the wet:dry conversion factor (GLMM: $t = 10.37$, $df = 260.22$, $p < 0.001$, $n = 313$).

We found a significant decrease of 1% water per year in moisture content over time in storage (GLMM: $z = -2.88$, $p = 0.003$, $n = 135$; $e^{-\beta} = 1.01$ CI = [1.00–1.02]; random effect var. ± SD = 0.003 ± 0.06). The lipid content, however, was not affected by storage length (GLMM: $z = 0.96$, $p = 0.336$, $n = 135$; random effect var. ± SD = 0.23 ± 0.47), but depended on the year of collection (GLMM, overall effect of year: $\chi^2 = 14.63$, $p = 0.02$). Sex and age did not influence tissue composition (GLMM: $z_{sex} = 1.52$, $p_{sex} = 0.129$; $z_{age} = 1.20$, $p_{age} = 0.231$; $n = 112$).

4. Discussion

Moisture content varied greatly among tissues. Although we only tested measured conversion factors against one empirical conversion factor of 4 (assuming 75% moisture content), these differences between organs regarding their water composition should preclude the use of a single conversion factor, no matter which one. The level of error

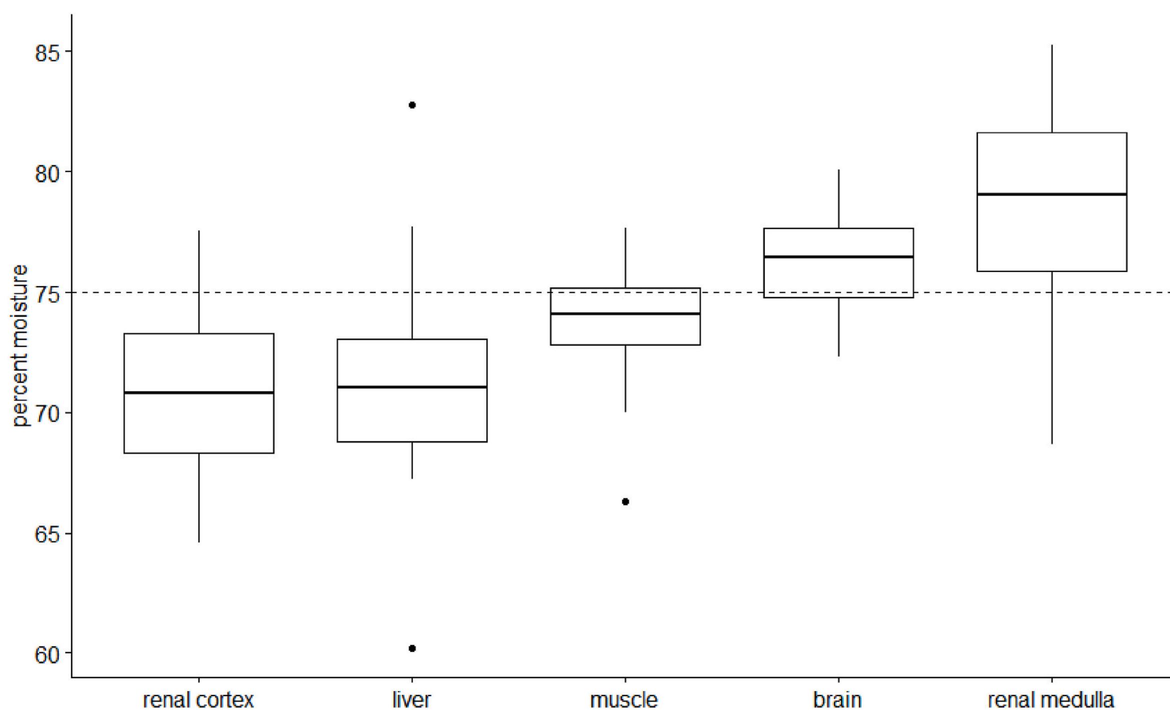

Fig. 1. Organ-specific moisture content from red fox tissues (box plot indicating median and quartiles). Dashed line indicates the most common empirical average moisture content for mammalian soft organs (75%).

