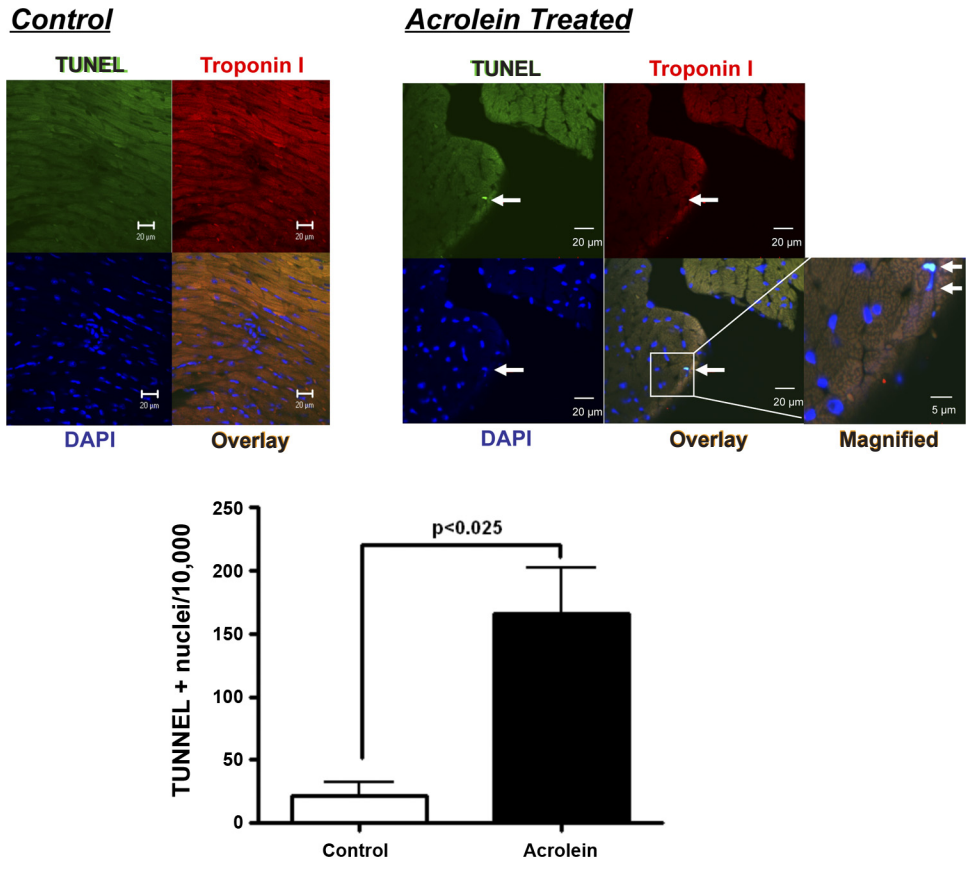

Fig. 5. Confocal microscopic images of terminal deoxytransferase-mediated dUTP nick-end labeling (TUNEL) staining in hearts from mice chronically fed acrolein or vehicle (control) and quantitation of TUNEL-positive nuclei. Myocytes were stained with anti-troponin I (red), and nuclei were stained with DAPI (blue). TUNEL-positive nuclei appear green-cyan on the overlaid images from the acrolein-exposed heart as shown in the magnified (zoom) image. Arrows denote a TUNEL-positive nucleus. Scale bar: standard magnification 20 μm; zoomed magnification 5 μm. n = 5/group.

cal studies have established that human subjects with HF are more vulnerable to the adverse cardiovascular effects of pollution exposure (5). Moreover, similar to our results obtained with acrolein exposure, environmental carbon monoxide also induces pathological remodeling in hearts of normal rats (1), supporting the idea that pollutant exposure could also lead to adverse changes in the heart in the absence of underlying cardiomyopathy.

One underlying mechanism for acrolein-mediated cardiac remodeling may be related to the induced abnormalities in eNOS function. Alterations in eNOS coupling and NO synthesis can contribute substantially to pathological cardiac remodeling (24, 36, 39). When electron transfer from its reductase to oxidase domains is normally coupled, eNOS is generally cardioprotective and antihypertrophic (39). However, during pathological hypertrophy and HF, both eNOS downregulation and uncoupling can occur, thereby augmenting superoxide generation, diminishing NO bioavailability, and increasing peroxynitrite formation (12, 24, 36, 39). In our study, acute acrolein exposure suppressed eNOS activation, whereas chronic acrolein exposure decreased overall eNOS abundance and reduced the eNOS dimer-to-monomer ratio, consistent with eNOS uncoupling. The biological relevance of these changes was demonstrated by the approximately twofold increase in protein-nitrotyrosine levels in the heart, indicative of increased peroxynitrite generation. Hence, disruption of eNOS function may be in part responsible for increased free radicals and oxidant stress induced by acrolein.

The observed cardiomyopathic phenotype may have resulted from both direct and indirect effects of acrolein. We have demonstrated that oral acrolein exposure induces protein-acrolein adducts in both plasma and myocardium with adduct abundance decreasing in a time-dependent manner following exposure. This suggests that consumed acrolein physically circulates to remote sites such as the heart to directly disrupt protein function, thereby secondarily inducing cardiac injury and inflammation. In our previous studies, we demonstrated that acrolein primarily modifies sarcomeric, cytoskeletal, and mitochondrial proteins in the context of acute exposure (19, 42). The time dependence of adduct levels in the current study suggests ongoing metabolic disposition and turnover of protein-acrolein adducts both systemically and in the heart. This is consistent with prior studies that have demonstrated lability of aldehyde-adducted proteins and degradation by the proteasome and lysosomes in minutes to hours (23, 26). Long-term exposure and/or reduced metabolic capacity for aldehyde detoxification may therefore enhance the adverse effects of acrolein. In this regard, we have previously shown that aldose reductase, the main aldehyde-reducing enzyme in the heart, is significantly downregulated in HF (33). Hence, the cardiotoxic effects of environmental acrolein may be heightened in subjects with preexisting HF.

In summary, we have shown that long-term environmental exposure to acrolein, at an amount within the range of human unsaturated aldehyde intake, induces DCM in the mouse. Primary features included the induction of myocardial inflam-

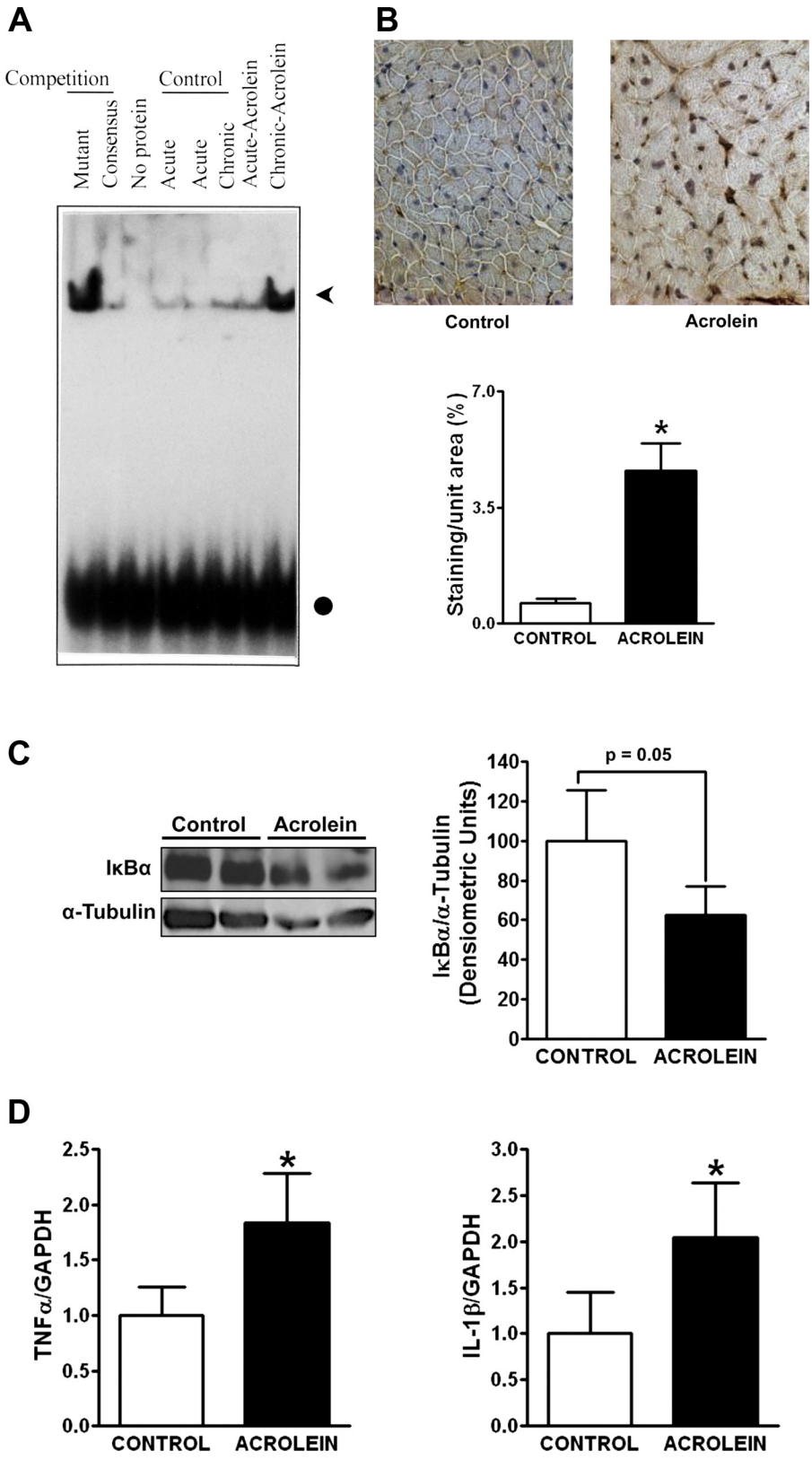

Fig. 6. Chronic acrolein exposure induces inflammation in the heart. *A*: EMSA to determine nuclear factor (NF)-κB DNA binding activity of pooled myocardial nuclear protein extracts from control and acrolein-fed mice. Acute control and acrolein-fed mice were given a single dose of 1 mg/kg vehicle or acrolein, and tissue was harvested at 24 h. Chronic control and acrolein-fed mice were administered daily vehicle or acrolein (1 mg/kg) for 48 days, and tissue was harvested 24 h after the final dose. NF-κB DNA binding is indicated by the arrowhead. The circle indicates unbound oligonucleotide probe. *B*: representative immunohistochemical stains for the activated p65 subunit of NF-κB in hearts from control mice and mice chronically fed acrolein, together with quantitation of staining intensity by image analysis. Note the nuclear localization of p65 in the acrolein-exposed mouse heart. *C*: WB and densitometry for inhibitor of κBα (IκBα) in hearts from control mice and mice chronically fed acrolein as in *A*. *D*: myocardial gene expression of tumor necrosis factor (TNF) and interleukin (IL)-1β by real-time PCR in the same hearts as in *B*. $*P < 0.05$ vs. control.

mation and oxidative/nitrative stress, which may represent responses to the formation of detrimental acrolein-protein adducts in the heart, together with myocyte hypertrophy and apoptosis. These results suggest that human exposure to acrolein can have analogous deleterious effects, especially in those with preexisting structural heart disease and/or reduced capacity for aldehyde detoxification. Moreover, our findings raise consideration of an underrecognized environmental basis for

idiopathic DCM related to aldehyde constituents of natural food and the pollutant mix.

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DISCLOSURES

There are no conflicts of interest to disclose.

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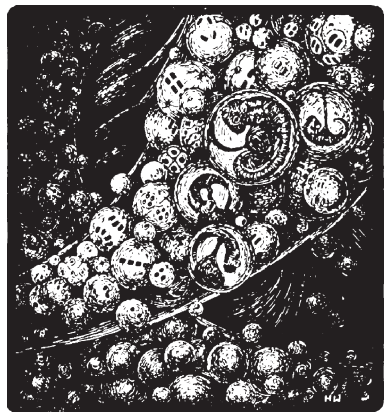

Assessment of protein and amino acid concentrations and labeling adequacy of commercial vegetarian diets formulated for dogs and cats

Kayo Kanakubo, BVSc; Andrea J. Fascetti, VMD, PhD; Jennifer A. Larsen, DVM, PhD

Objective—To determine measured crude protein (CP) and amino acid (AA) concentrations and assess labeling adequacy of vegetarian diets formulated for dogs and cats.

Design—Cross-sectional study.

Sample—13 dry and 11 canned vegetarian diets for dogs and cats.

Procedures—Concentrations of CP and AAs were determined for each diet. Values were compared with the Association of American Feed Control Officials (AAFCO) Dog and Cat Food Nutrient Profiles. Product labels were assessed for compliance with AAFCO regulations.

Results—CP concentration (dry-matter basis) ranged from 19.2% to 40.3% (median, 29.8%). Minimum CP concentrations for the specified species and life stage were met by 23 diets; the remaining diet passed appropriate AAFCO feeding trials. Six diets did not meet all AA minimums, compared with the AAFCO nutrient profiles. Of these 6 diets, 1 was below AAFCO minimum requirements in 4 AAs (leucine, methionine, methionine-cystine, and taurine), 2 were below in 3 AAs (methionine, methionine-cystine, and taurine), 2 were below in 2 AAs (lysine and tryptophan), and 1 was below in 1 AA (tryptophan). Only 3 and 8 diets (with and without a statement of calorie content as a requirement, respectively) were compliant with all pet food label regulations established by the AAFCO.

Conclusion and Clinical Relevance—Most diets assessed in this study were not compliant with AAFCO labeling regulations, and there were concerns regarding adequacy of AA content. Manufacturers should ensure regulatory compliance and nutritional adequacy of all diets, and pets fed commercially available vegetarian diets should be monitored and assessed routinely. (*J Am Vet Med Assoc* 2015;247:385–392)

Popularity of vegetarian and vegan diets for humans has increased for ethical, ecological, and health reasons, and this influences pet food choices for some families.[1,2] In addition, vegetarian diets are often used for veterinary patients with conditions such as hepatic encephalopathy, food allergies, and urate and cystine urolithiasis. However, for several reasons, vegetarian pet foods have been linked to concerns related to nutritional adequacy. Vegetarian protein sources are often poor sources of specific essential vitamins (vitamin D, vitamin A, niacin, and cobalamin), fatty acids (arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid), and minerals (calcium and potassium).[3] In addition, plants are highly variable in protein concentration and provide incomplete AA profiles for meeting the needs of pets. Therefore, vegetarian diets must be appropriately formulated and balanced, including the use of proper supplementation with purified sources of essential AAs when indicated.

Adequate protein and AA intake is an important consideration for both dogs and cats. Cats are more limited than dogs in their ability to conserve nitrogen and AAs in the face of inadequate dietary intake.[4] In addition, sulfur-containing AAs (methionine, cystine, and taurine) are found primarily in animal protein. Although it is not used for protein synthesis, taurine is a required dietary nutrient for cats and is important for several physiologic processes, including retinal function, cardiac function, reproduction, and growth.[5] Taurine is considered conditionally essential for dogs because they have the metabolic capacity to synthesize it when adequate concentrations of sulfur-containing AA precursors (methionine and cysteine) are available, except for specific breeds[6] and diseases associated with decreased taurine synthesis.[7] Taurine deficiency has also been identified in dogs fed low-protein diets for extended periods or fed diets limited in sulfur-containing AAs.[8,9]

Pet foods sold in the United States are regulated by both federal and state laws. Manufacturers are re-

Abbreviations

AA	Amino acid
AAFCO	Association of American Feed Control Officials
CP	Crude protein
DM	Dry matter
ME	Metabolizable energy

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sponsible for proper formulation and labeling of products to meet requirements set by the US FDA as well as those mandated by each state, many of which have adopted AAFCO model regulations for pet food.[10] Information from the label is often used by pet owners and veterinarians to assess pet foods; therefore, accuracy and compliance with regulations are expected.[11] However, to our knowledge, there have been no studies conducted on the incidence of noncompliance of any category of pet foods with AAFCO model regulations, although there is evidence that some diets may provide CP at concentrations below the minimum guaranteed analysis value.[12]

The primary objective of the study reported here was to measure CP and AA concentrations in commercial vegetarian foods formulated for dogs and cats and to compare those values with minimum required concentrations for the intended species and life stage as established by the AAFCO. A secondary objective was to compare the information on pet food labels with required components as established by the AAFCO. We hypothesized that all diets would meet all nutritional and labeling requirements.

Materials and Methods

Sample—Commercially available over-the-counter diets (foods distributed directly to the public without veterinary oversight) consisting of dry and canned products for dogs and cats that were labeled or marketed as vegan or vegetarian and available during June and July 2014 were obtained from local pet stores and online sources. Similarly labeled or marketed veterinary therapeutic dry and canned diets for dogs were obtained from a local veterinary clinic,[a] and 1 diet was donated by an employee of the University of California-Davis Veterinary Medical Teaching Hospital.

