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**Title: Mineral analysis of complete dog and cat foods in the UK
and compliance with European guidelines**

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1 **Abstract**

2 The mineral content of complete pet food is regulated to ensure health of the companion animal
3 population. A comprehensive analysis of adherence to these regulatory guidelines has not been
4 conducted. We measured mineral composition of a range of complete wet (n=97) and dry (n=80)
5 canine and feline pet food sold in the UK to assess compliance with EU guidelines. While a majority
6 of foods complied with ≥ 8 of 11 guidelines (99% and 83% for dry and wet food, respectively), many
7 failed to provide nutritional minimum (e.g. Cu, 20 % of wet food) or exceeded nutritional maximum
8 (e.g. Se, 76% of wet food). Only 6% (6/97) of wet and 39% (34/80) of dry food were fully compliant.
9 Some foods (20-30% of all analysed) had mineral imbalances such as not having the recommended
10 balance of Ca:P (between 1:1 to 2:1). Foods with high fish content had high levels of undesirable metal
11 elements such as arsenic. The study highlights broad non-compliance of a range of popular pet foods
12 sold in the UK with EU guidelines (95% and 61% of wet and dry foods, respectively). If fed exclusively
13 and over an extended period, a number of these pet foods could impact the general health of
14 companion animals.

15

1 Introduction

2

3 Companion animals, particularly dogs and cats, have requirements for essential minerals that need
4 to be supplied in their food (NRC, 2006). In the UK, wet pet foods (e.g. tins, pouches) are fed to 41%
5 of dogs and 77% of cats (PFMA, 2016), usually together with treats, snacks (including table scraps) or
6 other complementary foods. Thus, more than half of the companion animal population only consume
7 the main ration provided by their owners, with the remainder consuming a mixed, likely nutritionally-
8 unbalanced diet. In a pan-European Harris poll, 56% of UK pet owners bought branded pet foods,
9 higher than any other market in Europe (Grocer, 2010). Brand loyalty often results in the same
10 product being fed exclusively to the pet for long periods of time (months to years). It is therefore
11 imperative that such branded wet and dry foods, labelled as 'complete' are nutritionally replete and
12 balanced for macro- and micronutrients including sufficient, but not excessive, essential mineral
13 elements.

14

15 In countries of the European Union, guidelines established by the Fédération Européenne De
16 l'industrie des aliments pour Animaux Familiars (FEDIAF, the European Pet Food Industry Federation)
17 provides pet food companies with recommended concentrations of macronutrients (e.g. crude
18 protein, fat), micronutrients (e.g. specific vitamins and minerals) and amino acids (e.g. arginine,
19 lysine) (FEDIAF, 2013). Levels of incorporation or 'daily allowance' within a diet for each nutrient are
20 defined as *'the level of intake of a nutrient or food component that appears to be adequate to meet
21 the known nutritional needs of practically all healthy individuals. It reflects the minimum requirement
22 plus a safety margin for differences in availability between individual animals and for nutrient
23 interactions. In practice this would be translated as the levels of essential nutrients that healthy
24 individuals should consume over time to ensure adequate and safe nutrition'*. Such foods should
25 therefore have the correct proportions of essential macro- and micronutrients that are sufficient for
26 a daily ration (EU Regulation No 767/2009; art. 3(i), adapted to pet food) with a daily ration satisfying
27 all of an animal's energy and nutrient requirements without further complementary intake. These EU
28 guidelines were largely based upon the original National Research Council recommendations in the
29 USA, but for many minerals there is no specific recommendation and no 'safe upper limit (SUL)', i.e.
30 above which the micronutrients can become toxic (NRC, 2006). For toxic elements such as arsenic,
31 cadmium, mercury and lead, further legislation regarding acceptable concentrations in foods are
32 regulated by EU directive 2002/32/EC.

33

1 To our knowledge only one previous study, conducted in the USA, assessed total mineral content of
2 45 dry foods for dogs with reference to American recommendations (AAFCO; (Gagne et al., 2013)).
3 Thirteen percent did not comply with AAFCO guidelines for certain minerals and many diets had
4 excessive calcium. Long-term feeding of un-balanced or of diets deficient or excessive in certain
5 minerals can have adverse impacts on animal health (Davies, 2016, McDowell, 1992). For this reason,
6 we assessed, for the first time in the European Union, mineral composition of a broad range of dry
7 and wet foods for consumption by domestic dogs or cats. We referenced composition against 11 o
8 13 current EU guidelines for minerals (FEDIAF 2013). Briefly, we find widespread non-compliance;
9 only 6% and 39% of wet and dry foods, respectively complied with 11 of 13 FEDIAF guidelines. Many
10 individual products were mineral imbalanced (e.g. Ca:P ratio as low as 0.3:1 or as high as 3:1) or had
11 high concentrations of individual minerals (e.g. selenium $\geq 300 \mu\text{g } 100\text{g DM}^{-1}$; arsenic $\geq 1.1 \text{ mg } 100\text{g}$
12 DM^{-1}) that if fed exclusively for many months could impact the general health of companion animals.
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1 **Materials and Methods**

2 **Ethics:** This study was approved by the University of Nottingham School of Veterinary Medicine and
3 Science Research Ethics Committee.

4

5 **Selection of Pet foods:** Pet foods were bought locally from a range of commercial suppliers and pet
6 food supermarkets and were packaged in a mixture of sealed tins, pouches, cans or sealed bags. A
7 total of 177 different pet foods were included for analysis, all labelled as 'Complete'. The foods were
8 selected as representative of popular brands sold in the UK and including a range of main flavours
9 (e.g. beef, chicken, fish) largely aimed at adult pets (139 of 177) but also young (n=14) or senior (n=24)
10 animals. In total, 42 different brands were represented (dry food, n=21 brands; wet food, n=27
11 brands) with 113 products for cats (wet food, n=48; dry food, n=65) and 64 products for dogs (wet
12 food, n=49; dry food, n=15). Key nutritional information provided on the label was recorded such as
13 macronutrient content (percentage protein, fat, moisture, ash and fibre, as fed) alongside the country
14 of origin and batch number. Each product was designated as either a 'supermarket own-brand' food
15 or a 'prescription/veterinary/therapeutic' diet based upon the label and target population. Other
16 than these categories, all other products were designated as a 'standard' feline or canine food. Energy
17 content (gross, digestible, metabolic) was rarely provided on the label and was therefore calculated
18 using modified Atwater criteria ([Equations 1-7; Supplementary Information](#)). The concentration of
19 minerals added to each diet was also recorded, if provided (e.g. additional zinc, copper and iron; all
20 as µg/kg). Further information on the composition of the food was derived according to the
21 predominant labelling and checked according to the order of primary ingredients, e.g. main protein
22 source being beef, chicken or fish.