Table 1
Water and lipid content in tissues of red fox and associated wet-to-dry conversion factor (w:d CF = wet to dry Conversion Factor, w:LE CF = wet to lipid-extracted conversion factor). Foxes were trapped during winter from 2015 to 2018 in northern Manitoba, Canada.

Tissue	% moisture	n	se	% lipids	n	se	w:LE CF	n	se	w:d CF	n	se
Renal cortex	70.82	32	0.58	2.90	32	0.17	3.83	32	0.12	3.15	32	0.11
Liver	71.45	28	0.79	2.65	29	0.23	3.80	28	0.09	2.92	28	0.13
Muscle	73.92	38	0.35	2.92	38	0.18	4.34	38	0.09	3.92	38	0.11
Brain	76.07	29	0.38	7.97	29	0.49	7.73	29	0.21	4.16	29	0.14
Renal medulla	78.34	32	0.76	2.18	33	0.40	5.56	32	0.29	4.77	32	0.25

Table 2
Moisture and lipid difference between tissue pairs with z statistic and level of significance using samples of red foxes from northern Manitoba, Canada.

contrasted tissues	Difference in moisture content				Difference in lipid content			
	estimate	se	z value	Pr (>|z|)	estimate	se	Z value	Pr (>|z|)
Renal cortex - Brain	−5.26	0.69	−7.62	<0.001	−5.15	0.52	−10	<0.001
Renal medulla - Brain	2.21	0.85	2.61	0.063	−5.87	0.63	−9.25	<0.001
Liver - Brain	−4.57	0.87	−5.23	<0.001	−5.43	0.54	−10.04	<0.001
Muscle - Brain	−2.86	0.50	−5.73	<0.001	−5.15	0.56	−9.27	<0.001
Renal medulla - Renal cortex	7.47	0.95	7.85	<0.001	−0.72	0.41	−1.74	0.16
Liver - Renal cortex	0.69	0.98	0.71	0.952	−0.27	0.25	−1.11	0.39
Muscle - Renal cortex	2.40	0.66	3.64	0.002	0	0.28	−1.01	0.99
Liver - Renal medulla	−6.78	1.09	−6.21	<0.001	0.45	0.45	1	0.4
Muscle - Renal medulla	−5.07	0.82	−6.15	<0.001	0.72	0.47	1.54	0.21
Muscle - Liver	1.71	0.85	2.01	0.246	0.27	0.33	0.84	0.45

generated by using a single conversion factor across all soft internal tissues may cause substantial error (over 20% in most organs of our study for using an empirical conversion factor of 4), which may greatly affect comparisons within or between studies. For example, we found that measured THg values differed by 8%–50% compared to converted THg values, and by 72% in the brain when comparing THg in dry-lipid-extracted samples to converted dry mass. Tissue composition did affect the percent error we obtained when converting between wet and dry mass. The discrepancy between true and assumed tissue composition could then lead to inaccurate conversions, increasing with lipid content when converting from dry to wet, and increasing with water content when converting from wet to dry. The use of empirical conversion factors may, thus, lead to erroneous conclusions, with important consequences on subsequent management politics, particularly where public and environmental health are concerned (Cresson et al., 2017; Soerensen et al., 2023).

Although measuring tissue composition may be time-consuming, we strongly support and extend to terrestrial species Yang and Miyazaki's (2003) recommendation that researchers should report moisture content when studying trace element accumulation, at least using a sub-sample of their total sample batch. The difference in moisture between tissues was partly due to a negative relationship between moisture and lipid