Procedures—Information from the labels was compared with 9 AAFCO labeling requirements[13] (product and brand name, species specification, quantity statement, guaranteed analysis, ingredient statement, nutritional adequacy statement, feeding directions, name and address of manufacturer or distributor, and calorie content). The new labeling requirement for inclusion of the calorie content statement on all pet food labels was included in the AAFCO 2014 official publication.[13] However, the AAFCO recommended in that publication that enforcement be delayed 18 months for new products in development and 3 years for existing products.[14] Therefore, labels were assessed both including and excluding the statement of calorie content as a requirement. Information that was not provided on the label but was required for assessment was obtained from the product website or by contacting the manufacturer.

A sample of each diet was placed in a plastic bag, labeled with a number corresponding to the product, and submitted for analysis; all analytic laboratories were not aware of the commercial source for each sample submitted for analysis.

A sample of each of the canned diets was manually crushed within the plastic bag until a paste consistency was achieved, whereas dry diets were analyzed without any processing. Dry matter values were obtained by drying representative samples of each diet (20 g of canned diets and 5 g of dry diets) to a constant weight in a vacuum oven at 95° to 100°C.

In addition, 100 g of each canned diet and 50 g of each dry diet were stored in individual containers and frozen at –80°C. These samples were placed into a freeze-drier for 7 days prior to analysis, and canned diets then were manually crushed into a powder to ensure homogeneity. Approximately 5 g of each freeze-dried diet was submitted to a reference laboratory[b] for measurement of total nitrogen concentration via a combustion method.[15] This method was not included in the methods cited by the AAFCO[16]; however, results of a comparison study[17] with Kjeldahl analysis revealed that the combustion method had improved repeatability and reproducibility for SD ranges. Twenty diets were measured as single samples, and 4 diets were measured as duplicate samples in accordance with the laboratory's standard procedures. The laboratory's acceptable variance was 6.7%, and analytic variation for the 4 duplicate samples was 0.3%. Crude protein content was determined by use of the following equation: CP percentage = nitrogen percentage × 6.25.

For AA analysis, all freeze-dried samples were ground until they could pass through a 2-mm screen (80 mesh). Approximately 10 mg of each ground sample was hydrolyzed in a vacuumed-sealed glass ampule with 2 mL of 6M HCl at 115°C for 24 hours. The hydrolysate was then dried with nitrogen gas, and the resulting residue was reconditioned with lithium hydroxide loading buffer. This solution was filtered by use of a 0.45-µm polytetrafluorethylene syringe filter. The AA composition was determined in the filtrate by use of a norleucine internal standard with an automated high-performance liquid chromatography AA analyzer[c] at the Amino Acid Laboratory at the University of California-Davis, with methods described elsewhere.[18] Cystine and methionine concentrations were determined by use of performic acid oxidation with acid hydrolysis (hydrobromic acid method[19]), and tryptophan concentration was determined by use of a method described elsewhere.[20] All diets were measured as single samples. In addition to the internal standard used by the laboratory, a reference sample of purified casein was analyzed concurrently with each batch of sample diets; analytic variation was within 5%.

Measured CP and AA concentrations were compared with the minimum requirement in the AAFCO Dog and Cat Food Nutrient Profiles for the intended species and life stage.[13] Diets formulated for both dogs and cats were compared with the AAFCO food nutrient profiles for cats. Concentrations of CP and AA were corrected for energy density if the diet contained > 4,000 or > 4,500 kcal/kg of DM for canine or feline diets, respectively.[21] When assessing whether measured concentrations met the minimum values of the AAFCO food nutrient profiles, consideration was given to the allowed analytic variation for CP and lysine (AAFCO does not specify allowable variations for other AAs).[16]

Calorie content was obtained from the label or manufacturer; if calorie content was not provided or could not be obtained, it was calculated from the guaranteed analysis. For calculation of calorie content, measured CP and moisture concentrations were

used; modified Atwater values of 3.5 kcal/g for CP and nitrogen-free extract and 8.5 kcal/g for crude fat were used.[13] Ash concentration was obtained from the label or manufacturer or were estimated by use of the mean value of the ash concentrations measured for diets.

Statistical analysis—A Shapiro-Wilk test was used to confirm data were nonparametric. Spreadsheet software[d] was used to calculate descriptive statistics (median and range).

Results

Twenty-four diets were assessed, consisting of 13 dry diets (9 for dogs,[e–m] 3 for cats,[n–p] and 1 for both dogs and cats[q]) and 11 canned diets (8 for dogs,[r–y] 2 for cats,[z,aa] and 1 for both dogs and cats[bb]). One dry diet for dogs was donated; the other 23 diets were purchased. There were 21 over-the-counter diets for dogs or cats (or both) and 3 veterinary therapeutic diets for dogs. Dry diets represented 9 manufacturers, and canned diets represented 8 manufacturers. There were 2 dry diets that were manufactured at facilities outside the United States.

Only 3 diets (1 dry and 2 canned), including the statement of calorie content as a requirement, and 8 diets (4 dry and 4 canned), excluding the statement of calorie content as a requirement, were compliant with all pet food label regulations as established by the AAFCO. As indicated by the nutritional adequacy statement (or, when not provided, other label information), 14 diets (7 dry and 7 canned) were intended for adult maintenance, 9 diets (5 dry and 4 canned) were intended for all life stages, and 1 diet (dry) was intended for both growth and adult maintenance. Nutritional adequacy for the designated life stage or stages was substantiated by the formulation method to meet the AAFCO Dog and Cat Food Nutrient Profiles for all but 1 diet (1 dry diet for dogs); that diet successfully completed appropriate AAFCO-recognized animal feeding trials.

When label information was compared with the 9 AAFCO requirements, 20 diets (9 dry and 11 canned) met the requirement for product and brand name, 23 diets (13 dry and 10 canned) met the requirement for species specification, 18 diets (7 dry and 11 canned) met the requirement for quantity statement, 17 diets (6 dry and 11 canned) met the requirement for guaranteed analysis, 17 diets (6 dry and 11 canned) met the requirement for ingredient statement, 18 diets (7 dry and 11 canned) met the requirement for nutritional adequacy statement, 12 diets (7 dry and 5 canned) met the requirement for feeding directions, 20 diets (9 dry and 11 canned) met the requirement for name and address of the manufacturer or distributor, and 8 diets (2 dry and 6 canned) met the requirement for statement of calorie content. Of the diets that failed to meet the AAFCO labeling requirements, 4 had the product name outside of the principal display panel, 1 did not have a species-specification statement on the principal display panel, 6 did not have a quantity statement, 4 did not have a guaranteed analysis and 3 did not have an appropriate guaranteed analysis format (terms used and order of items), 5 had misspelled or duplicated words in the ingredient statement and 2 did not have an appropriate ingredient statement format (ingredients listed under 2 separate headings [ie, composition and additives]), 6 did not have a nutritional adequacy statement, 4 did not have feeding directions, 2 had misspelled words in the feeding directions, 6 did not have frequency or species specifications in the feeding directions, 4 did not have the name and address of the manufacturer, 14 did not have a statement of calorie content, and 2 did not have an appropriate calorie content format (not listed under a heading of calorie content or no information on method of determination). Both diets manufactured outside the United States did not meet 6 of the AAFCO labeling requirements (including not having a statement of calorie content), whereas some of the 22 diets manufactured within the United States did not meet up to 8 of the 9 requirements (including not having a statement of calorie content). Overall, 9 diets (4 dry and 5 canned) had labels with misspelled words.

None of the diets exceeded the maximum moisture percentage as reported on guaranteed analysis. Median measured moisture concentration of the diets was 4.8% (range, 3.3% to 7.8%) for dry diets and 69.9% (range, 61.4% to 74.3%) for canned diets.

Dried eggs were listed as an ingredient in 1 canned diet, whereas the other 23 diets listed only plant-sourced ingredients. Nineteen diets (11 dry and 8 canned) were supplemented with 1 or more AAs: methionine (7 dry and 4 canned), taurine (10 dry and 7 canned), lysine (7 dry and 2 canned), and tryptophan (5 dry and 0 canned); 1 dry diet was supplemented with both cystine and glycine. All 7 diets formulated for cats were supplemented with taurine. Two dry diets included a minimum taurine concentration claim in the guaranteed analysis (which is optional); both of these diets contained taurine in concentrations that exceeded the AAFCO minimum value. However, 1 of the 7 taurine-supplemented diets contained a measured taurine concentration that was 85% of the minimum listed in the guaranteed analysis.

Median measured CP concentration (DM basis) was 29.8% (range, 19.2% to 40.3%) for all diets. Measured CP concentrations were above the minimum requirement for the AAFCO Dog and Cat Food Nutrient Profiles (DM basis or corrected for energy density when necessary) for the intended species and life stage for 23 diets (12 dry and 11 canned). The dry diet for dogs that did not meet the minimum requirement contained 94% of the minimum required value but had completed an AAFCO-recognized animal feeding trial. One additional canned diet for dogs that exceeded 4,000 kcal/kg of DM contained only 91% of the reported minimum CP for the guaranteed analysis on an as-fed basis but met the AAFCO minimum CP on a DM basis when corrected for energy density. All other diets met the reported minimum CP for the guaranteed analysis.

Eighteen diets (10 dry and 8 canned) contained all AAs in concentrations that met or exceeded the minimum values for the AAFCO Dog and Cat Food Nutrient Profiles (DM basis or corrected for energy density when necessary) for the designated life stage (**Table 1**). Five diets (all for cats; 3 dry and 2 canned) provided 1 or more AAs at concentrations below the AAFCO minimum value. Of these 5 diets, 1 was below the AAFCO

Table 1—The AA concentrations of vegetarian dry and canned diets formulated for dogs and cats and values for the AAFCO Dog and Cat Food Nutrient Profiles.

AA	Median	Range	AAFCO	
			Growth and reproduction (minimum)	Adult maintenance (minimum)
Canine (n = 17)				
Arginine	1.66	1.08–2.83	0.62	0.51
Histidine	0.59	0.40–0.96	0.22	0.18
Isoleucine	1.05	0.84–1.81	0.45	0.37
Leucine	1.88	1.45–4.74	0.72	0.59
Lysine	1.40	0.99–2.47	0.77	0.63
Methionine-cystine	0.85	0.46–3.62	0.53	0.43
Phenylalanine-tyrosine	2.39	1.92–3.90	0.89	0.73
Threonine	1.13	0.90–1.53	0.58	0.48
Tryptophan	0.25	0.18–0.40	0.20	0.16
Valine	1.29	1.01–2.00	0.48	0.39
Taurine	0.19	0.11–0.30	—	—
Feline (n = 7)*				
Arginine	1.85	1.49–2.50	1.25	1.04
Histidine	0.77	0.68–0.88	0.31	0.31
Isoleucine	1.44	1.28–1.58	0.52	0.52
Leucine	3.41	0.43–4.81	1.25	1.25
Lysine	1.46	1.12–2.18	1.20	0.83
Methionine-cystine	1.63	0.59–3.14	1.10	1.10
Methionine†	0.62	0.51–1.32	0.62	0.62
Phenylalanine-tyrosine	3.20	3.00–3.88	0.88	0.88
Phenylalanine	1.89	1.80–2.22	0.42	0.42
Threonine	1.42	1.10–1.60	0.73	0.73
Tryptophan	0.36	0.16–0.41	0.25	0.16
Valine	1.72	1.51–1.80	0.62	0.62
Taurine (extruded)‡	0.18	0.15–0.18	0.10	0.10
Taurine (canned)§	0.12	0.11–0.15	0.20	0.20

Values reported are percentage DM.
*Includes results for 2 diets formulated for both dogs and cats. †Methionine is the only AA with a maximum allowed value, and only for feline diets (1.5% DM). ‡Values are for 4 extruded diets. §Values are for 3 canned diets.
— = Not applicable.

minimum requirements in 4 AAs (leucine, methionine, methionine-cystine, and taurine), 1 was below in 3 AAs (methionine, methionine-cystine, and taurine), 2 were below in 2 AAs (lysine and tryptophan), and 1 was below in 1 AA (tryptophan). An additional canned diet intended for both dogs and cats exceeded the AA minimum values for dogs but was below the minimum values for cats for 3 AAs (methionine, methionine-cystine, and taurine), despite inclusion of dried eggs as an ingredient. All of the canned diets formulated for cats (2 for cats and 1 for both dogs and cats) were below the AAFCO minimum value for taurine; dry diets for cats exceeded this value. Overall, of the diets that contained 1 or more AAs at concentrations below AAFCO minimum values, the AA concentrations ranged from 34% to 98% (median, 82%) of the minimum requirement stated in the AAFCO Dog and Cat Food Nutrient Profile. The 2 diets below the minimum value for lysine (98% and 93% of the minimum requirement) were within the analytic variation (20%) allowed by the AAFCO regulations; lysine was the only AA for which the AAFCO provided an allowance for analytic variation. All other AAs that did not meet the AAFCO minimum requirement exceeded the range of analytic variation provided by the laboratory.

Calorie content was provided on the label for 10 diets (4 dry and 6 canned). Calorie content was obtained from the product website for 2 diets (1 dry and 1 canned) and from the manufacturer (on a volume basis only [can or cup]) for 10 diets (6 dry and 4 canned). Calorie content information could not be obtained for 2 diets (both dry). Calorie content was calculated for 4 canned diets by use of the per-unit calorie content provided by the manufacturer, 4 dry diets by use of the modified Atwater factor and ash content provided by the manufacturer, and 4 dry diets by use of the modified Atwater factors and mean ash content calculated for dry diets (n = 8) for which the ash concentration was measured (5.76% on an as-fed basis). Median calorie content (DM basis) for all 24 diets was 3,758 kcal of ME/kg of diet (range, 2,915 to 4,316 kcal of ME/kg of diet). Median calorie content (DM basis) of the 17 diets for dogs was 3,725 kcal of ME/kg of diet (range, 3,233 to 4,316 kcal of ME/kg of diet) and of the 7 diets for cats or for both cats and dogs was 3,843 kcal of ME/kg of diet (range, 2,915 to 4,050 kcal of ME/kg of diet). One diet (canned maintenance diet for dogs) required adjustments in nutrient concentrations on the basis of the correction for calorie content.

Discussion

One objective for the present study was to assess product labeling by comparing diet labels with the AAFCO model regulations.[13] Although all pet foods must comply with federal labeling requirements,[22]

many states also mandate specific aspects of the label, often by adopting the AAFCO labeling and formulation requirements in full or in part.[10] Despite the fact all 24 diets were sold in most or all states, and even with exclusion of calorie content as a requirement, only 8 diets (including all 3 veterinary therapeutic diets) were compliant with all label regulations as established by the AAFCO.

There are 3 means of substantiating claims that pet foods are complete and balanced, and the label's nutritional adequacy statement must specify which method is used.[23] The first method is to formulate the diet to meet the AAFCO Dog and Cat Food Nutrient Profiles. The second method is to conduct a feeding trial by use of AAFCO-recognized protocols for the specified life stage; in the case of successful completion of an appropriate feeding trial, the pet food is exempt from meeting nutrient profiles. Third, if a food is a member of a nutritionally similar product family for which the designated lead product has successfully completed an AAFCO-recognized feeding trial, the label of the products for that food family may state that AAFCO feeding trials substantiate the claim of complete and balanced and the nutritional adequacy statements are indistinguishable. In both cases, the label will state that the product has passed animal feeding tests. When a product fails to meet 1 of the aforementioned 3 methods and is not clearly labeled on the principal display as a snack, treat, or dietary supplement, the product must contain a statement that indicates "intended for intermittent or supplemental feeding only." One diet in the present study had a nutritional adequacy statement that indicated it had successfully completed AAFCO-recognized animal feeding trials (which we confirmed by contacting the manufacturer) and was assessed as adequately formulated, although the CP concentration was 94% of the AAFCO nutrient profile minimum value; all AA concentrations exceeded the AAFCO minimum values. Of the 6 diets that did not have nutritional adequacy statements, none were labeled snack or treat, and they did not have a statement to indicate that the product was intended for intermittent or supplemental feeding only. Rather, the labels of those 6 diets included wording that indicated that they were intended to be complete and balanced (phrasing such as "100% complete" and "ideal maintenance"), which was inadequate.