23

24 **Assessment of compliance:** Complete foods should have, as defined by the EU, the correct
25 proportions of essential macro- and micronutrients that is sufficient for a daily ration (EU Regulation
26 No 767/2009; art. 3(i), adapted to pet food). The daily ration should satisfy all of an animal's energy
27 and nutrient requirements without further complementary intake (FEDIAF guidelines for dogs in
28 [Table S1](#)). These guidelines were largely based upon the original National Research Council minimum
29 recommendations in the USA ([Table S2](#)). Nevertheless, for many minerals there is no specific
30 recommendation and no 'safe upper limit (SUL)', i.e. above which the micronutrients can become
31 toxic (NRC, 2006). In the United States of America, pet food manufacturers also receive guidance from
32 the American Association of Feed Control Officials for maximum concentrations in pet food ([Table S3;](#)
33 ([AAFCO, 2015](#))). In the EU, aside from guideline quantities of macronutrients (crude protein, fat, ash

1 etc...) there are thirteen specific recommendations for macrominerals (calcium, phosphate, Ca:P,
2 potassium, sodium, chloride and magnesium) and trace minerals (copper, iodine, iron, manganese,
3 selenium and zinc). In addition, recommended maximum levels of 'undesirable' or heavy elements
4 (arsenic, cadmium, mercury and lead) in pet food are regulated by EU directive 2002/32/EC (**Table**
5 **S4**).

6
7 **Preparation of samples for elemental analysis:** To avoid trace ion contamination of the samples,
8 plastic utensils were used when preparing all food stuff and gloves were worn at all times. A known,
9 representative quantity (100-200g) of wet food (for small pouches and tins, all contents) or dry food
10 was first opened then emptied into a 250ml solvent-resistant container (Sarstedt, UK). Contents were
11 then freeze dried for 2 days and re-weighed to establish moisture content by difference. For larger
12 cans and dishes of wet food ($\geq 500\text{g}$), the whole sample was pre-mixed and a representative sample
13 (100-200g) was obtained for freeze-drying. Dried foods were ground to a consistent powder and
14 duplicate samples (100-200 mg) of this powder were acid-digested using standard techniques for
15 mineral analysis by inductively-coupled plasma mass-spectrometry (ICP-MS), a method widely
16 considered the gold-standard for mineral analyses of various fluids, tissues and other biological
17 composites such as foodstuffs (Goullé et al., 2005, Joy et al., 2015). In brief, after 1 hour incubation
18 in 3.0ml of 68% trace analysis grade (TAG) HNO_3 , 2.0ml 30% H_2O_2 (both Fisher Scientific UK Ltd,
19 Loughborough, UK), and 3.0ml milli-Q water (18.2 $\text{M}\Omega\text{ cm}$), acid-digestion was accelerated by
20 microwave-heating (Anton-Paar, 2000) for 45mins in 3.0 ml of 70% Trace Analysis Grade (TAG) HNO_3 ,
21 2.0 ml H_2O_2 and 3.0 ml milli-Q water (18.2 $\text{M}\Omega\text{ cm}$) (Fisher Scientific UK Ltd, Loughborough, UK). The
22 resulting solution was diluted to 15ml with milli-Q water. To control for unexpected sources of
23 contamination (e.g. in acids or water) duplicate blank tubes were run with each batch (containing all
24 liquids but no sample). Additionally, for every batch (to control for batch-to-batch variability) and/or
25 every 60 ICP-MS tubes (control for within-run drift), duplicate samples of a certified reference
26 material (CRM) were included. For all food digests, the CRM was NIST (National Institute of Standards
27 and Technology, Gaithersburg, MD, USA) 1577c (bovine liver). All single batch data for individual
28 elements were corrected to percentage recovery of the CRM (**Table S5**). Minimal between-batch
29 variability was accounted for in statistical models by including BatchID as a random effect. Data for
30 minerals not included on 1577c and thus not subjected to internal quality control were measured,
31 but are not reported (B, Li, Be, Al, Ba, Tl and U). Percentage recovery for nickel was relatively low and
32 is also not reported.

33

1 **Elemental analysis by ICP-MS:** Elemental analysis was by inductively coupled plasma-mass
2 spectrometry (ICP-MS; iCAPTM Q, Thermo Fisher Scientific Inc., Waltham, MA, USA) using a He
3 collision cell with 'kinetic energy discrimination' to reduce polyatomic interference in the analysis of
4 Ag, Al, As, B, Ba, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, S, Sr, Tl, U, V and Zn. Lithium,
5 Be and P were determined in standard (vacuum) mode and Se in 'hydrogen-cell' mode, with 'in-
6 sample switching'. Internal standards were Ge, Rh and Ir. Final foodstuff elemental composition is
7 presented after correction for blanks and batch variation (using the CRM as reference) as ppm (e.g.
8 major elements, 1ppm = 1mg/kg), ppb (e.g. trace elements, 1ppb = 1µg/kg) or per 100g of dry matter
9 [DM] as indicated in the text. For major and trace elements, recovery was >95% with <10% coefficient
10 of variation for each (n=17 separate analyses). Intra-assay variability for all elements was <2%.
11 Beryllium (Be), Lithium (Li), Silver (Ag), Titanium (Ti), Thallium (Tl) and Uranium (U) were identifiable
12 but the majority of results were close to limits of detection (LOD) and are not reported. Lead (Pb),
13 Caesium (Cs), Cadmium (Cd) were above LOD but below limits of quantification (LOQ) and are
14 therefore also not shown. Chromium (Cr), Molybdenum (Mo) and Vanadium (V) were above LOQ but
15 many results were close to LOQ and therefore these data are also not included.