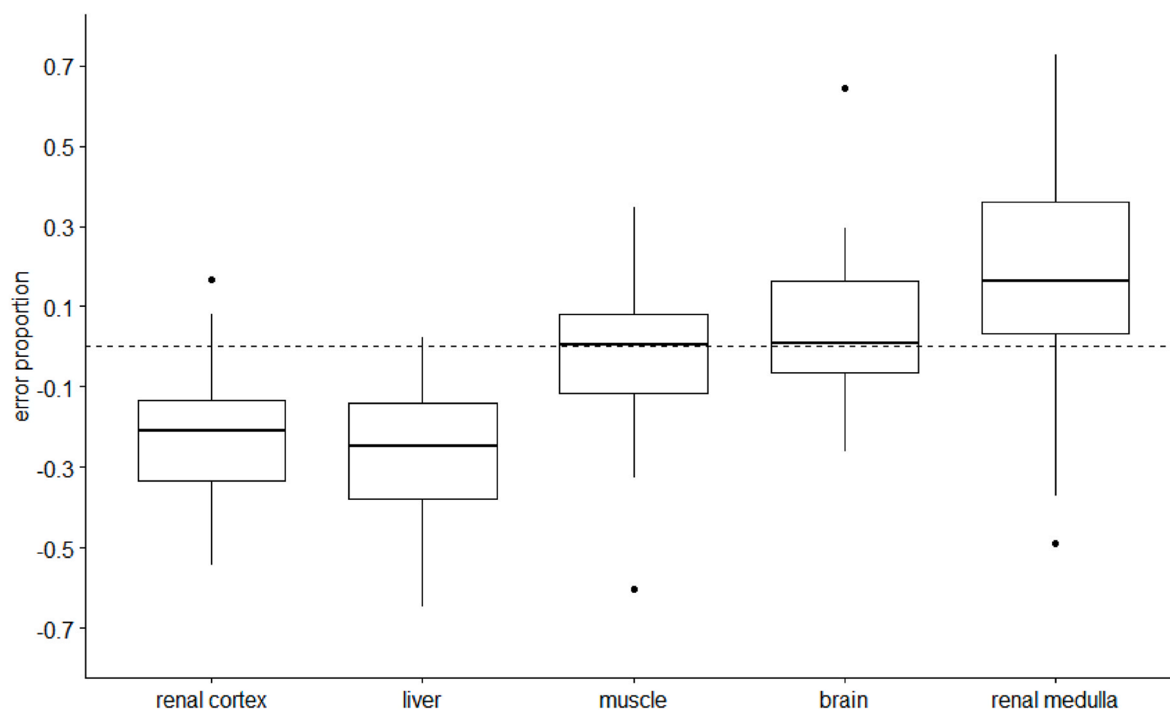

Fig. 2. Error associated with the use of a unique empirical moisture content (here 75%), when converting from dry to wet weight (box plot indicating median and quartiles). Dashed line indicates error = 0.

Table 3
Average mercury content measured in ng/g ("meas.") in different tissues of red foxes from northern Manitoba, Canada, and average empirical mercury content (ng/g) converted from mercury per dry mass unit (Hg converted dry:wet) or wet mass unit (Hg converted wet:dry) using an empirical conversion factor of 4 (i.e., assuming 75% moisture content). We provide mercury content in freeze-dried lipid-extracted samples to illustrate the effect of lipid extraction on mercury concentration.

Tissue	Hg wet (meas.)		Hg converted dry:wet		Hg dry (meas.)		Hg converted wet: dry		Hg lipid extracted		
	mean	se	mean	se	mean	se	mean	se	mean	se	n
renal cortex	473.99	68.40	380.85	61.64	1523.41	246.56	1895.96	273.61	1875.80	302.72	32
Liver	283.62	56.73	188.54	35.97	754.17	143.87	1134.48	226.93	1007.93	180.97	28
muscle	107.87	18.85	99.09	17.43	396.37	69.71	431.50	75.39	469.18	87.02	38
Brain	60.98	11.93	56.27	9.24	225.07	36.97	243.94	47.70	418.59	71.57	29
renal medulla	161.47	33.27	190.72	39.07	762.88	156.29	645.90	133.08	891.91	175.89	33

content. Such an inverse relationship between moisture and lipid content was also evidenced in fish and mammals (Cresson et al., 2017; Liwanag et al., 2012). However, in our study, the correlation between moisture and lipid content was weak for most organs and negligible in some (notably renal cortex and muscle). The weak pattern or absence thereof may be due to the generally low lipid content of fox internal tissues and the particularly low variation of lipid content in some specific tissues (renal cortex and muscle; Table 1); but lipid content, type, and lipid metabolism can strongly drive the biodistribution (and thus toxic effects) and toxicokinetics of lipophilic contaminants (e.g., Beckmen et al., 1999; Debier et al., 2003; Peterson et al., 2014). For example, in grey seals (*Halichoerus grypus*), as the blubber layer is depleted over the course of lactation, it becomes less able to retain PCBs, which leads to higher serum concentrations of this harmful contaminant (Debier et al., 2003). We thus recommend that studies provide the percent lipid in tissues, especially when working with lipid-rich tissues and lipophilic contaminants. Using tissues with a larger range of lipid content and accounting for the different types of lipids would provide a better understanding of the relationship between lipid, moisture content and wet to dry mass conversion factor.