The AAFCO Dog and Cat Food Nutrient Profiles provide minimum values for CP and essential AA concentrations (as well as a maximum value for methionine concentration in foods formulated for cats) for pet foods made with complex, nonpurified ingredients and to account for effects of processing and impacts on digestibility. Most (23/24) diets assessed in the present study met guaranteed analysis claims for minimum CP concentration, and most (23/24) diets exceeded CP minimum values for the AAFCO nutrient profiles; however, CP concentration was assessed with in vitro methods that provided an estimate of protein content calculated by use of the nitrogen concentration. As such, the calculated CP value provided no information related to protein quality, which is defined by the digestibility of the protein and the pattern and bioavailability of the AAs. It is generally recognized that plant protein sources have lower digestibility than do animal protein sources[24]; however, studies[25,26] of dogs have found equal total digestibility for soy-based protein when the soy product is adequately processed. Both animal and plant protein sources can vary in quality. Although protein digestibility was not assessed in the present study, short-term studies[27,28] revealed that animal-protein meals differ in their ability to support nitrogen retention in cats, with chicken and fish meals not differing from corn gluten meal, whereas meat meal is superior to corn gluten meal. Because digestibility, AA pattern, and AA bioavailability are not provided on product labels, protein quality cannot be assessed from a pet food's ingredient list or guaranteed analysis regardless of the fact that nutrients may be present in concentrations that satisfy the corresponding AAFCO nutrient profile. Investigators of 1 study[29] reported limitations of measured CP concentrations for the assessment of protein quality of pet food as evaluated with feeding trials on growing rats. They reported that the biological variables for assessment of protein quality (including weight gain, feed efficiency, protein efficiency ratio, net protein ratio, and net protein utilization) had poor correlation with measured CP concentrations.[29] Furthermore, the sum of essential AA concentrations was not correlated with measured CP concentration or biological variables (protein efficiency ratio and net protein ratio).[29]

Concentrations and proportions of AAs are arguably more important than is CP concentration per se, and AA bioavailability should also be considered. Dogs and cats differ from many other species in that they have obligatory bile acid conjugation with taurine rather than glycine, which is associated with variable losses of taurine through feces. Effects of intestinal bacteria on taurine loss appear to be substantial[30,31] and may be exacerbated by dietary factors. Studies[32,33] have revealed that cats fed canned versus frozen-preserved diets, or diets with soybean versus casein protein, had lower plasma taurine concentrations, even though the diets were equal in taurine content. The negative effect on taurine status appears to be secondary to augmented loss of bile acids through microbial degradation and accelerated cholecystokinin-mediated turnover of bile acids.[34] In addition, fiber likely increases taurine losses in the feces by influencing intestinal bacterial populations as well as through other effects on bile acid metabolism.[35] In the present study, the 3 diets for dogs that provided methionine-cystine concentrations closest to the AAFCO minimum value (8%, 25%, and 35% above the minimum value) were all canned diets that did not provide additional purified sulfur-containing AAs. In addition, all 3 canned diets for cats were too low in taurine concentration despite supplementation. Because plant-based diets are typically lower in sulfur-containing AAs and higher in fiber, these factors may contribute to an increased risk of taurine deficiency in both dogs and cats fed vegetarian diets, especially canned products and products that do not provide supplemental taurine or its precursors.

Processing of pet foods impacts protein digestibility as well as AA bioavailability. Conditions for ingredient rendering, extrusion cooking, and can retorting include application of heat, moisture, pressure, or

mechanical shear to inactivate food-borne pathogens, increase shelf-life, increase digestibility of certain nutrients (denaturation of protein, gelatinization of starch, and inactivation of trypsin inhibitors in vegetable protein), and promote desirable flavor and texture.[36] However, despite these beneficial effects of processing, some nutrients are lost during processing. Nonenzymatic browning of foods during processing as a result of Maillard reactions is considered a major factor that negatively affects the quality of protein. Depending on the exact conditions and nutrients present, variable AA losses occur (especially losses of lysine, methionine, cystine, and tryptophan).[37] Concentrations of 3 of the 4 AAs (all but lysine) were too low in some of the diets assessed in the present study.

Two diets for cats, including 1 diet with purified L-lysine in the ingredient list, did not meet the minimum concentration for lysine as per the AAFCO food nutrient profiles, but the values for these 2 diets were within the analytic variation allowed by the AAFCO. A third diet provided lysine at only 1% above the minimum AAFCO value. However, bioavailability is an important consideration. Acid hydrolysis of protein, which is required for the measurement of AAs in food, results in reversion of damaged (unavailable) lysine and falsely increases the estimate of bioavailable lysine. In 1 study,[38] measurement of total lysine overestimated by 87% the bioavailable lysine concentration of 20 diets formulated for cats (10 dry and 10 canned). Lysine is commonly the limiting AA in cereals, and the impact of processing on lysine availability together with a limited ability to accurately assess available lysine concentrations with routine methods is of particular concern for commercially available vegetarian pet foods.

Notably, 6 of the 24 diets assessed in the study reported here were inadequate in 1 or more AAs; 3 of these diets were too low in sulfur-containing AAs (methionine, methionine-cystine, and taurine). However, on the basis of the ingredient lists, all 3 of those diets were supplemented with taurine, and 2 of those 3 diets were also supplemented with methionine. This finding is similar to that in a study[39] conducted to investigate nutritional adequacy of 2 commercially available vegan diets for cats. The authors of that study[39] found that both diets had inadequate concentrations of taurine, methionine, methionine-cystine, arachidonic acid, and pyridoxine. One of the diets had additional deficiencies of CP, arginine, lysine, calcium, phosphorus, vitamin A, niacin, and vitamin B_{12}, despite label claims of nutritional adequacy and the fact that limiting AAs were listed in the ingredient list as additive supplements. Dietary deficiencies in sulfur-containing AAs and lysine could result in decreased food intake, low growth rate, and negative nitrogen balance in both dogs and cats.[40–46] Furthermore, dermatitis has been reported in dogs[47] and cats[48,49] with methionine and lysine deficiency, and retinal and cardiac dysfunction has been reported in dogs[8,9] and cats[50] with taurine deficiency.

Analysis of results of the study reported here indicated problems with compliance with labeling regulations in addition to concerns regarding adequacy of AA concentrations in commercially available vegetarian pet foods. Overall, only 5 of 21 over-the-counter diets, but all 3 of the veterinary therapeutic diets, met all requirements for labeling and nutritional adequacy (excluding the recently published regulation for a calorie content statement); however, the sample size was small. Another important limitation of this study was that samples were collected at 1 time point and from 1 batch of each product. The samples that were assessed for CP and AA concentrations may not have been representative because of variations in composition for each batch. In addition, although assay variability for both AA and CP analysis was low, substantial variations in results attributable to laboratory methods were possible. Regardless, all nutritional and labeling requirements should be met consistently, and manufacturers are responsible for quality assurance. It may be informative to measure the CP and AA concentrations across numerous batches to assess variation and more accurately determine the deviation from nutritional adequacy and regulatory compliance.

In the present study, we assessed only a limited number of essential nutrients in commercially available vegetarian pet foods. A more thorough evaluation of other essential nutrients is warranted, especially because important inadequacies of other nutrients in vegan pet foods have been reported.[39] In addition, there was no assessment of the animals while consuming the diets; evaluation of blood AA concentrations would provide valuable information for assessing the AA adequacy of pet foods.[51] Only 1 diet had a nutritional adequacy statement indicating that it had passed AAFCO feeding trials to substantiate a claim of complete and balanced for the specified life stages. Given that both the present study and a previous report[39] documented deficiencies of nutrients that were declared to have been included in purified form, this may be evidence that manufacturing errors occur or that diets are not formulated properly. Veterinary therapeutic diets may be more appropriate options for vegetarian pet foods because all 3 veterinary diets assessed in the study reported here met current nutritional adequacy and labeling requirements, compared with only 5 of 21 over-the-counter diets that met the nutritional adequacy and labeling requirements. In addition, the US FDA provides allowance for the marketing of veterinary therapeutic diets under the presumption that they are used only under the direction of a licensed veterinarian who is providing recommendations for appropriate use of the product and for monitoring of individual patients.[52] It may be prudent that such monitoring includes measurement of plasma AA and whole blood taurine concentrations as well as routine assessment of general health to more fully evaluate the status of pets eating vegetarian diets. Given the findings of the present study, this may be of even greater importance for dogs and cats eating canned vegetarian diets, in which case regular monitoring of taurine status in particular is strongly recommended. For all animals and regardless of diet, general routine monitoring and assessment are necessary for adequate nutritional evaluation and to enable clinicians to provide recommendations for individual animals.[53]

a. Sacramento Animal Hospital, Sacramento, Calif.
b. UC Davis Analytical Lab, University of California-Davis, Davis, Calif.

c. Biochrom 30, Biochrom Ltd, Holliston, Mass.
d. Microsoft Office Excel 2008, Microsoft Corp, Redmond, Wash.
e. Ami Dog, Ami, Padova, Italy.
f. Gourmet Fondue Veggie Cheese Burger Flavor, Evolution Diet Pet Food, Saint Paul, Minn.
g. Incredibly Delicious Gourmet Pasta, Evolution Diet Pet Food, Saint Paul, Minn.
h. Vegetarian Formula for Dogs, Dick Van Patten's Natural Balance Pet Foods, Pacoima, Calif.
i. Vegan Garden Medley Adult, Halo Purely for Pets, Tampa, Fla.
j. Nature's Recipe Healthy Skin Vegetarian Recipe, Big Heart Pet Brands, San Francisco, Calif.
k. Veterinary Diets HA Hypoallergenic Canine Formula, Néstle Purina, St Louis, Mo.
l. Veterinary Diet Canine Vegetarian, Royal Canin, Charles, Mo.
m. V-dog, V-dog Food, Sacramento, Calif.
n. Ami Cat, Ami, Padova, Italy.
o. Gourmet Fondue Veggie Cheese Burger Flavor, Evolution Diet Pet Food, Saint Paul, Minn.
p. Incredibly Delicious Gourmet Pasta, Evolution Diet Pet Food, Saint Paul, Minn.
q. Vegan, Wysong Corp, Midland, Mich.
r. AvoDerm Natural Vegetarian Formula, Central Garden and Pet Co, Walnut Creek, Calif.
s. Vegetable Stew Entrée, Evolution Diet Pet Food, Saint Paul, Minn.
t. Vegetarian Formula, Dick Van Patten's Natural Balance Pet Foods, Pacoima, Calif.
u. Vegan Garden Medley for Dogs, Halo, Purely for Pets, Tampa, Fla.
v. Nature's Recipe Stew Healthy Skin Vegetarian Recipe Cuts in Gray, Big Heart Pet Brands, San Francisco, Calif.
w. Organic Vegan Formula, PetGuard, Green Cove Springs, Fla.
x. Vegetarian Feast Dinner, PetGuard, Green Cove Springs, Fla.
y. Veterinary Diet Canine Vegetarian, Royal Canin, Charles, Mo.
z. Gourmet Entrée, Evolution Diet Pet Food, Saint Paul, Minn.
aa. Vegetable Stew Entrée, Evolution Diet Pet Food, Saint Paul, Minn.
bb. Vegetarian Dinner, Evanger's Dog and Cat Food Co, Wheeling, Ill.

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Influence of mercury and selenium chemistries on the progression of cardiomyopathy in pygmy sperm whales, *Kogia breviceps*

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HIGHLIGHTS

- More than half of stranded pygmy sperm whales exhibit signs of cardiomyopathy.
- Hg and Se balance and oxidative stress may influence progression of cardiomyopathy.
- Adults have significantly greater Hg:Se liver molar ratios than younger age classes.
- Hg:Se molar ratios were greater in males and increased with heart disease progression.
- Protein oxidation was greater in males and increased with heart disease progression.

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ABSTRACT

More than half of pygmy sperm whales (*Kogia breviceps*) that strand exhibit signs of cardiomyopathy (CMP). Many factors may contribute to the development of idiopathic CMP in *K. breviceps*, including genetics, infectious agents, contaminants, biotoxins, and dietary intake (e.g. selenium, mercury, and pro oxidants). This study assessed trace elements in *K. breviceps* at various stages of CMP progression using fresh frozen liver and heart samples collected from individuals that stranded along US Atlantic and Gulf coasts between 1993 and 2007. Standard addition calibration and collision cell inductively coupled plasma mass spectrometry (ICP MS) were employed for total Se analysis and pyrolysis atomic absorption (AA) was utilized for total Hg analysis to examine if the Se/Hg detoxification pathway inhibits the bioavailability of Se. Double spike speciated isotope dilution gas chromatography ICP MS was utilized to measure methyl Hg and inorganic Hg. Immunoblot detection and colorimetric assays were used to assess protein oxidation status. Data collected on trace elements, selenoproteins, and oxidative status were evaluated in the context of animal life history and other complementary histological information to gain insight into the biochemical pathways contributing to the development of CMP in *K. breviceps*. Cardiomyopathy was only observed in adult pygmy sperm whales, predominantly in male animals. Both Hg:Se molar ratios and overall protein oxidation were greater in males than females and increased with progression of CMP.

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1. Introduction

Greater than fifty percent of pygmy sperm whales (*Kogia breviceps*) that strand show signs of cardiac degradation and cardiomyopathy (CMP) (Bossart et al., 1985, 2007). Natural occurrence of CMP in *K. breviceps* is greater than all other mammal species studied to date. The idiopathic nature of this disease requires studying many of the possible factors that may contribute to the development of CMP in *K. breviceps*, including genetics, infectious agents, chemical toxins, biotoxins, contaminants, and nutritional abnormalities (e.g. trace elements, vitamins, and pro oxidants). This study focuses contaminant uptake and tissue distribution involving selenium (Se) and mercury (Hg) chemistries, along with a

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marker for protein oxidative stress, which may contribute to CMP onset and progression. Nutritional deficiencies of Se, accumulation of Hg, and imbalance of oxidants have been shown in humans, mice, hamsters, cattle, and dogs to play roles in the development and progression of CMP (Kennedy et al., 1987; Bartfay et al., 1998; Freeman et al., 1998; Kannan et al., 2004; Ichihara et al., 2006).

Marine mammals exhibit an Hg detoxification metabolism wherein Se is specifically involved in Hg detoxification pathways. Methyl Hg is demethylated to inorganic Hg (iHg) that can bind with elemental Se to form Hg Se crystals, which are stored in the liver and cannot be mobilized. Selenium binds strongly to Hg and has a greater affinity for Hg than sulfur due to a low solubility product constant ($K_{sp} = 1 \times 10^{-59}$ at 298 K). This mechanism ap pears to be uniquely self limiting in marine mammals; measured Hg:Se molar ratios near or less than 1 are typical. In other species (e.g. birds) that are more sensitive to Hg contamination, Hg:Se mo lar ratios can exceed 10:1(Scheuhammer et al., 2008). Relating the Hg and Se chemistries and oxidation measurements to CMP in pyg my sperm whales allows for a first look into whether the Hg detox ification pathway could play a role in CMP progression by sequestering bioavailable Se that may otherwise by channeled into chemoprotective pathways, for example in selenoprotein/sele noenzyme synthesis, and Se antioxidant biochemistry.

Marine mammals can serve as key sentinel species for environ mental health since they are long lived, top predators that utilize many of the same ocean resources as humans (Wells et al., 2004; Bossart, 2006). Increased human activity in recent decades has accelerated inputs of Hg into the marine environment resulting in larger amounts entering marine food chains. Mercury concentra tions have been well documented in marine mammal species that have strong interactions with humans through sharing coastal water habitats, subsistence hunting, or being caught as fisheries by catch, while little is known about pygmy sperm whales that rarely have interactions with humans until stranding (Wells et al., 2004; Booth and Zeller, 2005). During 2003, an increased number of pygmy sperm whales stranded along the southeastern coasts of the United States and renewed marine mammal commu nity interest in understanding health problems associated with the species (Berini, 2009). Pygmy sperm whales are pelagic odontoce tes that feed at higher trophic levels than mysticetes and other marine organisms, translating to relatively higher accumulation of toxic metals such as Hg. Pygmy sperm whales primarily con sume a cephalopod diet that is high in polyunsaturated fatty acids (PUFAs) and free radicals. Lacking sufficient antioxidant defenses, PUFAs can turn rancid very rapidly and can impact cellular mem brane structure and function (Kennedy et al., 1987). Stomach con tents of pygmy sperm whales have revealed their main prey items are squid in the family Histioteuthidae, which are predatory squid that concentrate Hg and other contaminants in the food chain (Bustamante et al., 2006; Santos et al., 2006). It is uncertain if the restrictive diet of *K. breviceps* is playing a role in the develop ment and progression of CMP by requiring an excess in antioxi dants, while simultaneously adding large enough doses of Hg that can contribute to oxidative damage and affect Se biochemistry response. This working hypothesis was studied by analyzing Hg and Se chemistries in conjunction with protein oxidation in the context of cardiac disease state.

2. Materials and methods

2.1. Study population and sample collection

Pygmy sperm whale samples were collected from animals that stranded from 1993 to 2007 along the United States Atlantic and Gulf of Mexico coasts. All animals that are part of this study were either euthanized or freshly dead in order to minimize postmortem degradation of samples. Liver tissue from individuals ($n = 30$) were collected according to NISTIR 6279 for the National Marine Mam mal Tissue Bank (NMMTB) housed at the National Institute of Stan dards and Technology (NIST), Charleston, SC and stored under cryogenic conditions (approximately −150 °C) (Becker et al., 1999). Additional heart ($n = 11$) and liver ($n = 32$) samples donated for use in this project were collected by the Center for Coastal Envi ronmental Health and Biomolecular Research (CCEHBR) NOS/ NOAA, Charleston, SC and stored at −80 °C until analysis. Fresh heart tissue was available for collection at necropsies from some individual animals ($n = 36$) for histology preparation and histopa thological evaluation. Individual animal life history and gross pathology data for this project was generated by the collaborating institutions and communicated in the form of necropsy reports. Animals were divided into three age classes based on the following criteria: calf, dependent animal that is observed with its presumed mother or <200 cm; subadult, independent sexually immature ani mals 200 270 cm; adult, independent animals with observed indi cation of sexual maturity or >270 cm (Bossart et al., 1985).