16

17

1 Results

2 **Standardisation of mineral detection:** Analysis of 12 veterinary diets with a stated guaranteed
3 analysis for 5 minerals were compared against values determined by ICP-MS at The University of
4 Nottingham, as described above. Excellent comparisons were achieved with a highly-significant
5 correlation between estimates of mineral composition (Figure S1). Furthermore, a number of food
6 samples were analysed repeatedly within-run (8 technical replications) yielding a coefficient of
7 variation of <5% for elements at low (e.g. arsenic) or high (e.g. selenium) concentration.

8
9 **Macronutrient and energy composition of the diets:** As expected, foods fed to cats had greater crude
10 protein content and lower carbohydrate, than foods fed to dogs (Table 1). Total mineral content
11 (reflected as declared ash on the label) also tended to be increased in foods fed to cats (Table 1). On
12 an as-fed basis, fat content was similar between wet foods, but increased in dry foods fed to cats
13 versus dogs (Table 1). Foods fed to cats had higher mean energy density per se, but after correction
14 for dry matter content, were similar (Table 1). The declared ash content of pet food (corrected to
15 DM) correlated well with measured total mineral content, but considerable variability was noted
16 particularly for 'standard' wet diets (Figure S2). Foods designated as 'supermarket own brand' tended
17 to have higher ash content than 'prescription or therapeutic' diets (Figure S2).

18
19 **Mineral (elemental) composition of the diets:** Overall elemental composition of foods for cats or
20 dogs was broadly similar (i.e. no significant difference for total major or trace elemental composition;
21 Table 2). Corrected for moisture content, wet pet food had higher sulphur, calcium, phosphorus,
22 sodium, potassium, strontium and rubidium but lower manganese relative to dry food (Table 2).
23 Foods (wet or dry) fed to cats had lower magnesium but higher potassium than foods fed to dogs
24 (Table 2). When all foods were represented according to the mandatory FEDIAF guideline (Table S1,
25 11 of 13 guidelines were tested; chloride and iodine were not measured) some interesting outliers
26 were noted: for example, two foods had Ca:P \leq 0.25 whereas three others had a Ca:P ratio of \geq 2.5
27 (Figure 1a). Most foods complied with guidelines for elemental iron due largely to the exceptionally
28 wide acceptable range (Figure 1b). A number of foods, particularly wet food, were either above or
29 below FEDIAF guidelines for copper (Figure 1c) and the majority of wet foods exceeded the legal
30 maximum for selenium (Table S1; 56.8 $\mu\text{g}/100\text{g DM}$), which had a very narrow acceptable range
31 (Figure 1d).

32

1 **Compliance of foods with European legislation:** Analysis of all feline or canine foods revealed broad
2 non-compliance with all EU (FEDIAF) Guidelines; 94% (91/97) and 61% (46/80) of wet and dry foods,
3 respectively failed to comply with all guidelines (Figure 2a,b). While a majority of foods complied with
4 ≥ 8 of 11 guidelines (Dry food, 99% (79/80); Wet food, 83% (81/97)) many, particularly wet food, failed
5 to either provide the minimum (e.g. for copper, 20 % of wet food; Figure 1c) or exceeded the
6 maximum (e.g. for selenium, 76% of wet food; Figure 1d) levels of at least one essential mineral. Many
7 had marked mineral imbalance (e.g. 29% of wet, 20% of dry for Ca:P ratio; Figure 1a) and some
8 individual products had markedly variable mineral composition (e.g. five diets represented in Figure
9 2c). Repeated analysis of a subset of wet foods with a different batch-ID again showed broad non-
10 compliance in line with the original dataset, indicating that our analysis was not an isolated single-
11 batch effect (Figure 2d). Pictorial representation of compliance of all wet and dry foods analysed,
12 according to FEDIAF guidelines is presented in Supplementary information (Figure S3, dry foods;
13 Figure S4, wet foods).

14
15 **Undesirable metal elements in food:** Most undesirable metal elements in foods analysed (e.g.
16 beryllium, thallium, lead, cadmium, uranium) were below detectable or quantifiable levels. However,
17 some foods, particularly those with fish, were relatively high in the metalloid, arsenic (Figure 3a).
18 Further examination revealed that only those foods that declared incorporation of higher levels of
19 fish derivatives, as oppose to fish oil, had high levels of arsenic with three being above a safe upper
20 limit (SUL) for organic As, two above the legal limit for food stuffs (Figure 3b). Indeed, As levels tended
21 to increase steeply when ≥ 30 -40% fish was declared (Figure 3c). Furthermore, a subset ($\times 10$) of
22 foods low or high in As were sent for direct analysis of mercury (Hg), which correlated well with As
23 content (Figure 3d).

24

25

1 Discussion

2

3 This study, for the first time in the EU, has objectively and comprehensively assessed mineral
4 composition and mineral balance of a large number of complete, wet and dry pet foods bought in
5 pet-food supermarkets in the UK. The results suggest broad non-compliance with current EU
6 guidelines for pet food, in that 92% of wet and 61% of dry foods did not comply with all
7 recommendations. For a few individual products, mineral content often either far exceeded or did
8 not meet nutritional requirements. A number had such unbalanced mineral content (e.g. Ca:P) that
9 exclusive, longer-term feeding of these products could compromise general well-being of companion
10 animals. Finally, we found that diets with high fish content also had relatively high arsenic content.
11 Given that chronic arsenic intake in human epidemiological studies is associated with albuminuria,
12 proteinuria and increased mortality from kidney disease (Zheng et al., 2014) then one must question
13 why a high proportion of these diets are fed to domestic cats, whom are prone to chronic kidney
14 disease (Lawson et al., 2015).