The percent moisture we report differs somewhat from moisture contents reported elsewhere for red fox livers and muscles (Kalisinska et al. 2009; 2012). Our study intended to represent the fate of samples collected in the field in classic ways encountered in many ecology long-term projects. In that context, variability in tissue composition and how this composition affects levels of contaminants may arise because of factors researchers cannot control for. For example, in our case, we could not control the conditions in which carcasses were kept before we received them. The differences between the percent moisture in our red foxes, and those from other studies could be due to sample-storage conditions (from the field to the archive of a lab), material (e.g., freezer quality), or laboratory methods (Binkowski, 2012). Further research is thus required to understand the relative effects of these variables on tissue composition determination, and how they may affect the relationships we have determined. In addition, we treated renal cortices and medullas separately due to the large anatomical and physiological differences of these kidney tissues and the fact that they accumulate mercury differently (Dainowski et al., 2015, Chapter 5), and found that although lipid content was relatively similar, moisture content differed largely between them. Renal medulla and cortex should be treated separately.

We neither found a difference between sexes nor an effect of age on tissue composition, but our age data were biased towards juveniles and sex ratio was unbalanced for some years (Warret Rodrigues et al., 2023), which may have lowered the statistical power of these variables and prevented us from detecting an effect. Diet, age, sex and reproductive status among other factors can at least partially explain tissue composition, specifically lipids (Lockyer et al., 1985; Cobos and Díaz, 2015).