2.2. Sample preparation

Fresh frozen tissue was cryogenically homogenized to produce a uniform sample composition of fresh frozen powder for analysis. Liver samples from the NMMTB were homogenized in class 100 clean room conditions at NIST, Charleston, SC using cryogenic pro cedures developed by Zeisler et al. (1983) and Pugh et al. (2007). Left ventricle heart and liver tissue samples donated by CCEHBR/ NOS/NOS were cryogenically homogenized using a bench top 6850 Freezer/Mill (SPEX SamplePrep, Metuchen, NJ). Samples were placed in vials with a stainless steel impactor, capped, placed in the mill, submerged in LN_2, and shaken at 10 Hz for 3 min. Homoge nized powder was transferred into pre cleaned polypropylene jars and stored at −80 °C.

Acid assisted microwave digestion using PTFE pressurized ves sels was utilized to decompose liver and heart tissue samples prior to performing inductively coupled plasma mass spectrometry (ICP MS) analyses of total Se. Sample digestion methods are described in detail in the Supplementary Material. Tissue samples were ali quotted directly in nickel weigh boats and weighed for total Hg (THg) measurements. Sample preparation methods for MeHg and iHg were described in detail by Davis et al. (2007).

Hematoxylin and eosin (H&E) stained histology slides for each animal case were evaluated independently without knowledge of life history or chemical analyses data by a veterinary pathologist and assigned a heart score according to criteria put forth by Bossart et al. (2007). Heart histology and gross pathology was used to cat egorize heart disease in the following three stages: no pathological findings (NPF), myocardial degeneration (MCD), and cardiomyopa thy (CMP).

2.3. Analytical techniques

2.3.1. Instrumentation

Total Se mass fraction measurements were collected using a Thermo Electron X Series II ICP MS (Bremen, Germany) with a standard low volume glass impact bead spray chamber (Peltier cooled at +3 °C), concentric glass nebulizer, and operating in colli sion cell mode utilizing 8% H_2 in 92% He as the collision cell gas. The collision cell ICP MS working conditions were optimized with a 10 ng/g 68 element tuning solution and a Se calibrant prior to sample analysis. The mass fraction of THg was determined with a direct mercury analyzer DMA 80 (Milestone Scientific, Shelton, CT) by pyrolytic sample decomposition, catalytic reduction to

Hg^0, and trapping on a gold amalgamation trap. The Hg was then thermally desorbed and the Hg atomic absorbance was measured at 254 nm. Methyl Hg and iHg measurements were made using a Thermo Trace GC Ultra gas chromatograph (ThermoFinnigan, Aus tin, TX) equipped with a 30 m DB 5MS + DG 250 μm i.d. capillary column coated with a 0.25 μm thick film of (5% Phenyl) meth ylpolysiloxane (J & W Scientific, Folsom, CA). The GC was coupled to a Thermo Elemental X7 quadrupole ICP MS (Winsford, UK) by a Thermo GC/ICP MS commercial interface.

2.3.2. Calibration methods and sample measurements

An analytical quantification and validation scheme using the method of standard additions was employed for Se mass fraction measurements in liver and heart samples (Christopher et al., 2005). A NIST interlaboratory comparison exercise control mate rial, QC03LH3 pygmy sperm whale liver homogenate was used to build matrix matched standard addition calibration curves for li ver tissue analysis by spiking at different concentration levels. Cal ibration curve slopes were used to assign Se concentrations in unknown samples that were unspiked. Single point standard addi tion methods were used for heart tissue analysis since a whale heart CRM is not available and calibration curves from one tissue (e.g. liver) are not transferable to another tissue type (e.g. heart) to produce accurate data. Single point methods avoid matrix inter ferences by splitting a single sample and spiking one of the sample splits. The spike was prepared from SRM 3149 Selenium Standard Solution (NIST, Gaithersburg, MD).

Total Hg concentrations were determined by external calibra tion utilizing SRM 1946 Lake Superior fish tissue (NIST, Gaithers burg, MD) and QC03LH3 pygmy sperm whale liver homogenate by aliquotting different masses of the certified reference materials (CRMs) in nickel sample boats. The slope and intercept from the established calibration curves for the CRMs were used to calculate the concentration in the heart, liver, and control material samples. MeHg and iHg were measured using double spike speciated iso tope dilution methods as described in detail by Davis et al. (2007). Details of quality assurance are provided in the Supple mentary Material. Reported concentrations for Se, THg, and MeHg are presented as mass fraction values, expressed in μg/g on a wet mass fraction basis.

2.4. Protein oxidation

Protein oxidation status was examined in liver samples from 30 individual pygmy sperm whales that were banked in the NMMTB, have been cryogenically homogenized, and were prepared for anal ysis under anoxic conditions. Only NMMTB livers were measured for protein oxidation since sample collection and storage integrity minimized outside oxidation exposure to the samples. Oxyblot™ Protein Oxidation Detection Kit (Chemicon International, Temecu la, CA) was used for immunoblot detection and quantification of proteins that have been modified by free radicals. Methods for pro tein oxidation and calculating the oxidation ratio are described in detail in the Supplementary Material. Normalizing for varying pro tein concentrations between animal samples was achieved by per forming a Bio Rad protein assay (Bio Rad Laboratories, New York), which is based on the Bradford method using bovine serum albu min as a standard protein (Bradford, 1976).

Cayman's GSH assay kit (Cayman Chemical Company, Ann Ar bor, MI) was used to measure total free glutathione by measuring both total GSH and GSSG. The kit utilized glutathione reductase for enzymatic recycling to quantify GSH (Tietze, 1969; Eyer and Pod hradský, 1986; Baker et al., 1990). Variation between 96 well plates was normalized by running QC03LH3 on each plate and dividing the sample concentration by the QC03LH3 concentration on the plate to produce a relative sample concentration that accounted for inter plate variability to facilitate among sample, be tween plate comparisons.

2.5. Statistical analyses

Statistics were performed using JMP 7 (SAS Institute Inc., Cary, North Carolina) and Microsoft Excel (Redmond, Washington). Data was first tested for normality using a Shapiro Wilk goodness of fit test and equal variance was tested with the Levene median test. Pearson's correlation analyses were carried out to determine if mo lar concentrations between Hg and Se were linearly associated within each tissue; and to determine whether Hg and Se correlated with protein oxidation levels within liver tissue. Analysis of vari ance (ANOVA) was used to analyze the relationship of trace ele ment concentrations, molar ratios, and protein oxidation with the factors of age class, gender, and heart disease stage. Tukey hon estly significant difference (HSD) tests and least squares (LS) mean plots were performed on statistically significant ($p < 0.05$) data to determine how the means varied within a factor.

3. Results and discussion

3.1. Concentrations of trace elements in liver and heart tissue

Summary data for Se, THg, and MeHg concentrations, % MeHg, and Hg:Se molar ratios determined in pygmy sperm whale liver and heart tissue are presented in Table 1. The US Environmental Protection Agency (USEPA) reference doses for THg and MeHg in edible fish tissue are 0.300 μg/g, wet mass fraction and 0.100 μg/ g, wet mass, respectively (USEPA, 1999). All pygmy sperm whale liver and heart samples analyzed reflect THg and MeHg concentra tions that were over USEPA action limits, which may reflect levels that are potentially hazardous to pygmy sperm whale health. Odontocetes are exposed to high levels of Hg, primarily in the MeHg form, through squid, crustacean, and fish consumption. Oce anic squid in the family Histioteuthidae comprise 80% of the pyg my sperm whale diet (Santos et al., 2006). In *Histioteuthis reversa*, MeHg represents 83% of the THg (0.015 ± 0.005 μg/g, wet mass fraction) in whole squids (Bustamante et al., 2006). While Hg con centrations in squid may appear relatively low, dietary Hg expo sure allows biomagnification up the food chain along with bioaccumulation by individuals yielding high Hg concentrations in pygmy sperm whales. Similar to pygmy sperm whales, squid is the major food item for long finned pilot whales (*Globicephala melas*) and short finned pilot whales (*Globicephala macrorhyn chus*). Methyl Hg concentrations in *K. breviceps* liver tissues were comparable to both pilot whale species (Caurant et al., 1996; Bus tamante et al., 2003). Many humans consume squid and the diet of Faroe islanders, indigenous people in the Arctic, and some people in Japan consists partly of marine mammal tissue, which poses possible health risks from the dietary intake of Hg mainly in the more toxic MeHg form (Myers and Davidson, 1998; Wagemann et al., 1998; Booth and Zeller, 2005; Endo et al., 2005; Bustamante et al., 2006).

Previous studies on trace elements in for *K. breviceps* include small data sets which primarily examined total element concentra tions (Supplementary material, Table S2). The data set in this study is the largest and most comprehensive to date for *K. breviceps* assessing element speciation, and it is the first to present data for heart tissue. Pearson's correlation analysis indicated there were no correlations between heart and liver tissue concentrations of Se ($p = 0.422$, $r = 0.270$) or Hg ($p = 0.931$, $r = 0.030$), which was unex pected since internal organ correlations have been found for these elements in other odontocete species (Meador et al., 1999; Yang et al., 2002; Bryan et al., 2007). Selenium concentrations were

Table 1
Trace element concentrations (μg/g, wet mass fraction), % MeHg, and Hg:Se molar ratios in heart and liver from pygmy sperm whales.

	THg	MeHg	% MeHg	Se	Hg:Se
Heart					
n	11	11	11	11	11
Mean	1.409	1.786	92.761	2.389	0.242
SD	0.523	0.819	3.861	0.704	0.115
Range	0.301–2.482	0.248–3.360	81.950–95.840	1.405–3.792	0.084–0.522
Liver					
n	62	7	7	62	62
Mean	11.537	1.102	33.306	9.444	0.416
SD	10.627	0.507	14.019	4.399	0.266
Range	0.385–56.888	0.156–1.650	17.030–51.540	2.005–21.551	0.009–1.039

approximately 4 times greater in the liver than the heart, and THg concentrations were approximately 8 times greater in the liver than the heart. Methyl Hg reflected 92.761 ± 3.861% of the THg in heart tissue and showed no trend with increasing THg concentra tions, while 33.306 ± 14.019% of THg in the liver is MeHg and per cent MeHg exponentially decreased with increasing THg concentrations (Supplementary material, Fig. S3). At low THg con centrations the metal is mainly in the MeHg form and at greater THg concentrations demethylation is occurring resulting in iHg as the predominant form. Increasing THg concentrations in con junction with decreasing percent MeHg verified that the liver is the site of Hg deposition. Similar exponential decrease trends in percent MeHg relative to increasing THg were additionally ob served in pilot whale and striped dolphin (*Stenella coeruleoalba*) livers and the authors of these studies also concluded that the liver is the site of Hg demethylation and iHg storage (Palmisano et al., 1995; Caurant et al., 1996). Heart tissue was comparable to blood and skeletal muscle tissue in other cetacean species regarding THg concentrations, percent MeHg, and no relationship between per cent MeHg and increasing THg. Since the heart is muscle tissue and pumps blood, it was anticipated that Hg concentrations and elemental species would behave similar to skeletal muscle and blood. Comparable to percent MeHg in *K. breviceps* heart tissue, bottlenose dolphins (*Tursiops truncatus*) were found to have 0.512 ± 0.363 μg/g THg and 91% MeHg in blood (Bryan et al., 2005, 2007). In other studies that examined percentage MeHg to THg in liver and muscle tissue, pilot whale and beluga (*Delphin apterus leucas*) percent MeHg ranged from approximately 3 33% in liver and 78 97% in muscle (Caurant et al., 1996; Wagemann et al., 1998).

A strong positive correlation existed between Se and THg con centrations in liver ($p < 0.001$, $r = 0.773$) and no correlation was present between Se and THg concentrations in heart ($p = 0.498$, $r = 0.229$). Strong positive correlations between Se and Hg concen trations in pygmy sperm whale tissues were expected since this has been reported commonly in internal organs, such as liver and kidney, of other marine mammal species (Becker, 2000). The mean molar ratio of Hg:Se in liver was 0.416 ± 0.266 (0.009 1.039). Law et al. (2001) found a Hg:Se molar ratio of 0.32 in a one pygmy sperm whale liver, emphasizing the need for greater sampling ef fort. Many individuals fell close to having 1:1 molar ratios between Hg and Se in liver. The 1:1 molar ratio relationship between Hg and Se in liver was first noted in marine mammals in 1973 by Koeman et al. and has since been observed in several marine mammal spe cies trace element studies (Koeman et al., 1973; Becker, 2000; Scheuhammer et al., 2008). In *K. breviceps* liver, Hg:Se molar ratios increased in individual animals as a function of increasing THg mo lar masses as illustrated in Fig. 1. Studies on fish eating bird species have shown onset of neurological and birth defects once the Hg:Se molar ratio exceeded 1(Scheuhammer et al., 2008). To date, delete rious health impacts have not been reported in marine mammals

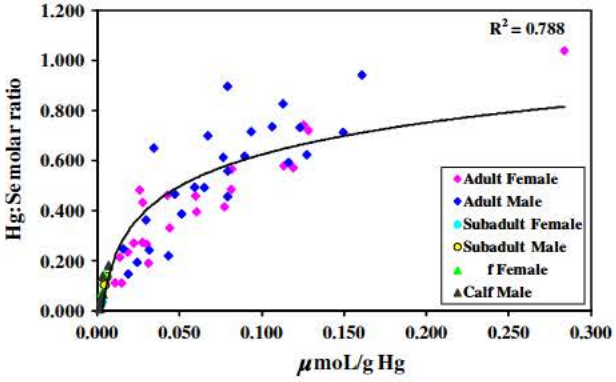

Fig. 1. Relationship between THg molar mass and Hg:Se molar ratio in livers of pygmy sperm whales, partitioned as a function of gender and age class.

in conjunction with high Hg concentrations suggesting that marine mammals have developed a mechanism to deal with high Hg in take. Selenium aids in detoxifying Hg by forming mercury selenide crystals which could limit Se bioavailability for seleno amino acid formation, protein synthesis, and metalloprotein binding. Seleno proteins, selenoenzymes, and Se binding proteins play important roles in antioxidant biochemistry. Unlike many enzymes that can be recycled once used, each time a selenoenzyme is used it must be broken down and synthesized all over again making the Se accessible to bind to free Hg. Selenium bioavailability may be re stricted in the presence of high Hg concentrations measured in pygmy sperm whales. Some studies suggest that all Hg binds to Se and the quantity of non Hg bound Se can be calculated by the molar difference of the two elements ($Se_{nmol/g}$ $Hg_{nmol/g}$) (Drasch et al., 2000; Falnoga et al., 2006). Pygmy sperm whales with an Hg:Se molar ratio greater than 1 had negative values for the quan tity of non Hg bound Se signifying that all bioavailable Se may be bound to Hg. Total trace element concentrations do not give much insight into the actual mechanisms or pathways by which Se and Hg interact and how the Hg sequestering mechanism influences Se bioavailability and biochemistry, but allow associations to be made between concentration data, animal life history information, and heart disease state.

3.2. Protein oxidation in pygmy sperm whale liver

Proteins are a key target of pro oxidants and free radicals. Immunoblot was used to assess overall protein oxidation since this method allowed immunodetection and quantification of carbonyl groups that were introduced to proteins which were modified by oxidation. Oxidation ratio differences were not detected in correla tion to Se or Hg concentrations. Total free GSH and GSSG assays measured antioxidants in a specific pathway that aid in protecting

cells from pro oxidants and free radicals at the level of interception (Arteel and Sies, 2001). Glutathione can function to detoxify xeno biotics and this correlation was examined with Hg and Se concen trations in pygmy sperm whale liver. Correlations were not observed between Hg concentration and GSH states, which may be a limitation of the assay only measuring free GSH and not other pools of GSH such as that bound to proteins. While this study could not examine the GPx activity in *K. breviceps* liver tissue, GPx pro duces GSSG during the reduction of hydroperoxides and GSSG is reduced to two GSH molecules. Activity of GPx is thought to be di rectly proportional to the rate of GSSG produced (Reed, 1990). Glu tathione peroxidase contains the seleno amino acid selenocysteine in its polypeptide chain. Although not statistically significant, there is a positive correlation between Se concentration and GSSG ($p = 0.064$, $r = 0.349$) in pygmy sperm whale liver (Supplementary material, Fig. S4). Humans suffering from chronic liver disease have been found to have positive and highly significant correlations be tween Se concentrations and GPx activities and the study con cluded that these correlations may be well correlated with GSH and Se accessibility (Czuczejko et al., 2003).

The liver is secondarily affected in pygmy sperm whales with CMP and hepatic congestion was observed in these animals. Exam ining protein oxidation status in the liver relative to CMP was of key interest for this study since the liver is the primary organ for selenoprotein synthesis and xenobiotic uptake, detoxification, and storage or excretion. To date, there is no literature available that examines oxidative stress markers in relationship to diseases affecting marine mammals. Effects of dietary fat intake on anti oxi dative state have been studied in captive bottlenose dolphins. Ani mals fed a high fat fish diet resulted in blood serum lipid peroxidase levels that were significantly higher than animals fed a low fat fish diet and the study concluded that decreased antiox idative states may be strongly influenced by high amounts of PU FAs and fat in diet (Kasamatsu et al., 2001). The mainly squid diet of pygmy sperm whales is relatively low fat (approximately 2% total lipid) when compared to a fish diet, however squid have PUFA levels greater than most fish species (Kirsch et al., 1998; Iver son et al., 2002; Recks and Seaborn, 2008). Pygmy sperm whales are potentially exposed to oxidative stress by ingesting large amounts of PUFAs and Hg. PUFAs are prone to oxidation because of multiple double bonds that rapidly transform into epoxides. Mercury promotes free radical formation through univalent redox reactions and has a very high affinity for thiol groups, which can lead to oxidative stress and lipid peroxidation (Ganther, 1980). Antioxidants, such as selenoproteins, glutathione, and GPx, defend against oxidants by prevention, interception, and repair of oxida tive damage. Oxidative stress can impair protein function, damage DNA, and damage membrane lipids which can lead onset set of dis ease (Arteel and Sies, 2001).