15

16 **Macronutrient and energy composition of the diets:** On an as-fed basis (cats and dogs) the wet foods
17 in our dataset had greater crude protein, fat, ash and moisture content than dry foods, with the latter
18 having higher energy, fibre and carbohydrate (reported on the label as nitrogen-free extract [NFE]).
19 Corrected for moisture content, energy density of wet and dry foods was similar. In accord with being
20 obligate carnivores, feline diets had more crude protein than canine diets. A recent study estimated
21 that feral felids consumed approximately 52% crude protein, 46% fat, 11% ash and only 2% N-free
22 extract (Plantinga et al., 2011). These values, with the exception of fat, approximate to feline wet
23 foods in this study (see [Table 1](#)) and are consistent with animals that would be engaging in far greater
24 physical activity than a relatively sedentary housecat. Dry feline and canine pet food has a more
25 carbohydrate-rich content than wet food. Canines have evolved away from their wolf ancestors,
26 partially through adaptation toward consumption of a carbohydrate-rich, omnivorous diet (Axelsson
27 et al., 2013). Felines, on the other hand, have remained remarkable true to their lineage as obligate
28 carnivores from arid environments (Driscoll et al., 2007); the majority of their essential nutrients (e.g.
29 arginine, taurine, arachidonic acid, retinol and niacin) can be obtained readily from eating other
30 animals together with efficient gastrointestinal extraction and retention of water from solid food
31 (Montague et al., 2014). Guideline feed amounts on pet food labels are on an as-fed (i.e. weight of
32 food) basis. Owners can become confused by labelling information which may contribute to
33 companion animal obesity, since dry foods that are predominantly fed have much higher energy

1 density than wet foods (German, 2006). In addition, 40% (71 of 177) of foods analysed in our study
2 had $\geq 10\%$ ash on a dry matter basis, nine with ash $\geq 14\%$. Higher ash intake is associated with CKD in
3 cats (Hughes et al., 2002).

4

5 **Compliance with EU guidelines:** In 2010 a European scientific advisory board was established to
6 ensure that current state-of-the-art knowledge on complete nutrition for health, well-being and
7 longevity could be implemented for companion animals across EU states, via the industry's relevant
8 regulatory body, FEDIAF (FEDIAF, 2013). Their recommendations cover what is considered optimal
9 intake of macronutrients such as protein and fat, micronutrients such as specific amino acids, trace
10 elements and other minerals and vitamins to meet requirements for maintenance of good health.
11 Minimum requirements are based on published values (NRC, 2006) or unpublished industry research.
12 Nutritional maximums reflect the level in food that is not associated with any adverse effect, where
13 known (FEDIAF, 2013). Consideration of these recommendations in light of our study where 11 of 13
14 recommendations were referenced, we found that the majority were broadly compliant (i.e. with ≥ 8
15 of 11 guidelines), particularly dry food, but many individual foods were far outside the recommended
16 range (e.g. Ca:P ratio of 0.06 or 2.96:1). If fed over long periods of time, these products could
17 compromise optimal health of companion animals (Schoenmakers et al., 2000).

18

19 Seventy-six percent of wet foods had greater than the maximum concentration (legal) of selenium,
20 with seven having more than 3-fold the nutritional maximum (i.e. 3-fold more than $56.8 \mu\text{g}\cdot 100\text{g DM}^{-1}$
21 ¹). Selenosis is rare in companion animals (Burk and Hill, 2015), but nevertheless has been reported
22 (Yu et al., 2006). We acknowledge that the current study has not assessed bioavailability of ingested
23 selenium, which can be variable between companion animals, but rather has simply assessed
24 concentration in the foods – for which the EU guidelines are applicable. Our highly variable results
25 for total measured selenium in pet food do call into question the evidence base for designating such
26 a tight recommended range. Some diets also had ≥ 2 -fold the legal maximum for dietary copper (4.66
27 vs. recommendation, $2.80 \text{ mg}\cdot 100\text{g DM}^{-1}$] or less than half the nutritional minimum (0.18 vs.
28 recommendation, $0.50 \text{ mg}\cdot 100\text{g DM}^{-1}$). Similarly, although copper toxicity is also rare in companion
29 animals (Wills and Simpson, 1994), excess copper in the diet can reduce bioavailability of zinc and
30 iron (Gulec and Collins, 2014). Copper deficiency can directly impact reproductive performance and
31 cause coat/hair instability (Zentek and Meyer, 1991). Relatively high levels of certain trace minerals
32 can either lead to toxicosis *per se* or significantly affect bioavailability of other trace minerals through
33 gastrointestinal interactions, in particular between calcium, iron, copper, zinc, magnesium and

1 phosphate (Forbes, 1983). The mineral balance of complete diets is therefore important if they are
2 to be fed exclusively over long periods of time. Excess calcium intake ($\approx \geq 2.5\text{g}\cdot 100\text{g DM}^{-1}$; 32 foods in
3 our study) can lead to joint, limb and skeletal deformities (Hazewinkel, 1989).

4

5 **Arsenic:** Whilst the primary outcome of this study was to assess mineral composition of a range of
6 wet and dry pet foods with reference to compliance against EU recommendations, it did not escape
7 our attention that certain other undesirable elements such as arsenic were particularly high in certain
8 foods (see **Figure 4**), particularly in diets for domestic cats. Indeed, three foods were above World
9 Health Organization recommendations for safe upper limits for organic arsenic, two were above the
10 current legal maximum for animal feedstuffs. We estimate that, if fed exclusively, annual intake
11 (assuming modified Atwater criteria for intake per kg metabolic bodyweight) for the five foods with
12 highest arsenic content would yield a total exposure of between 800-6500 mgs arsenic per year.
13 Arsenic is water soluble and accumulates in organs such as the liver and kidney, where it is relatively
14 toxic either by itself (Yu et al., 2006) or via interactions with other catalytic metals such as iron and
15 copper (Valko et al., 2005). High urinary arsenic is associated with increased risk of CKD (Chen et al.,
16 2014). Certain soil and water sources are relatively contaminated with an abundance of arsenic
17 (Robles-Osorio et al., 2015), due to its inclusion in agrochemicals such as phosphate fertilisers
18 (Jayasumana et al., 2015). Arsenic may therefore bio-accumulate in the tissues of animals such as fish
19 that inhabit these waters (Rosso et al., 2013) and in the animals that consume these fish sources such
20 as the domestic cat. It remains to be determined if relatively high, long-term arsenic intake
21 predisposes the domestic cat to chronic kidney disease or whether high intake of other metals that
22 are also prevalent in fish may also have an effect, such as mercury (Storelli et al., 2007).