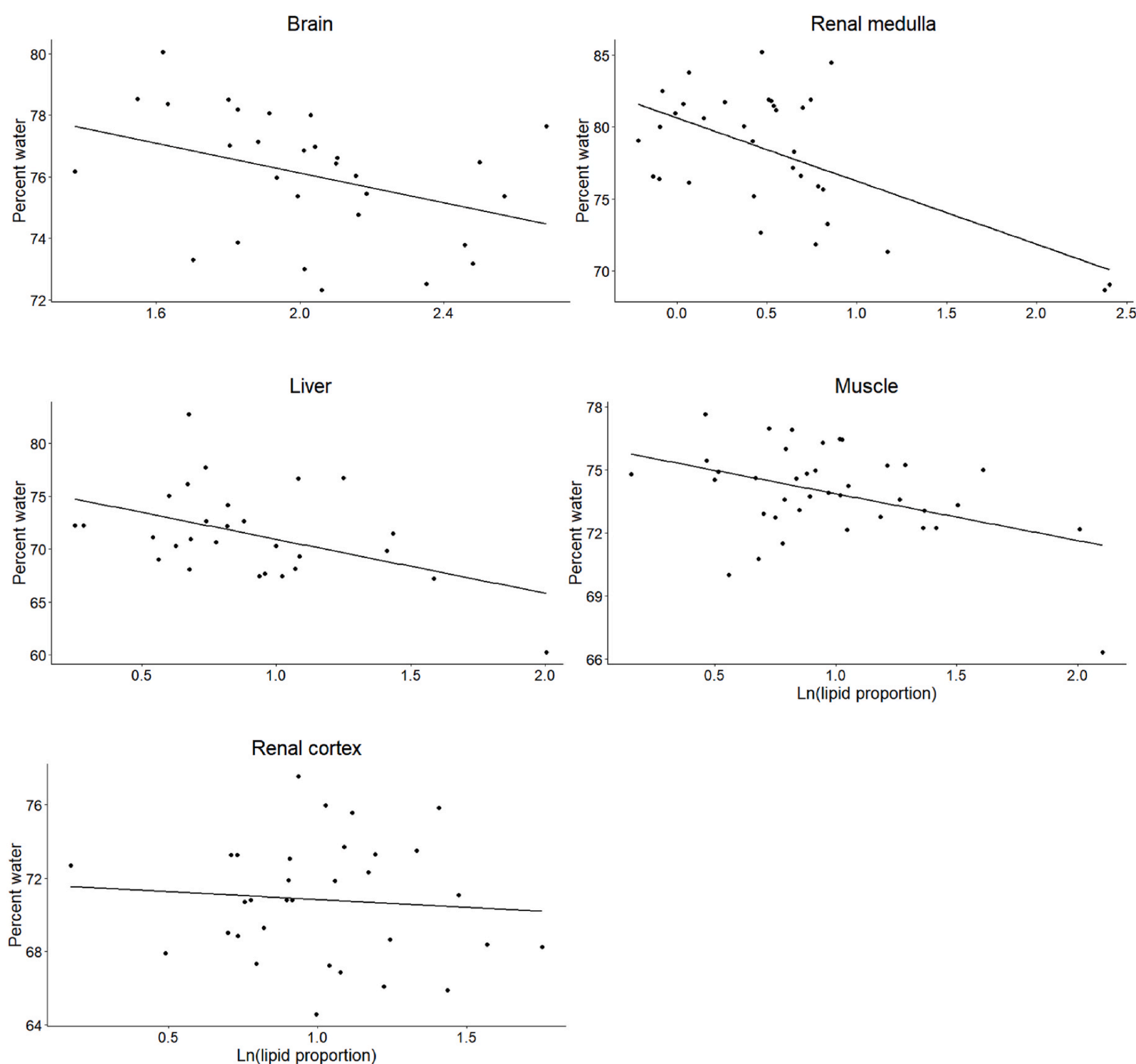

Fig. 3. Scatter plot of the correlation between moisture and lipid content for brain (r = −0.46, n = 29), renal medulla (r = −0.34, n = 33), liver (r = −0.37, n = 28), muscle (r = −0.025, n = 38) and Renal cortex (r = −0.03, n = 32) of red foxes trapped in northern Manitoba, Canada.

Individual traits should, thus, always be considered during exploratory analyses at least, since they may affect tissue composition.

Finally, we found an important effect of storage time on tissue composition, with a yearly loss of 1% moisture. Freezing, freeze-thaw cycles and frozen storage all negatively affect the water-holding capacity of muscle tissue by disrupting fiber and changing protein structures, thus contributing to moisture loss over time (Leygonie et al., 2012; Zhang et al., 2017). It is, thus, unsurprising that the longer samples remain frozen, the more likely moisture loss will be large enough to produce detectable changes in solute concentration, including contaminants (Zhang et al., 2017). In addition, changes in the myofibrillar structure are already detectable after one freeze-thaw cycle (Zhang et al., 2017). As frozen-storage time increases, the opportunity for samples to undergo freeze-thaw cycles also increases (e.g., sample relocation for material maintenance, researchers taking samples out for subsampling for research needs), which may exacerbate the loss of moisture in samples. The change in moisture content over time is likely to preclude direct comparisons of trace element concentration in tissues between years. Therefore, we believe it a better practice to work on dry tissues when working with multi-year data, or at least researchers should measure the composition of their tissues and apply a correction factor accounting for water loss.

5. Conclusion

We provide evidence that diverse factors, including some related to standard laboratory practices, may affect tissue composition, thus generating sources of variation that may greatly affect conclusions regarding trace element and contaminant accumulation in wildlife tissues. Most health guidelines are expressed on a wet mass basis (Cresson et al., 2017), yet water content may vary within species, for example due to storage condition, therefore values may not be directly comparable. Dry mass analyses should be standard, but we, at least, advise systematically reporting tissue composition to minimize discrepancy due to water loss during storage. Although some studies may not need a level of precision that warrants detailed knowledge of tissue composition (e.g., low level of contamination, low precision of lab equipment, comparison of tissue with substantial differences in contaminant concentration), tissue composition should ideally always be calculated (at least using a subsample of analyzed tissues) and reported regardless, as other studies may require this information for meaningful comparisons.

Author contributions

C.W.R conceived the idea and designed the methodology with

support from J.D.R.; C.W.R. led data collection with support from F.W., D.A. and J.D.R.; D.A. supervised the mercury analyses. J.D.R. and F.W. obtained the funding; C.W.R. analyzed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116727.

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