3.3. Trace element concentrations and protein oxidation in relation to age class, gender, and heart disease stage

3.3.1. Age class

Animals were grouped into age classes based on total body length and observations of sexual maturity. Pygmy sperm whale adults can range in age from 4 to 22 years old, which may mask some statistical patterns due to the broad range of ages in the adult age class. Adult animals have THg and Se concentrations in liver that are significantly greater ($p < 0.0001$) than younger age classes. Selenium concentrations in liver are greater in calves and subad ults than THg concentrations and this pattern reverses in adult ani mals (Fig. 2). Mercury increases little with age in fast growing calf and subadult pygmy sperm whales because of growth dilution, while in adults as growth slows Hg continuously bioaccumulates, prey are larger in size, and quantities of food ingested increase.

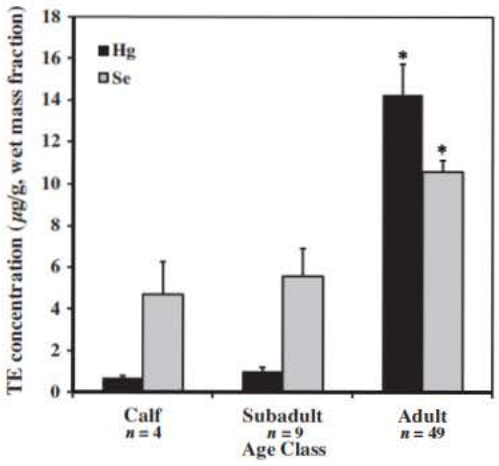

Fig. 2. Mean (+SE) THg and Se concentrations in pygmy sperm whale liver, partitioned as a function of age class. Significantly different mean Hg and Se concentrations between age classes are denoted with an asterisk ($p < 0.0001$).

Mercury has been shown to accumulate in liver in relationship to age in bottlenose dolphin (Meador et al., 1999). In heart tissue, Se concentrations are greater in adults than calves ($p = 0.151$) and THg concentrations are significantly greater in adults than calves ($p = 0.016$). When heart tissue concentrations were com pared within an age class, Se is greater than THg in calves and adult pygmy sperm whales, which does not reflect the pattern that was observed in adult liver tissue. As a comparison, significant differ ences in Se concentrations between age classes were not observed in bottlenose dolphins (Bryan et al., 2007). Age plays a role in the molar relationship between Hg and Se in liver. Adult animals bio accumulate higher concentrations of Hg, their Hg:Se molar ratios are significantly greater ($p < 0.0001$) than younger age classes, and their Hg:Se molar ratio approaches one (Fig. 1). Age class dif ferences were not observed for protein oxidation.

3.3.2. Gender

Full factorial ANOVA showed no significant differences in Se ($p = 0.590$) and Hg ($p = 0.097$) concentrations in liver between adult males and females. Statistically significant differences be tween genders have also not been observed for Se concentrations in bottlenose dolphins and humans; and Hg concentrations in hu mans (Drasch et al., 2000; Bryan et al., 2007). In adult *K. breviceps*, Hg:Se molar ratios were greater in males than females ($p = 0.072$) (Fig. 3A). Bottlenose dolphin and pilot whale females, more specif ically lactating pilot whale females, exhibit greater Hg concentra tions and Hg:Se molar ratios in liver tissue than males (Caurant

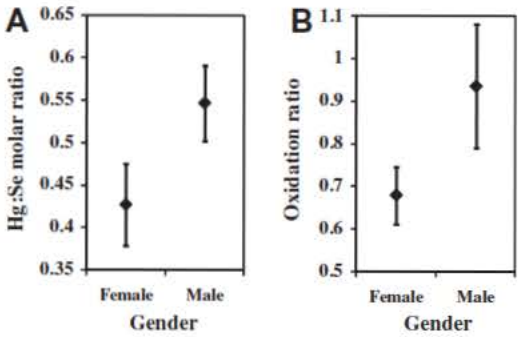

Fig. 3. Mean (±SE) (A) Hg:Se molar ratios in livers of adult females ($n = 22$) and males ($n = 22$) and (B) immunoblot oxidation ratios in livers of females ($n = 13$) and males ($n = 17$).

et al., 1996; Meador et al., 1999; Bryan et al., 2007). Adult females are thought to have greater bioaccumulation of Hg than adult males due to increased dietary consumption to keep up with the energy demands of gestation and lactation (Bryan et al., 2007). The same gender pattern was expected to prevail in pygmy sperm whale females, especially since they reach sexual maturity at a younger age of approximately 3.5 years old than bottlenose dol phin females, which become sexually mature at 5 10 years old. Since age class differences were not observed for protein oxidation, all age classes were included in statistical analyses of gender and protein oxidation. Immunoblot oxidation ratio gender patterns were similar to Hg:Se molar ratio gender patterns (Fig. 3). While not statistically significant ($p = 0.160$), males have a greater mean oxidation ratio than females. Total GSH means were not different between male and females.

3.3.3. Heart disease stage

Only a subset of 5 of the 11 heart samples measured for Hg and Se content possessed the complementary histological information needed to assign a heart disease score, preventing statistical com parisons of trace element concentrations in heart tissue relative to heart disease stage. A heart score was assigned for 36 of 62 liver samples. Animals that have NPF have significantly lower ($p = 0.004$) Se concentrations and lower ($p = 0.094$) Hg concentra tions than animals affected with MCD and CMP. Selenium concen trations increase in conjunction with increasing Hg concentrations and both relate to MCD and CMP in pygmy sperm whales. While not statistically significant ($p = 0.236$), animals with MCD and CMP have greater liver Hg:Se molar ratios than animals with NPF (Fig. 4A). Mercury concentrations drive the overall Hg:Se ratio when Hg is present in greater concentrations than Se. Selenium may be bound to Hg to detoxify Hg and is no longer bioavailable in heart disease affected animals.

Of the 30 pygmy sperm whale livers analyzed for protein oxida tion, heart scores were able to be assigned to 21 individual ani mals. Although not statistically significant ($p = 0.453$), animals affected with CMP have greater mean immunoblot oxidation ratios than animals with NPF or MCD (Fig. 4B, and supplemental Fig. S1). Gender may be interrelated with heart disease stage in adult ani mals, since prevalence of CMP was greater in males and males had both greater Hg:Se and oxidation ratios (Fig. 3), which were both elevated in animals with CMP (Fig. 4). Overall, stage of CMP progression did not relate to reduced or oxidized glutathione con centrations (data not shown); these measurements provide com plementary information about selenium specific oxidation pathways, and not an overall protein oxidation status. These find ings point out that it is difficult to determine whether oxidative stress directly or indirectly plays a role in CMP progression. Other studies have pointed out that redox equilibrium is essential to bio logical systems and that imbalance due to oxidative stress or swinging the pendulum too far in the direction of reductive stress can result in similar deleterious health effects (Rajasekaran et al., 2007).

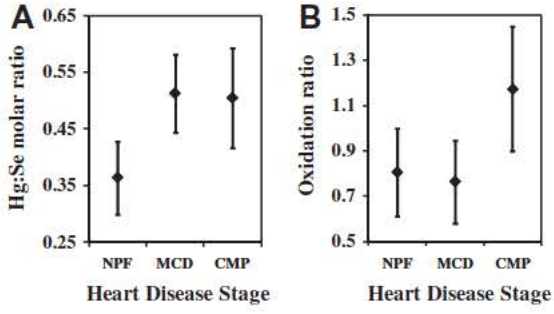

Fig. 4. Mean (±SE) (A) Hg:Se molar ratios (NPF ($n = 15$), MCD (n 13), CMP ($n = 8$)) and (B) immunoblot oxidation ratios (NPF ($n = 8$), MCD ($n = 9$), CMP ($n = 4$)) in pygmy sperm whale livers as a function of heart disease stage.

Pygmy sperm whales could possibly be a species that are more susceptible to developing CMP and environmental factors put them at greater risk for disease onset. Pygmy sperm whales may perhaps serve as an indicator species to tie human health concerns back with how trace elements and oxidative stress can play a role in CMP. Myocardial degeneration was observed in subadult and adult pygmy sperm whales, while CMP was only observed in adult animals. In human epidemiological studies, cardiac disease usually presented in adulthood (Virmani, 2004). Cardiomyopathy was more prevalent in adult male pygmy sperm whales and males were found to have greater Hg:Se molar ratios and immunoblot oxida tion ratios, which may relate to prevalence of CMP. Human men are 2 3 times more likely than women to develop dilated CMP, and men are affected 1 1.5 times more frequently than women with hypertrophic CMP (Virmani, 2004). In human population studies examining benefits of fish consumption with heart disease risk, findings showed that greater Hg exposure reduces benefits of fish consumption relative to heart disease risks (Rissanen et al., 2000). Many marine mammal studies have put forth the idea that Se status may be impacted by sequestration chemistry wherein Se binds Hg in the process of detoxifying Hg. This study represents an initial attempt to study Hg speciation and detoxification chemistry in marine mammals and its impact on development of CMP, but a range of complementary studies needs to be performed to ascer tain the ultimate biochemical impact of this detoxification mecha nism and its role in CMP progression and more generally, in antioxidant and Se biochemistry.

Disclaimer

Certain commercial products and instruments are identified in this paper to adequately specify the experimental procedures. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology. Nor does it im ply that the items mentioned are the best for the intended purpose.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.05.051.

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Pathobiochemistry

Selenium protein identification and profiling by mass spectrometry: A tool to assess progression of cardiomyopathy in a whale model

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ABSTRACT

Non-ischemic cardiomyopathy is a leading cause of congestive heart failure and sudden cardiac death in humans and in some cases the etiology of cardiomyopathy can include the downstream effects of an essential element deficiency. Of all mammal species, pygmy sperm whales (*Kogia breviceps*) present the greatest known prevalence of cardiomyopathy with more than half of examined individuals indicating the presence of cardiomyopathy from gross and histo-pathology. Several factors such as genetics, infectious agents, contaminants, biotoxins, and inappropriate dietary intake (vitamins, selenium, mercury, and pro-oxidants), may contribute to the development of idiopathic cardiomyopathy in *K. breviceps*. Due to the important role Se can play in antioxidant biochemistry and protein formation, Se protein presence and relative abundance were explored in cardiomyopathy related cases. Selenium proteins were separated and detected by multi-dimension liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS), Se protein identification was performed by liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS), and Se protein profiles were examined in liver (n = 30) and heart tissue (n = 5) by SEC/UV/ICP-MS detection. Data collected on selenium proteins was evaluated in the context of individual animal trace element concentration, life history, and histological information. Selenium containing protein peak profiles varied in presence and intensity between animals with no pathological findings of cardiomyopathy and animals exhibiting evidence of cardiomyopathy. In particular, one class of proteins, metallothioneins, was found to be associated with Se and was in greater abundance in animals with cardiomyopathy than those with no pathological findings. Profiling Se species with SEC/ICP-MS proved to be a useful tool to identify Se protein pattern differences between heart disease stages in *K. breviceps* and an approach similar to this may be applied to other species to study Se protein associations with cardiomyopathy.

1. Introduction

This study focuses on how selenium (Se) is associated with a primary dilated cardiomyopathy in pygmy sperm whales, *Kogia breviceps*. Studying cardiomyopathy in pygmy sperm whales is of particular interest since greater than 50% of the whales that strand are affected by this disease. Determining the etiology may be relevant to humans since the clinically the cause(s) of non ischemic cardiomyopathy commonly cannot be determined, even in cases that lead to end stage heart failure or heart transplantation [1,2]. The natural occurrence of cardiomyopathy in *K. breviceps* is greater than all other mammal species studied to date warranting further examination of factors that are associated with the onset of cardiomyopathy in this whale species. The presence of cardiomyopathy in pygmy sperm whales was first identified and described in 1985 by Bossart et al. [3]. Cardiomyopathy in *K. breviceps*, as in human, is a myocardial disease that results in deterioration of cardiomyocyte number, size and function, and diminished left and/or right ventricular function. Pygmy sperm whales present pathology of a mixed form comprising dilated and hypertrophic cardiomyopathy [1].

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Selenium is an essential micronutrient for animals that is primarily acquired through diet. The major biological role of Se is in antioxidant defense, but it can be toxic at high concentrations [4]. Total Se concentration in blood reflects total Se status in an individual indicating whether Se concentrations are balanced, toxic, or deficient. However, total Se concentration provides little information about the element's bioactivity since the biological function of Se is primarily mediated by incorporation or association with proteins. The liver is the predominant site of seleno amino acid formation, Se protein synthesis, and excretion, therefore, making liver one of the tissues of choice for which to study Se speciation [5,6]. Additionally, the liver is affected secondarily in late stage cardiomyopathy through hepatic congestion.

Etiology of cardiomyopathy in pygmy sperm whales is currently idiopathic. Nutritional deficiencies of Se have been shown in humans, mice, dogs, and cattle to play roles in development and progression of cardiomyopathy [7 11]. Humans that suffer from Keshan disease have congestive cardiomyopathy caused by a combination of dietary deficiency of selenium and presence of Coxsakievirus or patients that are on a ketogenic diet to treat intractable epilepsy are often nutritionally deficient in Se and either experience cardiomyopathy or are at greater risk for heart disease development [9,12,13]. There have been relatively few studies on altered selenoprotein synthesis and research addressing whether Se roles in cardiomyopathy are correlative or causitive. Many factors may contribute to development and progression of cardiomyopathy, such as genetics, infectious agents, biotoxins, and dietary intake (vitamins, Se, Hg, pro oxidants). These factors could act singly or additively and may be interconnected to one another. Other studies have shown that multiple dietary factors can interact, such as deficiency of vitamin E and Se, to further progression of myocardium degeneration [14 16].

Many prior marine mammal studies have put forth the idea that Se status may be impacted by sequestration chemistry wherein Se binds Hg in the process of detoxifying Hg [17 19]; however the ultimate biochemical impact of this detoxification mechanism has been less studied. To properly address Se bioavailability and determine whether Se activity is truly deficient due to Hg robbing *K. breviceps* of bioactive Se leading to onset of cardiomyopathy, presence of selenoenzymes, selenoproteins, and Se containing proteins must first be characterized in terms of abundance and form. Here, methods have been developed for the complex matrices of pygmy sperm whale liver and heart tissue to extract, purify, detect, and identify Se proteins. This study seeks to utilize mass spectrometry to identify changes in Se protein presence and abundance within animals exhibiting various states of cardiomyopathy.

2. Materials and methods

2.1. Sample collection

Since 1998, the National Institute of Standards and Technology (NIST) has cryogenically banked liver tissue from stranded individual *K. breviceps* in the National Marine Mammal Tissue Bank (NMMTB) housed at the National Institute of Standards and Technology (NIST), Charleston, SC. Liver samples obtained for the NMMTB were collected, processed, and frozen by trained field collectors during animal necropsies and stored in the specimen according to NISTIR 6279 [20]. Frozen heart samples were available from some of the same individual animals that had liver samples in the NMMTB and were donated for use in this project by the Center for Coastal Environmental Health and Biomolecular Research/National Ocean Service/National Oceanographic and Atmospheric Administration (CCEHBR/NOS/NOAA), Charleston, SC. Heart tissue samples for analytical analyses were placed in polypropylene centrifuge tubes and stored at −80 °C. Fresh heart tissue that was collected at the necropsy for histology preparation and histopathological evaluation was fixed in 10% neutral buffered formalin and tissue was sectioned at 5 μm for slide preparation. Individual animal life history and gross pathology data for this project was generated by necropsy principal investigators at collaborating institutions.

The liver for QC03LH3 pygmy sperm whale liver homogenate was collected in 1994 from a female (MMES9469SC 8) that stranded and tissues were donated to NIST, Charleston, SC by CCEHBR/NOS/NOAA for use in making an interlaboratory comparison exercise control material. QC03LH3 was used throughout method development and for Se protein identification in *K. breviceps* due to sample abundance and integrity of sample collection, homogenization, and storage.

2.2. Heart disease stage assignment

Hematoxylin and eosin (H & E) stained histology slides for each animal case were evaluated independently and blind of protein and chemical analyses data by a veterinary pathologist and assigned a heart score according to criteria put forth by Bossart et al. [1] and the Dallas cardiomyopathy criteria in humans [21]. Heart histology and gross pathology was used to categorize cardiomyopathy progression in the following three stages: no pathological findings (NPF), myocardial degeneration (MCD), and cardiomyopathy (CMP). Descriptions of each stage are provided in the Supplementary materials along with histology images.