23

24 **Limitations of the study:** Our analysis has not been exhaustive. Rather, we present a snap-shot of
25 current popular foods bought in UK pet supermarkets. Although owners primarily feed their pets
26 complete diets, a proportion of nutritional intake may come from complimentary foods – for which
27 we have made no assessment of mineral composition. A number of surveys have indicated that up to
28 50% of dog owners and approximately 20% of cat owners supplement the daily ration with such treats
29 (PDSA, 2013). However, complimentary foods are rarely nutritionally-balanced products, especially
30 with respect to mineral composition. For example, high-protein ‘jerky’ supplements are virtually
31 devoid of calcium and high in phosphorus.

32 By design, this study has not considered bioavailability of minerals. It is possible that a non-compliant
33 diet with, for example, high copper or selenium does not cause copper or selenium toxicosis due to

1 mineral or food interactions in the gastrointestinal tract that prevent absorption. Nevertheless, the
2 EU guidelines, for which we assessed compliance against, refer to concentrations in foods taking into
3 account margins of safety and thus should, at least, be adhered to. Mineral bioavailability and
4 metabolism is complex. Gut absorption of microminerals such as zinc or copper can be markedly
5 reduced if the diet has relatively high fibre or phytate content; high dietary calcium can inhibit
6 gastrointestinal iron and zinc absorption (Forbes, 1983). For animals with sub-clinical disease, such as
7 early-stage kidney disease, variation in hormonal status (e.g. parathyroid hormone) or vitamin status
8 (e.g. Vitamin D) can profoundly influence mineral uptake from the gut (Llach and Massry, 1985).

9

10 In conclusion, this study is the first to sample a wide range of wet and dry pet foods, designated as
11 'complete' and widely available to consumers in the UK, for mineral composition. A majority were
12 non-compliant according to current European recommendations (FEDIAF, 2013). Many had either
13 insufficient, excessive or an inappropriate balance of minerals which, if fed exclusively for a long
14 period of time, could underpin a host of clinical diseases in dogs and cats including skeletal,
15 neurological, or dermatological disease. Furthermore, foods with relatively high levels of fish or fish
16 derivatives (i.e. $\geq 14\%$) also had high levels of undesirable metal elements such as arsenic, which
17 bioaccumulate in internal organs and may contribute toward a plethora of sub-clinical disease states
18 (Ercal et al., 2001, Mertz, 1981). The data suggest a need for better compliance to current
19 recommendations for mineral nutrition of companion animals in order to safeguard animal health,
20 and for a better understanding of the mineral requirements of cats and dogs. For consumers, the
21 study can offer a number of suggestions to limit the impact of such variability in pet foods: 1) weigh
22 100g of wet and/or dry food to grasp how much you are feeding and feed accordingly, 2) feed a diet
23 of mainly dry with some wet food, 3) choose foods with a declared as-fed mineral content (i.e. % ash
24 on the label) of not more than 10% for dry food (three of our foods exceeded this value) and not more
25 than 2% for wet food (sixty of our foods exceeded this value), 4) make the feeding of foods with a
26 high fish content a rarity rather than the norm and 5) vary the types of food fed over extended
27 periods.

28

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4 disclose.

5

6 **Author contributions**

7 D.S.G., C.W. and M.D. designed research; L.J., R.A., C.D., C.W. & D.S.G. conducted the research; D.S.G.
8 and M.D. co-wrote the manuscript. All authors critically evaluated the paper. D.S.G. conducted the
9 statistical analyses. D.S.G. and M.A.J.D. have primary responsibility for its final content.

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1 **Table 1: Macronutrient and calculated energy content of complete cat and dog food**

| Composition (all % per DM ⁻¹) | CAT (n=113) Complete foods | | DOG (n=64) Complete foods | | Statistics P-value | |
|--|-------------------------------|-------------------------|------------------------------|-------------------------|-----------------------|-------|
| | Wet (n=48) Mean (SD) | Dry (n=65) Mean (SD) | Wet (n=49) Mean (SD) | Dry (n=15) Mean (SD) | Wet | Dry |
| Crude Protein ¹ | 49.5 (11.0) | 34.6 (4.4) | 41.7 (9.6) | 23.3 (4.9) | <.001 | <.001 |
| Fats and Oils | 24.8 (8.64) | 16.2 (5.4) | 26.4 (6.6) | 12.9 (5.8) | 0.28 | 0.03 |
| Fibre | 0.61 (0.75) | 3.06 (1.67) | 0.82 (0.67) | 3.47 (2.18) | 0.14 | 0.43 |
| Ash | 11.2 (2.8) | 8.29 (1.57) | 10.1 (3.1) | 7.34 (2.45) | 0.06 | 0.06 |
| N-free extract ² | 2.29 (2.37) | 34.6 (6.5) | 4.49 (4.45) | 48.5 (.8) | 0.003 | <.001 |
| Moisture (% as fed) | 80.3 (3.2) | 8 (0) | 77.1 (3.9) | 8 (0) | 0.06 | - |
| Energy content (kcal per 100g) | | | | | | |
| Gross Energy ³ | 113 (22) | 476 (30) | 131 (21) | 447 (31) | <.001 | 0.002 |
| Digestible Energy ⁴ | 98.9 (19.4) | 405 (28) | 114 (18.2) | 379 (26) | <.001 | 0.001 |
| Metabolisable Energy (ME) | 60.7 (22.2) | 379 (27) | 70.8 (23.4) | 355 (27) | 0.03 | 0.003 |
| Atwater ME ⁵ ⁶ | 83.7 (19.0) | 360 (28) | 99.1 (17.5) | 346 (29) | <.001 | 0.10 |
| Atwater DM ⁷ | 392 (59) | 381 (32) | 386 (63) | 362 (33) | 0.66 | 0.04 |

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Table 1: Macronutrient content were derived from the stated composition on the label each product. Products were clearly labelled as being a complete diet, for a cat or dog and were either wet food or dry kibble. Energy densities were calculated according to modified Atwater criteria as outlined in Equations 1-7, supplementary information. All data were normally distributed and were analysed by 1-way ANOVA within each type of food to test for differences in composition of foods fed to cats or dogs. A formal comparison between wet or dry food was not considered of value. A P-value of <0.05 was accepted as indicating statistical significance.

1 **Table 2: Elemental composition of complete, wet cat and dog food**

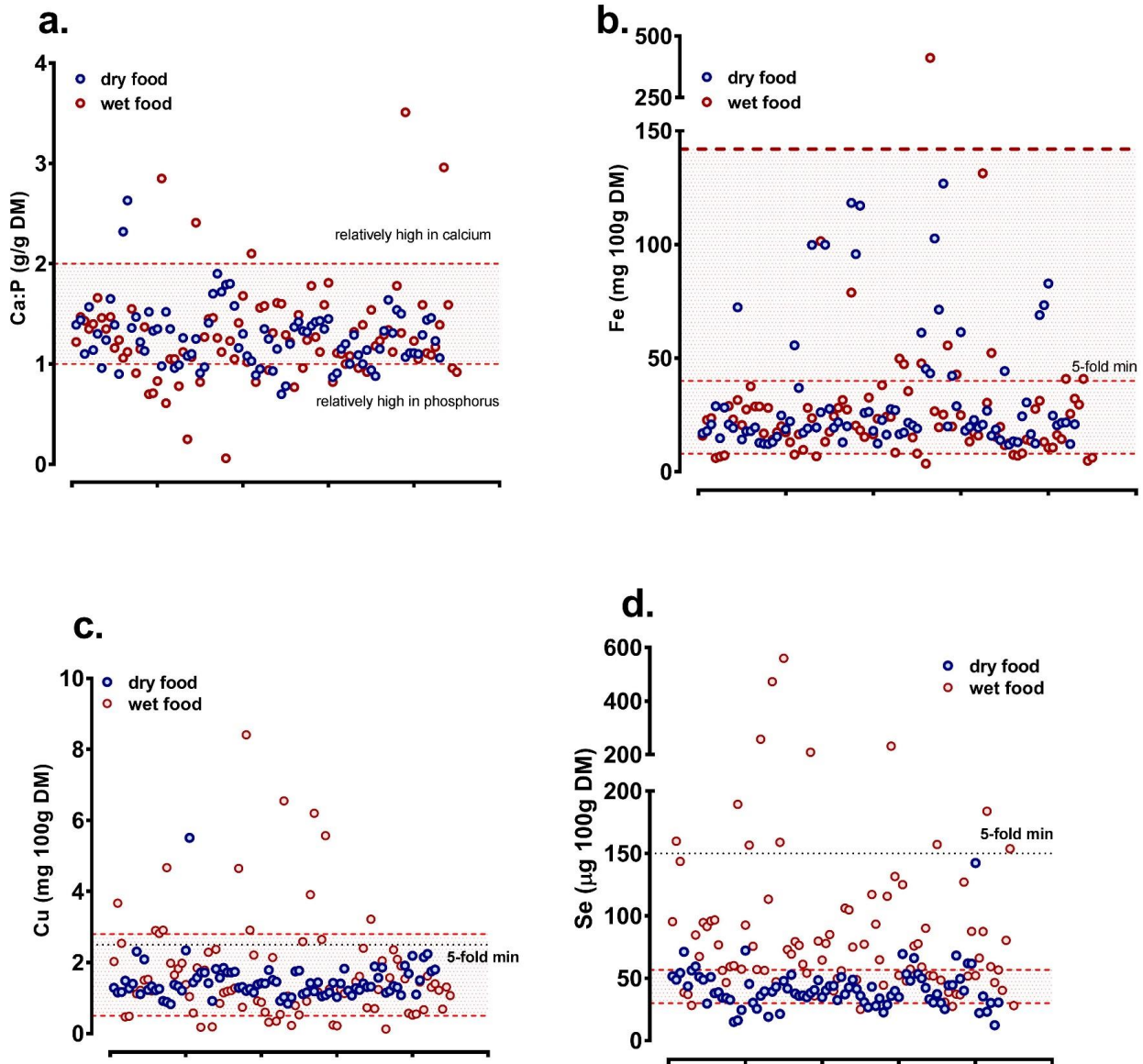
| Major element (all ppm, mg/kg DM) | CAT (n=113) Complete foods | | DOG (n=64) Complete foods | | Statistics P-value | |
|--|-------------------------------|----------------------------|------------------------------|----------------------------|-----------------------|-----------------|
| | WET (n=48) Median (IQR) | DRY (n=65) Median (IQR) | WET (n=49) Median (IQR) | DRY (n=15) Median (IQR) | Cat vs. Dog | Wet vs. Dry |
| Sulphur | 7269 (6348,8442) | 4989 (4124,6447) | 5211 (4442,7528) | 3302 (2925,3923) | 0.01 | <.001 |
| Calcium | 17423 (13580,25565) | 14125 (11442,19456) | 19766 (15147,27387) | 12860 (6290,17813) | 0.21 | <.001 |
| Phosphorus | 15715 (13297,19300) | 12365 (10730,14194) | 15672 (10471,21473) | 9555 (5051,13016) | 0.59 | <.001 |
| Sodium | 11906 (6437,17135) | 5692 (4233,6319) | 10180 (6350,14148) | 4093 (2743,4375) | 0.63 | <.001 |
| Magnesium | 1048 (847,1195) | 1110 (915,1357) | 1353 (1080,1762) | 1208 (1129,1360) | <.001 | 0.86 |
| Potassium | 13376 (9943,18670) | 7686 (7174,8615) | 14857 (10102,19474) | 6254 (5567,7593) | <.001 | <.001 |
| TOTAL MAJOR | 73070 (61513,82443) | 47352 (41733,54338) | 70925 (54602,86870) | 41224 (25416,46955) | 0.07 | <.001 |
| Trace element (all ppb, µg/kg DM) | | | | | | |
| Zinc | 154 (127,178) | 176 (143,208) | 170 (137,223) | 180 (145,214) | 0.03 | 0.21 |
| Manganese | 19.7 (11.3,24.3) | 59.6 (37.8,69.4) | 23.3 (15.9,35.4) | 57.9 (36.9,75.7) | 0.05 | <.001 |
| Strontium | 15.0 (10.2,33.8) | 13.9 (11.4,17.0) | 20.2 (14.0,27.6) | 10.9 (9.34,13.6) | 0.04 | <.001 |
| Rubidium | 8.45 (6.8,11.9) | 5.55 (4.16,6.76) | 9.32 (5.76,12.5) | 4.68 (2.98,6.20) | 0.002 | <.001 |
| Molybdenum | 0.75 (0.46,0.88) | <0.64 (LOQ) | <0.64 (LOQ) | <0.64 (LOQ) | - | - |
| Chromium | <0.48 (LOQ) | <0.48 (LOQ) | <0.48 (LOQ) | 0.60 (0.43,1.28) | - | - |
| Vanadium | <0.16 (LOQ) | <0.16 (LOQ) | <0.16 (LOQ) | 0.19 (0.14,0.47) | - | - |
| Cobalt | 0.08 (0.05,0.11) | 0.08 (0.06,0.13) | 0.10 (0.06,0.14) | 0.08 (0.07,0.14) | 0.007 | 0.28 |
| TOTAL TRACE | 427 (339,610) | 501 (365,634) | 478 (433,617) | 537 (438,820) | 0.60 | 0.13 |