2.3. Sample preparation for trace element and protein analysis

Fresh frozen tissue was cryogenically homogenized to produce a uniform sample composition of fresh frozen powder for analysis. Liver samples were homogenized in class 100 clean room conditions at NIST, Charleston, SC using cryogenic procedures developed by Zeisler et al. and Pugh et al. [22,23]. Left ventricle heart tissue was cryogenically homogenized using a bench top 6850 Freezer/Mill (SPEX SamplePrep, Metuchen, NJ). Samples were placed in vials with a stainless steel impactor, capped, placed in the mill, submerged in LN_2, and shaken at 10 Hz for 3 min. Homogenized powder was transferred into sterile polypropylene jars (Nalge Nunc International, Rochester, NY) and stored at −80 °C until analysis.

RIPA lysis buffer (Pierce, Rockford, IL) with 1X concentration Halt™ protease inhibitor cocktail and 5 mM ethylenediamine tetracetic adic (EDTA) was placed in 6 mL aliquots into polypropylene centrifuge tubes. Approximately 0.5 g homogenized sample was weighed into each centrifuge tube with buffer solution and vortexed. The centrifuge tubes were laid on ice and rocked for 15 min. The tubes were then centrifuged for 3 min at 1500*g*. The supernatant was pulled off in 1.5 mL aliquots and placed into protein LoBind microcentrifuge tubes (Eppendorf, Hauppauge, NY). A Microfuge 22R (Beckman Coulter, Fullerton, CA) was used to centrifuge microcentrifuge tubes for 15 min at 14,000*g* and 4 °C. The supernatant from each microcentrifuge tube was filtered with a 0.2 μm PTFE membrane filter (SunSri, Rockwood, TN) into a glass auto sampler vial. All samples were kept on ice between steps to prevent protein degradation. Protein extraction was controlled and optimized by protein concentration measurements to ensure that tissue cells were thoroughly lysed. Total protein concentration was measured at 595 nm according to the Bradford method [24] using a Coomassie Plus assay kit (Pierce) and a DU800 spectrophotometer (Beckman Coulter). Bovine serum albumin (Pierce) was used as the calibration standard.

2.4. Analytical methods

2.4.1. Instrumentation

Total Se mass fraction measurements were made using a Thermo Electron X Series II ICP MS (Bremen, Germany) with a standard low volume glass impact bead spray chamber (Peltier cooled at +3 °C), concentric glass nebulizer, and operating in collision cell mode utilizing 8% H_2 in 92% He as the collision cell gas. Detailed total Se measurement methods and statistical analyses were described by Bryan et al. [25]. Fig. 1 outlines the multiple steps and instrumentation used for Se

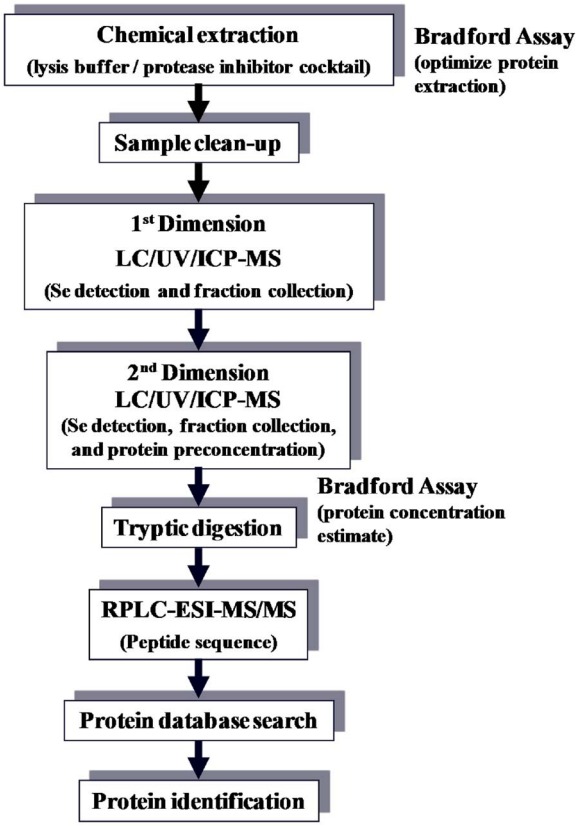


Fig. 1. Flow chart of selenium species separation, detection, and identification methods. 1st dimension LC – size exclusion chromatography (SEC); 2nd dimension LC – strong anion exchange (SAX).

separation, detection, and identification. Liquid chromatography coupled to UV spectrophotometer and Thermo Elemental X7 quadropole ICP MS (Winsford, UK) (LC/UV/ICP MS) were used for ^{80}Se separation, detection, and fraction collection. A DX 600 (Dionex, Sunnyvale, CA) ion chromatography system consisting of GP50 gradient pump, AS autosampler (cooled to 4 °C) (150 µL injection loop), and AD25 UV/Vis absorbance detector (set at 280 nm) was used for chromatographic separation. The chromatographic system was coupled to the ICP MS by 0.010 in. internal diameter (id) PEEK tubing. Once proteins were detected by the UV absorbance detector and presence of Se was identified by ICP MS, the chromatographic system was uncoupled from the ICP MS and the chromatographic system was set in line with a fraction collector (Foxy 200, ISCO, Lincoln, NE) to collect protein fractions. Liquid chromatography coupled to a Surveyor LC pump and autosampler and LTQ ESI MS/MS (ThermoFisher, Waltham, MA) were utilized for peptide sequencing.

2.4.2. Instrument calibration methods and quality assurance

Collision cell ICP MS working conditions were optimized with a 10 ng/g 68 element tuning solution (High Purity Standards, North Charleston, SC) and a Se calibrant prior to sample analysis and coupling to other instruments. LTQ ESI MS/MS signal intensity was optimized using ThermoFisher Xcalibur software tune methods with angiotensin (Sigma, Sigma Aldrich, St. Louis, MO). Commercial availability of selenoprotein standards is limited to GPx1 from bovine erythrocytes (84.5 kDa) and TrxR from rat liver (55 kDa 67 kDa) (Sigma Aldrich). The glutathione peroxidase standard along with procedural blanks was taken through the same analysis steps as unknown pygmy sperm whale samples. QC03LH3 and GPx standard were used throughout method development and sample analyses for method validation, reproducibility, and protein identification. The GPx standard was used to verify protein recovery along each chromatographic separation and verify that the selenoprotein could be properly identified by peptide sequence.

2.4.3. Protein separation

Protein separation steps are outlined in Fig. 1. The 1st dimension of protein separation was carried out by LC, protein presence was detected by UV/Vis, and Se containing peaks were detected by ICP MS. Then Se containing fractions were collected from each of the 1st dimension Se containing protein peak elution times for 2nd dimension LC protein separation, ICP MS Se detection, and fraction collection when Se species began to elute. Mobile phase compositions, chromatographic programs, and sample injection volumes are outlined in the Supplementary materials (Table S1) for each LC separation. Liquid chromatography first dimension separation was carried out on a size exclusion column (SEC) that had an effective separation range of 1 kDa to 300 kDa since the column has notable tolerance of complex matrices. Strong anion exchange liquid chromatography (SAX/UV/ICP MS) was used for second dimension separation additional clean up of Se species. Replicate injections and LC separations were performed in order to collect multiple fractions from the same sample. Strong anion exchange fractions from within each SEC Se containing protein peak were then combined and pre concentrated using Centrifugal Filter tubes (Millipore, Billerica, MA) which have a 3 kDa molecular weight cutoff. An Avanti J 20 XPI centrifuge (Beckman Coulter) was used to spin down samples for approximately 20 min at 7000*g* and 4 °C. Pre concentrated sample that did not pass through the filter and contained proteins greater than 3 kDa was removed from the top chamber of the tube and placed in protein LoBind microcentrifuge tubes for measurement of total protein concentration or tryptic digestion.

2.4.4. Protein identification

Selenium containing protein fractions collected required protein alkylation and tryptic digestion in order to prevent formation of disulfide bridges and to obtain peptides of a suitable length for identification by LC ESI MS/MS. Bradford assay was used to estimate total protein concentration in each protein fraction in order to decide how much sample was required for digestion. Protein was weighed out and 0.2% RapiGest (Waters, Milford, MA) in 50 mmol/L Tris buffer (pH 8.0) along with 200 mmol/L 1, 4 dithio DL threitol (DTT) (Fluka, Sigma Aldrich) solution was added to the protein. Samples were heated at 60 °C for 30 min followed by 30 min at 37 °C. Then 200 mmol/L iodoacetamide (Sigma, Sigma Aldrich) solution was added and samples were incubated at room temperature in the dark with vortex mixing for 1 h. The alkylation reaction was stopped with the addition of 200 mmol/L DTT solution and samples were again incubated at room temperature in the dark with vortex mixing for 30 min. Trypsin (Promega, Madison, WI) was added to alkylated protein to achieve a ratio of trypsin:protein (g/g) of 1:50 to 1:100 and incubated for 20 h at 37 °C. Digestion was stopped and RapiGest was cleaved by adding 25.5% trifluoroacetic acid (TFA) (Supelco, Bellefonte, PA) to bring sample pH < 2 prior to incubation at 37 °C for 60 min. After incubation, 0.1% FA (aq) formic acid (Fluka, Sigma Aldrich) was added and samples were centrifuged at 14,000*g* for 10 min at 4 °C to precipitate RapiGest. The supernatant was removed and transferred to protein LoBind microcentrifuge tubes which were stored frozen (−20 °C) until analysis.

Reverse Phase Liquid chromatography electrospray ionization tandem mass spectrometry (RPLC ESI MS/MS) was used for peptide sequencing and protein identification. Mobile phase compositions, chromatographic program, and sample injection volume are outlined in the Supplementary materials (Table S2) for chromatographic separation of peptides. SEQUEST software (ThermoFisher, Waltham, MA) was used to compare peptide fragmentation patterns to theoretical fragmentation of peptides. The FASTA database was created by downloading all currently known protein sequences that contain the amino acid selenocysteine (U) from all species in the UniProtKB/Swiss Prot database (downloaded from www.expasy.org on 06/10/08) since there is no

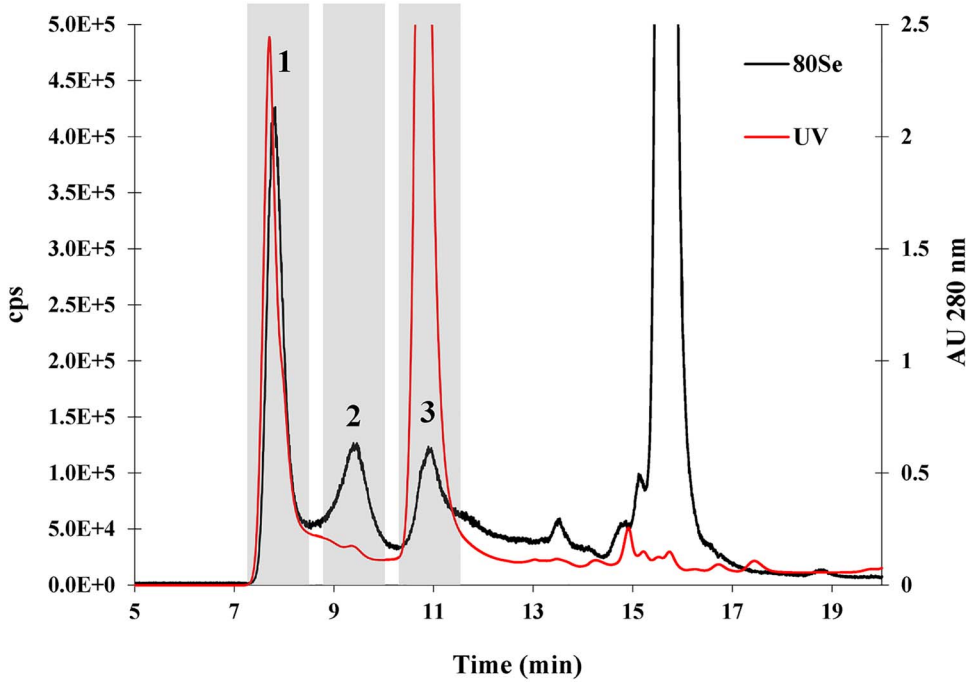


Fig. 2. SEC/UV/ICP-MS chromatogram (^{80}Se intensity) of QC03LH3 pygmy sperm whale liver homogenate overlaid with UV absorption profile. Numbers indicate Se containing protein peaks.

protein database available for pygmy sperm whales. In addition, a custom made database was created that contained FASTA sequences from known selenoproteins and Se containing protein in humans and bovines. A list of proteins included in the custom database can be found in the Supplementary materials (Table S3). Scoring and cutoff criteria were set in SEQUEST for database searches to decide that a MS/MS data set indicated the presence of a protein in the sample. Monoisotopic precursor and fragment mass types were allowed along with two missed cleavage sites, cysteine alkylation, and methionine oxidation. The mass range of the peptides was 200 *m/z* to 6000 *m/z*. The XCORR versus charges state values were set at 1.50, 2.50, and 3.00; and the delta CORR cutoff value was 0.100. Maximum peptide probability was set at 0.05. This probability limit minimized the false positive rate of randomly matching a peptide in the database.

2.4.5. Selenium profiling

Liver samples from animals in the NMMTB, heart samples donated by CCEHBR/NOS/NOAA, and QC03LH3 were taken through sample preparation, separated by size exclusion chromatography, and Se species were detected by ICP MS in a single batch to eliminate variation in ICP MS response between batches and instrument drift was monitored over the course of the single sequence. Chromatograms were plotted for each sample to compare Se maximum peak intensities with total trace element concentrations and heart disease stage. Statistical analyses were performed using JMP 7 (SAS Institute Inc., Cary, NC) and Microsoft Excel (Redmond, Washington). Pearson's correlation analyses were carried out to determine if total trace element concentrations were linearly associated with individual Se peak maximum intensities. Analysis of variance (ANOVA) was used to analyze the relationship between individual Se peak maximum intensities and heart disease stage. Variations between heart disease stage mean maximum intensity within a peak were examined with least squares (LS) mean plots.

3. Results

3.1. Quality assurance

A single Se containing protein peak for the GPx1 standard from bovine erythrocytes had a retention time on the SEC column of approximately 9.5 min (Supplementary materials Fig. S7). The GPx1 standard from bovine erythrocytes had 97.56% protein sequence coverage with bovine GPx1 and a list of matched peptides with their probability scores can be found in Supplementary materials (Table S4). As a selenoprotein, glutathione peroxidase uniquely contains the selenoamino acid selenocysteine, which is coded for in a peptide sequence as a "U". The active site of GPx contains the selenocysteine residue that carries out the catalytic function of redox reactions [26]. The selenocysteine containing selenopeptide GKVLLIENVASLUGTTVR was identified in the GPx standard from bovine erythrocytes (Supplementary materials Fig. S8).

3.2. Selenium protein separation and identification

Sample separation has been identified as the limiting step in Se speciation of complex biological samples that contain a variety of Se proteins. Selenium in proteins containing selenoamino acids is covalently bound therefore the metal is less likely to separate from the protein of interest during protein separation and clean up steps. However, Se is not covalently bound in proteins that transport Se or Se small molecules and is more easily lost during some preparations, such as SDS PAGE, resulting in loss of detection of presence of these proteins by ICP MS. Liquid chromatography coupled to ICP MS was the most effective tool for separating intact Se proteins and retaining their native composition during Se separation and Se detection. Two dimensions of liquid chromatography were used for further separation and purification of Se proteins in the complex matrix of QC03LH3 pygmy sperm whale liver homogenate to improve protein identification with tandem mass spectrometry.

Fig. 2 shows a SEC/UV/ICP MS chromatogram of the pygmy sperm whale liver homogenate QC03LH3. Proteins and smaller molecules elute from the size exclusion column according to molecular weight resulting in larger proteins eluting first before smaller proteins. There were three prominent protein peaks containing Se from QC03LH3 that were separated by the size exclusion column. The UV peaks coinciding with Se peaks shows the absorbance for all the proteins that elute at the same time, but the selenium proteins were the only ones of interest in this study. The large Se peak with a retention time of approximately 16 min contained both organic and inorganic small molecule Se species and was not evaluated in this study.

Tandem mass spectrometry was used to identify proteins that were

Table 1
QC03LH3 Se protein peptide matches, species, % protein sequence coverage, and peptide probability (P) value.