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3 **Table 2:** Data are presented as median (IQR; 1st to 3rd quartile) for each element. Duplicate, freeze-dried
4 homogenized samples (100-200mgs) were analysed by ICP-MS, and a mean value established. Since elemental
5 composition is presented as parts (ppm, ppb) per unit dry matter, a formal statistical comparison was
6 conducted as a 2 (cat vs dog) × 2 (wet vs dry) factorial ANOVA, with the interaction term included. If necessary,
7 data were log₁₀ transformed prior to analysis in order to normalize residual errors, to avoid distributional bias.
8 Statistical significance was accepted at P<0.003 (Bonferroni correction for 14 elements above LOQ). Data for
9 total trace elements includes As, Co, Cr, Cs, Cu, Fe, Mn, Mo, Rb, Se, Sr, V and Zn.

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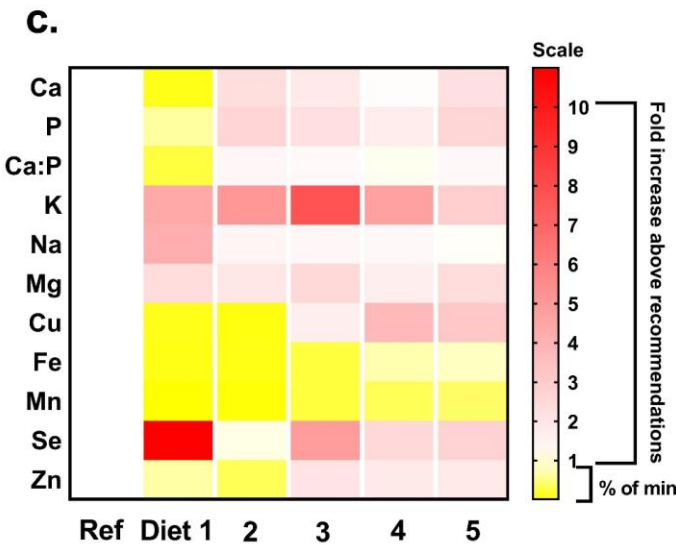
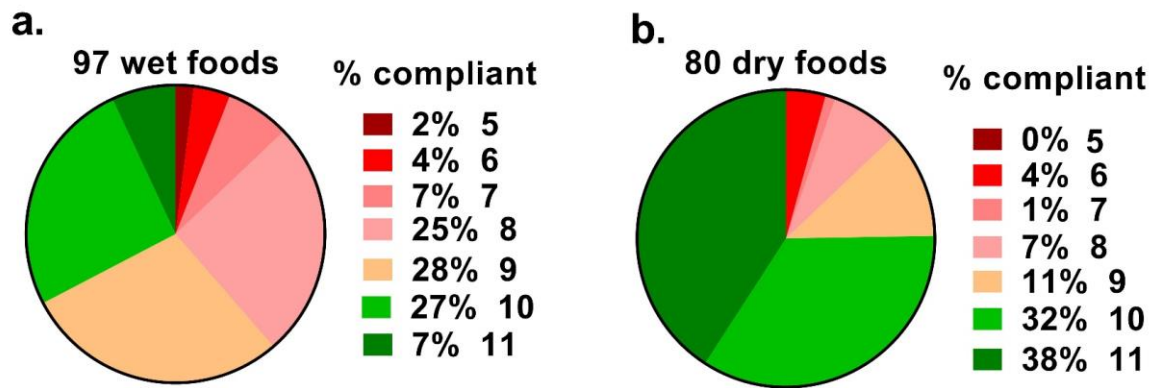
1 **Figure 1: Calcium to Phosphorus, Copper, Iron and Selenium content of complete, wet and dry pet foods**



2
3 **Figure 1: a-d.** Total elemental matter (Calcium [Ca], Phosphorus [P], Iron, [Fe], Copper, [Cu] and Selenium, [Se])
4 were measured in duplicate samples of freeze-dried, homogenized and nitric acid-digested pet food by ICP-
5 MS. Each dot represents a single wet (red shaded dots) or dry (blue shaded dots) food. Lower and upper dotted
6 lines represent nutritional minimum and maximum, respectively as recommended by FEDIAF. Black dotted line
7 represents incorporation at 5-fold nutritional minimum, since few recommendations for a feline nutritional
8 maximum exists. Data for each product are plotted individually along the x-axis, with elemental density on the
9 y-axis.