Protein	Species	% protein sequence coverage	Peptides	P value
Glutathione peroxidase 1	Bovine	54.63%	CEVNGEK	8.32E-05*
			SAAALAAAAPR	1.38E-04
			NEEILNCLK	5.70E-05*
			FITWSPVCR	3.56E-04*
			FLVGPDGVPVR	1.22E-05
			FLVGPDGVPVRR	5.88E-05*
			GLVVLGFPCNQFGHQENAKNEEILNCLK	1.06E-11
			YVRPGGGFEPNFMLFEK	5.13E-07
			FLTIDIEPDIETLLSQGASA	8.23E-04
			AHPLFAFLR	3.24E-06
	Human	30.35%	GLVVLGFPCNQFGHQENAK	5.56E-11*
			VLLIENVASLUGTTVR	2.93E-09*
			YVRPGGGFEPNFMLFEK	2.62E-09*
			NDVAWNFEK	3.79E-07*
	Common marmoset	4.48%	NDVAWNFEK	9.04E-06
	Mouse	5.47%	DYTEMNDLQKR	5.34E-07
	Pig	8.74%	EALPTPSDDATALMTDPK	1.09E-10
	Rabbit	8.50%	YVRPGGGFEPNFMLFQK	1.44E-05
Glutathione S-transferase A1	Bovine	8.11%	AILNYIATKYNLYGKDMK	4.83E-09
Glutathione S-transferase P	Bovine	7.62%	FQDGDLTLYQSNAILR	4.85E-09
	Long-tailed hamster	7.62%	FEDGDLTLYQSNAILR	5.63E-07
	Pig	5.31%	PPYTITYFPVR	1.35E-04
Glyceraldehyde-3-phosphate dehydrogenase	Bovine	37.24%	AENGKLVINGK	2.47E-03*
			LEKPAKYDEIKK	3.50E-05*
			GAAQNIIPASTGAAK	1.09E-07*
			VVDLMVHMASKE	7.73E-05*
			IVSNASCTTNCLAPLAK	1.11E-09*
			AITIFQERDPANIK	3.51E-04
			VPTPNVSVVDLTCR	4.77E-04*
			LTGMAFRVPTPNVSVVDLTCR	3.09E-05*
			RVIISAPSADAPMFVMGVNHEK	8.69E-05
Glyceraldehyde-3-phosphate dehydrogenase	Human	20.00%	WGDAGAEYVVESTGVFTTMEK	5.00E-13*
			VKVGVNGFGR	4.73E-05*
			LISWYDNEFGYSNR	2.93E-06*
			RVIISAPSADAPMFVMGVNHEK	2.36E-05*
	Greater Egyptian jerboa	5.79%	WGDAGAEYVVESTGVFTTMEK	9.63E-10
Metallothionein-1A	Bovine	29.51%	GASDKCSCCA	2.19E-06
			CAQGCVCK	9.66E-04
			CAQGCVCKGASDK	1.85E-06
			CAQGCVCKGASDKCSCCA	2.65E-13
Metallothionein-2	Human	16.39%	GASDKCSCCA	3.89E-07*
Metallothionein-3	Human	17.65%	SCCSCCPAECEK	1.24E-05*
	Wild yak	17.65%	SCCSCCPAECEK	4.34E-06
Metallothionein-4	Dog	29.03%	CAQGCICKGGSDKCSCCA	2.87E-08
Phospholipid hydroperoxide glutathione peroxidase	Human	6.09%	YGPMEEPLVIEK	5.14E-06*
Selenium-binding protein 1	Bovine	5.30%	GGPVQVLEDQELK	1.26E-06
			TKLLLPSLISSR	8.77E-07*
	Human	7.84%	GGPVQVLEDEELK	6.94E-06*
			LTGQLFLGGSIVK	3.66E-07
			EEIVYLPCIYR	4.62E-06*
Thioredoxin reductase 1	Human	1.54%	FLIATGERPR	2.79E-04*
3-mercaptopyruvate sulfurtransferase	Human	9.76%	AGQPLQLLDASWYLPK	2.81E-08
			ALVSAQWVAEALR	7.53E-05*

* P value was calculated from the custom made human and bovine protein database since peptides associated with these values were not identified from the SwissProt database.

present in QC03LH3 pygmy sperm whale liver from selenium containing protein fractions separated by liquid chromatography and detected by ICP MS. Only QC03LH3 and GPx1 standard from bovine erythrocytes were taken through the complete sequence of steps for protein identification. Table 1 provides percent sequence coverage in each protein and a list of peptides matched with the respective probability scores. Peptide matches used for identification of Se proteins in pygmy sperm whales were made assuming that a high degree of protein homology exists between species because the proteome for *K. breviceps* has not been sequenced. Protein matches between pygmy sperm whales and species for which protein databases were available indicated that peptide sequences for many proteins are likely highly conserved between species. Some Se protein sequences in *Kogia* spp. could be greatly different from those sequenced in other animals therefore preventing identification of some Se proteins in pygmy sperm whales. Additionally, the likelihood of false positive identifications is increased. Low abundance Se proteins that contribute to the ^{80}Se intensity may not have been identified since peptides for these proteins were not detectable at such low concentrations relative to greater abundance proteins. Several Se proteins were identified by a single peptide MS/MS spectrum match and should be verified by complementary means. Western blots with antibody against selenoproteins and Se containing proteins would be the next step in protein verification.

Selenium proteins were identified in the three prominent SEC/ICP MS protein peaks containing Se from QC03LH3 pygmy sperm whale liver homogenate (Fig. 2). Greater molecular weight selenoproteins, selenium containing, and selenium binding proteins were found in peaks 1 and 2; and proteins that fall into each of these Se protein classifications are identified in Table 2. Metallothioneins were identified in peak 3, which are small low molecular weight selenium binding

Table 2
Properties and functions of Se proteins identified in QC03LH3 pygmy sperm whale liver.

Protein	Protein Class	Species	Sequence Length	MW (kDa)	Known function
Glutathione peroxidase 1	Selenoprotein	Bovine	205 AA	22.7	protects hemoglobin in red blood cells from oxidative breakdown
		Human	201 AA	21.9	
		Common marmoset	201 AA	21.8	
		Mouse	201 AA	22.3	
		Pig	206 AA	22.6	
		Rabbit	200 AA	21.9	
Glutathione S-transferase A1	Se-binding	Bovine	222 AA	25.5	conjugation of reduced glutathione to hydrophobic electrophiles
Glutathione S-transferase P	Se-binding	Bovine	210 AA	23.6	conjugation of reduced glutathione to hydrophobic electrophiles; aids in detoxification with xenobiotic metabolism
		Long-tailed hamster	210 AA	23.6	
		Pig	207 AA	23.5	
Glyceraldehyde-3-phosphate dehydrogenase	Se-binding	Bovine	333 AA	35.9	involved in metabolic switch under oxidative stress allowing cells to produce more NADPH
		Human	335 AA	36.1	
		Greater Egyptian jerboa	363 AA	39.4	
Metallothionein-1A	Se-binding	Bovine	61 AA	6.0	high cysteine residue content binds heavy metals
Metallothionein-2	Se-binding	Human	61 AA	6.0	high cysteine residue content binds heavy metals
Metallothionein-3	Se-binding	Human	68 AA	6.9	binds heavy metals
		Wild yak	68 AA	6.9	
Metallothionein-4	Se-binding	Dog	62 AA	6.2	binds heavy metals
Phospholipid hydroperoxide glutathione peroxidase	Selenoprotein	Human	197 AA	22.2	protects cells against membrane lipid peroxidation and oxidative damage
Selenium-binding protein 1	Se-binding	Bovine	472 AA	52.6	selenium-binding protein; involved in sensing reactive xenobiotics in the cytoplasm
		Human	472 AA	52.4	
Serum albumin	Se-containing	Bovine	607 AA	69.3	most abundant protein in plasma, regulates colloidal osmotic pressure of blood, acts as a plasma carrier by non-specific binding
		Human	609 AA	69.4	
Thioredoxin reductase 1	Selenoprotein	Human	649 AA	70.9	reduces thioredoxin using NADPH
3-mercaptopyruvate sulfurtransferase	Se-binding	Human	297 AA	33.2	transfers sulfur-containing groups (Se can substitute) to thiol compounds; participates in cysteine metabolism

proteins. Select LC ESI MS/MS peptide spectra of Se proteins identified from SEC peaks after SAX can be found in the Supplementary materials. Table 2 highlights the properties and functions of Se protein classes (selenoproteins, Se containing proteins, and Se binding proteins) identified in QC03LH3 pygmy sperm whale liver. Serum albumin was included in Table 2 since this protein can contain selenomethionine, transport small Se molecules, and was identified in several sample fractions. Serum albumin is a high abundance protein in many tissues, including liver, making it difficult to eliminate during sample preparation.

3.3. Selenium profiling in pygmy sperm whale liver and heart tissue

Selenium species profiling was performed along with total Se and Hg concentration measurements on 30 frozen liver samples from the NMMTB and 5 frozen heart samples donated by CCEHBR/NOS/NOAA. Heart disease stages were assigned from histologic heart tissue preparations to complement 21 animals with liver samples from the NMMTB and for all 5 animal heart samples. Total Se (9.19 ± 4.00 μg/g, wet mass fraction) was positively correlated with the magnitude of certain Se protein species peaks in pygmy sperm whale livers. The maximum peak heights (cps) for peaks 2 and 3 increase significantly as total Se concentrations increase (Fig. 3B and C). In contrast, maximum peak heights for peak 1 do not change in relationship to increasing total Se concentrations (Fig. 3A). Fig. 4 shows a representative comparison between the SEC/ICP MS chromatogram for liver samples from individuals at different stages of heart disease progression. Selenium protein profiles differ in liver in relationship to stage of cardiomyopathy progression. To assess the differences in Se protein peak height intensities between heart disease stages, mean individual peak intensities were calculated at each disease state as a percentage of the total sum of the three peak height intensities for that stage (Fig. 5). Animals with no pathological findings had Se protein percentages among peaks that were equivalent. Whales with cardiomyopathy had the greatest peak 3 intensities when compared to other heart disease stages. Animals with myocardial degeneration and cardiomyopathy had greater peak 3 intensities than peak 1 intensities. While peak 2 intensities increased significantly (ANOVA, $p = 0.020$) with increasing total Se, the peaks mean percentage remained constant relative to heart disease stage. Pygmy sperm whales with NPF have significantly (ANOVA, $p = 0.020$) lower total Se concentrations ((7.159 ± 1.255) μg/g, wet mass fraction) than animals with MCD ((10.502 ± 1.183) μg/g, wet mass fraction) or CMP ((11.759 ± 1.775) μg/g, wet mass fraction) [25]. Fig. 6 shows a comparison between the SEC/ICP MS chromatograms for all heart samples at different stages of heart disease progression. Peak patterns in heart tissue were similar to liver tissue while ^{80}Se count rates were an order of magnitude lower in heart tissue than liver tissue (Figs. 2 and 6). The heart tissue chromatograms have several small Se peaks and have inferior peak resolution compared to liver tissue possibly due to poorer sample collection and storage conditions that may have allowed protein degradation, relative to specimens preserved using strict NMMTB protocols. The number and quality of heart tissue samples prevented further statistical analyses.

4. Discussion and conclusion

Cardiomyopathy in pygmy sperm whales is a chronic, progressive disease in which varying degrees of cardiac degeneration occur leading to the terminal state of advanced cardiomyopathy [1]. This appears to be the first study to assess Se protein profiles with LC/UV/ICP MS in this mammalian species affected by a non ischemic cardiomyopathy. For whales with MCD and CMP, Se peak patterns showed that low

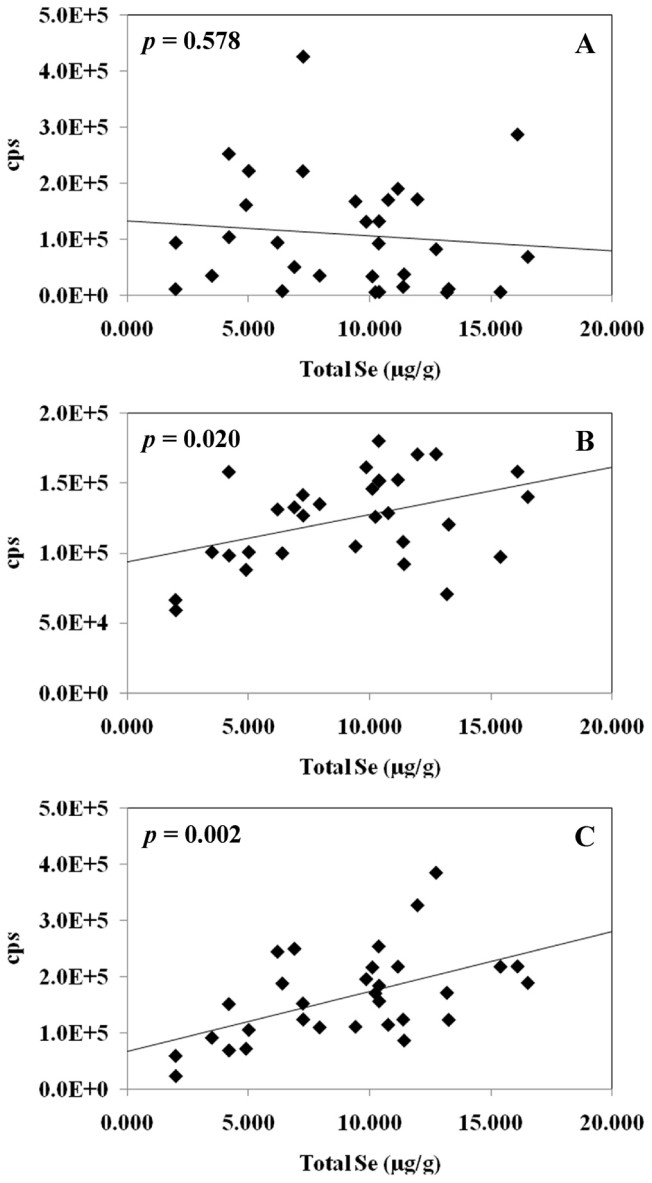


Fig. 3. Effect of the total selenium concentration on the intensity (cps) of selenium species in individual peaks in pygmy sperm whale livers (n = 30). Relationship between maximum peak intensity and total Se concentration for peaks 1 (A), 2 (B), and 3 (C). Data points are fitted with linear trend lines and data was collected in a single day.

molecular weight proteins, such as metallothionein, were in greater abundance than animals with NPF. Once more, the relative abundance of high molecular weight Se proteins increased parallel to increasing total Se concentration. These findings may be a model for Se related non ischemic cardiomyopathy in humans.

These Se associated proteins, glutathione peroxidase, selenium binding protein, and metallothioneins, have critical functions in the protection from oxidative damage, metal detoxification, detecting xenobiotics, and binding xenobiotics. These protective roles are important to pygmy sperm whales because these animals are continuously exposed to oxidative stress and contaminants in the marine environment [25,27].

4.1. Glutathione peroxidase 1

The protective effect of GPx is of particular importance when an organism in under oxidative stress [5]. Glutathione peroxidase 1 is a cellular or cytosolic enzyme that prevents lipid peroxidation of cell membranes by reducing pro oxidants such as hydrogen peroxide and organic hydroperoxide consequently protecting cells from oxidative damage [28]. The selenoprotein glutathione peroxidase 1 protein was identified in QCO3LH3 by several separation schemes utilizing single (SEC) and two dimensional separations (SEC and SAX). Glutathione peroxidase is the most extensively studied selenoprotein for which protein functions and structure have been widely characterized and this protein has been identified in many animal species [4]. While GPx activity has been studied in tissues from bottlenose dolphins (*Tursiops truncatus*) [29,30] and ringed seals (*Pusa hispida*) [31], this is the first study to separate and identify GPx1 at the protein level in a marine mammal species.

4.2. Selenium binding protein 1

Selenium binding protein 1 (SBP1) was identified in QC03LH3 pygmy sperm whale liver and selenium binding proteins may act in sensing reactive xenobiotics in the cytoplasm [32]. Pygmy sperm whales are exposed to PCBs and high concentrations of PCBs have been measured in many other marine mammal species [33]. Rats that have been exposed to toxic coplanar polychlorinated biphenyls (PCBs) have shown up regulation of selenium binding protein [32]. Exposure to chemicals known to be peroxisome proliferators, such as dibutyl phthalate, Wy 14 643, and ciprofibrate, have been shown in a mouse model to decrease abundance of selenium binding proteins [34]. Studies of the effects of different chemicals illustrate that regulation of selenium binding protein expression may be chemical dependent. Porat et al. [35] have suggested that selenium binding protein mediates the intracellular transport of Se. Selenium deficiency may limit SBP1 expression therefore reducing biological function of the protein.

4.3. Metallothioneins

Metallothioneins (MTs), which are Se binding proteins, were the only Se proteins identified in peak 3 of the size exclusion chromatography separation (Fig. 2). Additional low abundance Se proteins with similar molecular weights to MTs may have been present that minimally contributed to Se intensity in peak 3, but their peptides were undetectable for identification. Metallothioneins are low in molecular weight and rich in cysteine residues. Metals bind easily to MTs due to the thiol groups (SH) in the cysteine residues [36]. Selenium has a high binding affinity for cysteine. Metallothionein peptide fragments, identified in Se containing peak 3 and shown in Tables 1 and 2, contain several cysteine residues in each fragment. Metallothioneins are synthesized in a high capacity in tissues that uptake, store, and eliminate metals such as liver [37]. Both essential and toxic trace elements can induce MTs through chelation of cysteine residues. Metallothioneins act in maintaining homeostasis and detoxification by restricting availability of metal cations at harmful sites [36]. Metallothioneins have been proposed as biomarkers to assess marine organisms for exposure and impact of toxic metals in the marine ecosystem [37]. Kwohn et al. [38,39] were the first to isolate and identify MT1 and MT2 in striped dolphins (*Stenella coeruleoalba*). Metallothioneins have been detected in liver and associated with metals in several marine mammal species including sperm whales (*Physter macrocephalus*), bottlenose dolphins, striped dolphins, pilot whales (*Globicephala melas*), narwhals (*Monodon monoceros*), Dall's porpoises (*Phocoenoides dalli*), and California sea lions (*Zalophus californianus*) [40 43].