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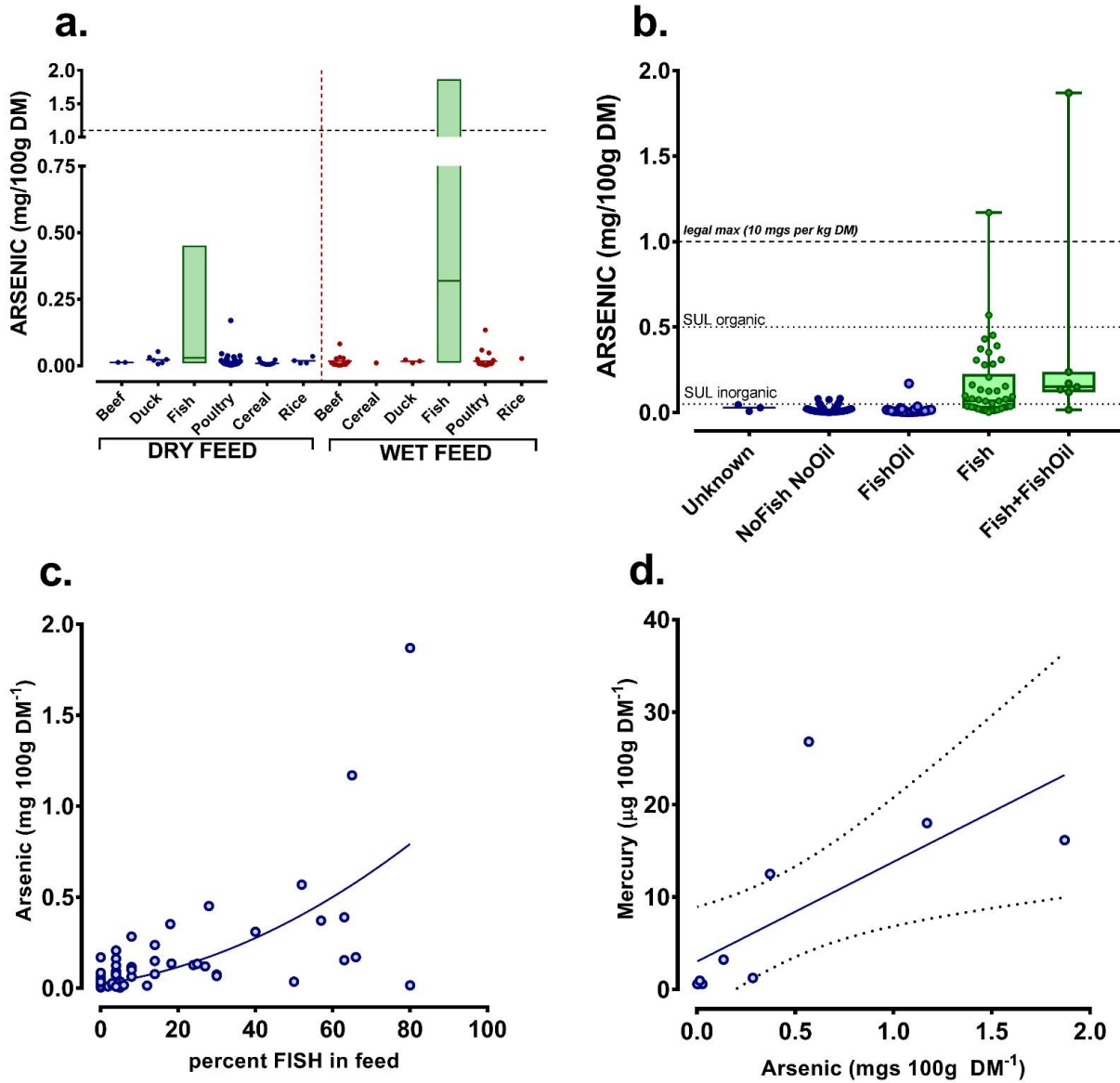
1 **Figure 2: Compliance of complete, wet and dry pet foods with European guidelines**



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3 **Figure 2:** All wet (a) and dry (b) foods analysed were assessed against current European (FEDIAF) guidelines
4 for individual elements (nutritional minimum and maximum). The percent of total that complied with all 11 of
5 13 guidelines (chloride and iodine were not assessed) are represented as green (e.g. 6 of 97 = 7% wet foods).
6 c, Five individual diets are represented to show broad non-compliance and marked variability in elemental
7 content. Ref, optimal nutritional content for each element (i.e. mid-way between nutritional minimum and
8 maximum). Scale to 1 is percent below minimum (e.g. 0.20 is 5-fold less than nutritional minimum), scale 1-10
9 is fold-content above nutritional maximum. d, A subset of the original dataset (Batch 1) was purchased for a
10 second time (Batch 2) with a different batch-ID to assess repeat compliance.

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2 **Figure 3: Arsenic in wet and dry pet food.**



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4 **Figure 3:** Arsenic in wet and dry foods was analysed by ICP-MS (see Methods). **a)** Foods were classified
5 according to main declared ingredient and are presented as dot-plots for visual clarity or box-plots (line at
6 median, box represents range). Upper horizontal dotted line is legal maximum (10 mg/kg DM). Vertical dotted
7 line separates dry from wet food. **b)** comparison of all foods declaring incorporation of fish derivatives (FISH)
8 or fish oil (FishOil), none (NoFishNoOil) or both (Fish+FishOil). Three diets remained unknown as to fish or
9 fishoil incorporation. **c)** scatterplot of the percent incorporation of fish derivatives vs. arsenic in food. Line is
10 non-linear (quadratic) regression (r^2 , 0.47). **d)** Ten samples of food with low ($\times 5$) or high ($\times 5$) arsenic levels
11 were sent for Direct Mercury Analysis (DMA-80) at The British Geological Survey (Keyworth, UK). Solid Line is
12 linear regression with dotted lines 95% C.I. and the equation for the line is $y=10.78 \cdot X+3.031$; $F_{1,9}=8.4$, $P=0.01$.
13