Since the Se binding proteins metallothionein were the only Se proteins identified in peak 3, increased relative abundance of MTs suggest their potential utility as a biomarker for onset of early stages of heart disease leading to cardiomyopathy in pygmy sperm whales. Metallothioneins have been proposed in other mammal studies as biomarkers of exposure for metal pollution [41] and findings in this study could lead to another application of MTs as biomarkers for non ischemic cardiomyopathy. Metallothioneins have been suggested in

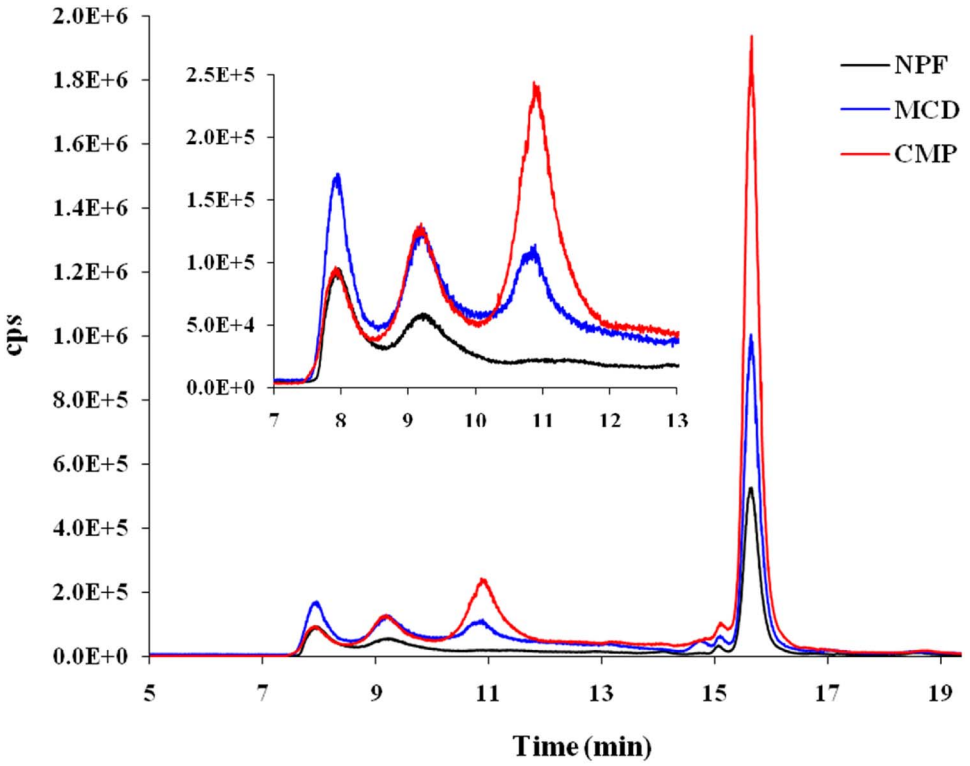
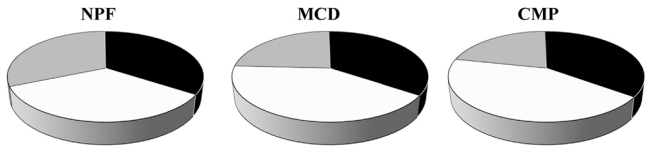


Fig. 4. Pygmy sperm whale liver SEC/ICP-MS ^{80}Se profiles for a representative individual at each heart disease stage; no pathological findings (NPF), myocardial degeneration (MCD), cardiomyopathy (CMP). Inset shows protein peak region of the SEC/ICP-MS chromatogram.



Heart Disease Stage	Peak 1	Peak 2	Peak 3
NPF (n = 8)	31.3 ± 6.2 %	34.4 ± 3.2 %	34.3 ± 4.0 %
MCD (n = 9)	24.0 ± 5.9 %	34.7 ± 3.1 %	41.3 ± 3.8 %
CMP (n = 4)	21.4 ± 8.8 %	35.4 ± 4.6 %	43.2 ± 5.6 %

Fig. 5. Pygmy sperm whale liver Se protein distribution in each protein peak as a percentage (mean ± SE) of the sum of the three Se containing protein peak heights relative to heart disease stage (n = number of whales at disease stage); no pathological findings (NPF), myocardial degeneration (MCD), cardiomyopathy (CMP).

playing a cardioprotective role by regulating metal homeostasis and anti oxidant response [44 46]. Recently, a human study found that individuals with the MT1A genetic polymorphism are predisposed to developing cardiovascular disease when there is an imbalance between oxidant production and antioxidant defenses [47]. In our study, MT1A was specifically identified in pygmy sperm whales liver. Variations in Se protein profiles in tissues of pygmy sperm whales at different heart disease stages may lend insight into how Se protein presence and relative abundance changes throughout this type of cardiomyopathy disease progression.

4.4. Selenium concentration

Since peaks 2 and 3 increased in intensity relative to total Se concentration increase in liver, there could be similar Se incorporation mechanisms between proteins eluting in these peaks. Total Se and Hg concentrations have been shown to be closely positively correlated in liver. However, total Hg (10.452 ± 8.744 μg/g, wet mass fraction) does not have a significant correlation with the concentration of Se protein species in individual peaks in pygmy sperm whale livers (total Hg measurements were discussed in Bryan et al. [25]). This may be due to hydrogen selenide aiding in detoxifying methyl Hg and binding to inorganic Hg forming mercury selenide (HgSe) crystals, which are small inert inorganic molecules that are stored in the liver [48]. Thus, there appears to be sequestering of the bioavailable Se pool for Hg detoxification rather than for interactions involving protein formation.

4.5. Selenium species profiling in pygmy sperm whale liver and heart

Relative peak heights of Se protein species in Se profiles were related to cardiomyopathy progression in pygmy sperm whales. Differences in Se protein distribution among tissues and sub cellular fractions have previously been identified and suggested that these proteins are involved in several metabolic pathways [49,50]. Further biological importance of Se proteins was recognized when preferential routing of Se for formation of specific Se proteins was discovered with insufficient Se intake [49]. Selenium protein profile differences between animals with no pathological findings and animals affected by cardiac disease indicate a potential altered metabolic pathway of protein homeostasis. Peak 2 Se protein relative intensities do not change as a function of heart disease progression suggesting that Se proteins in this peak are not related to heart disease and that peak 2 Se protein abundance increases as a consequence of total Se concentration increasing. Peak 1 intensities are not affected by total Se concentration therefore the Se proteins found in this peak could actually remain stable throughout heart disease progression even though peak 1 appears to be of greater intensity in NPF animals (Fig. 4). Given that total Se concentrations are greater in whales with CMP, accumulation of total Se in conjunction with increases in peak 3 intensities may indicate that in the presence of heart disease correlated Se proteins found in peak 3 are up regulated and involved in the progression of the disease state.

4.6. Selenoproteins and human heart failure

Selenoproteins are required for normal cardiac health, in particular with regards to oxidative stress, which can be associated with progressive human chronic heart failure [51]. The role of micronutrients in heart failure is also being increasingly recognized. With wider

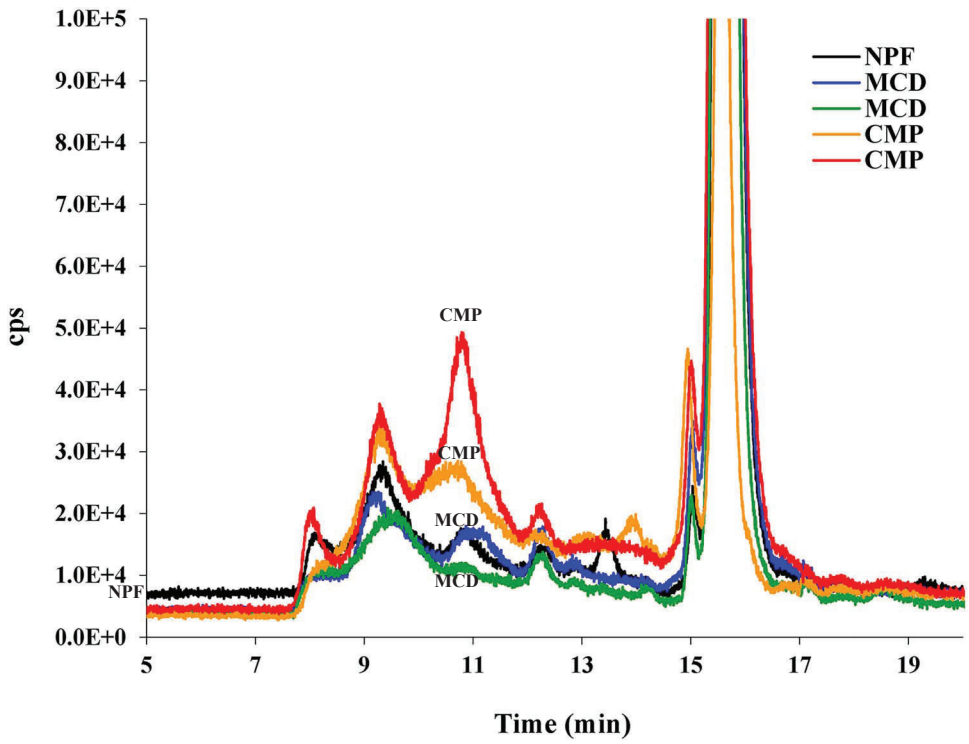


Fig. 6. SEC/ICP-MS ^{80}Se profiles for 5 individual pygmy sperm whale hearts with different heart disease stages; no pathological findings (NPF), myocardial degeneration (MCD), cardiomyopathy (CMP).

applications in higher cardiac risk populations of various forms of bariatric surgery and supplemental nutrition it becomes increasingly important to understand the pathophysiology of co factors such as Se [52]. This is in addition to the understudied role of environmental exposures such as Se and Hg in the development or progression of heart failure. For example, a reversible dilated cardiomyopathy secondary to Se deficiency has been long recognized (i.e., Keshan disease) [53].

Profiling Se species with SEC/ICP MS was a useful tool in identi fying differences in Se protein containing peak patterns between stages of dilated cardiomyopathy disease progression. For whales with MCD and CMP, Se peak patterns showed that low molecular weight proteins were in greater abundance than animals with NPF. Relative abundance of high molecular weight Se proteins increased parallel to total Se concentration increasing. Protein identification and profiling was the first step in gaining insight to how selenium proteins are related to cardiomyopathy in pygmy sperm whales.

Many of the factors that can contribute to onset and progression of cardiomyopathy in pygmy sperm whales may not stand alone but rather act collectively and require further investigation. Methods developed and used in this study to identify and profile Se proteins in *K. breviceps* at various stages of cardiomyopathy progression could be applied to other species that are affected by cardiomyopathy to gain further in sight into disease progression and the role of Se in non ischemic car diomyopathy.

Disclaimer

Certain commercial products and instruments are identified in this paper to adequately specify the experimental procedures. Such identi fication does not imply recommendation or endorsement by the National Institute of Standards and Technology. Nor does it imply that the items mentioned are the best for the intended purpose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jtemb.2017.05.005

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Review

Naturally Occurring Food Toxins

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Abstract: Although many foods contain toxins as a naturally-occurring constituent or, are formed as the result of handling or processing, the incidence of adverse reactions to food is relatively low. The low incidence of adverse effects is the result of some pragmatic solutions by the US Food and Drug Administration (FDA) and other regulatory agencies through the creative use of specifications, action levels, tolerances, warning labels and prohibitions. Manufacturers have also played a role by setting limits on certain substances and developing mitigation procedures for process-induced toxins. Regardless of measures taken by regulators and food producers to protect consumers from natural food toxins, consumption of small levels of these materials is unavoidable. Although the risk for toxicity due to consumption of food toxins is fairly low, there is always the possibility of toxicity due to contamination, overconsumption, allergy or an unpredictable idiosyncratic response. The purpose of this review is to provide a toxicological and regulatory overview of some of the toxins present in some commonly consumed foods, and where possible, discuss the steps that have been taken to reduce consumer exposure, many of which are possible because of the unique process of food regulation in the United States.

Keywords: toxin; natural; environmental; exposure; processing; cooking; food

1. Introduction

Historically, we have learned that everything is toxic; it is only the dose that separates the toxic from the non-toxic. Even water is toxic if a large amount (4–5 liters) is consumed in a relatively short

time (2–3 hours). The pathogenesis of water intoxication includes hyponatremia, followed by cerebral edema, seizures and death.

Like water, too much of a good thing such as the antioxidant vitamin A, can have acute toxic effects leading to hepatotoxicity [1] or chronic high levels can have a pro-oxidant effect [2]. Something as innocent as licorice, when consumed in large amounts may be harmful. For example, Bannister and associates reported hypokalemia leading to cardiac arrest in a 58-year-old woman who had been eating about 1.8 kg of licorice per week [3]. This licorice intoxication (dubbed "glycyrrhizism" after glycyrrhizic acid, the active component of licorice), has an effect resembling that of aldosterone, which suppresses the renin-angiotensin-aldosterone axis, resulting in the loss of potassium. Clinically, hypokalemia with alkalosis, cardiac arrhythmias, muscular symptoms together with sodium retention and edema, and severe hypertension are observed. The syndrome may develop at a level of 100 g licorice per day but gradually abates upon withdrawal of the licorice [4].

Recently, public health and social agendas have become more proactive in food toxicology, such as regulating (or outright banning) trans fats or "endocrine disruptors" in foods on the basis of public safety, including a suggestion of removing the generally recognized as safe (GRAS) status for salt [5]. These agendas lose sight of the basic principle of toxicology that "the dose makes the poison" and that demanding "safety *per se*" or "safe at any dose", for all foods and ingredients is a non-starter and as a concept, was abandoned with the adoption of the Federal Food and Drug Act (FFDCA) in 1958. For their part, the regulators can limit amounts of potentially toxic substances allowed in food and in those circumstances where setting limits is not effective and public health policy makers provide the public with sufficient information (e.g., label information), where possible, to protect the consumer from reasonably foreseeable problems. Labeling requirements by the FDA provide the consumer with helpful information about content of fats, carbohydrate, protein, potential allergens, caloric value, *etc.*, but do not provide information about toxins that may be inherent in the foods or formed during processing. Because some food toxins cannot be removed from foods and others may be created during processing or cooking, consumption of small quantities of food toxins is unavoidable. The purpose of this review is to illustrate the potential risks of these toxins when consumed at concentrations normally present in foods and the steps taken by regulators to mitigate exposure where possible. Although regulatory information from countries other than the United States is included, FDA legislation is emphasized. Readers from other countries are advised to consult regulations for their specific region, because regulations and regulatory practices in other countries may differ from those in the United States.

2. Regulatory Accommodation

Foods are regarded as such because they are edible—they cannot be unpalatable or toxic—and; foods must have nutritional, hedonic or satietal value—otherwise there would be no point in consuming them. Therefore, in the absence of a spontaneous change or contamination, the concept of a toxic food *per se* would seem to be an oxymoron. How then, could a food be toxic and still be considered a food—there are two principal means: (1) an ordinarily non-toxic food has become toxic, if even for a small subpopulation; and (2) over-consumption of an ordinarily non-toxic food. This shift between toxic and non-toxic or toxic only for a select group has the potential for creating headaches for regulatory agencies charged with protecting the health of the public, but as the reader will see in the

following pages, the FDA and other regulatory agencies have created some thoughtful and pragmatic solutions for achieving a balance of acceptable risk and unavoidable circumstances.

The large diversity of acceptable foods made it difficult for the framers of the Federal Food Drug and Cosmetic Act (FFDCA) to define what a food could be, so they settled on the pragmatic definition provided in §201(f) [6]:

> The term "food" means (1) articles used for food or drink for man or other animals, (2) chewing gum, and (3) articles used for components of any such article.

The framers are to be congratulated on their realistic approach, but a little interpretation is required. In the first clause "…articles used for food..." includes what humans and animals will eat as such (including eggs, meat, kohlrabi, Velveeta® cheese and angel food cake). The third clause "articles used for components of any such article," are simply those substances used to make food (defined in the first clause)—therefore, anything approved for addition to food, becomes a part of the food. The second clause was more of a political consideration than anything else, as there was some disagreement whether chewing gum was swallowed or expectorated; the swallowers prevailed and chewing gum is regulated as a food. Had the majority determined that chewing gum was expectorated (as is evident on a sidewalk outside of any theater or church), it would have been classified with breath mints (which are not swallowed) and are therefore regulated as a cosmetic, whose function is to "…promote attractiveness…" of the body [6]. It has also been ruled by the FDA that proposed dietary supplements (which are regulated as a subset of foods) meant to be held in the mouth, followed by expectoration, are not dietary supplements, because they are not swallowed.

The definition of food has generally held since the 1958 definition, although it was changed slightly in the 7th Circuit in 1983, to now indicate that a food is something consumed "…primarily, for [it's] taste, aroma or nutritive value." This court decision did not radically change the definition of food from the original context, but in this particular case, prohibited the use of a food extract for therapeutic intent (*i.e.*, amylase isolated from kidney beans as an inhibitor of carbohydrate breakdown and marketed for weight loss—so-called "starch blockers").

In general, the law prohibits the sale of food "if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food" (in practice, "fitness" can be quite subjective). Also, some foods which are ordinarily safe to eat may become unsafe, as described in §402 of the FFDCA [7]:

> §402. A food shall be deemed to be adulterated—(a) (1) If it bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health…

The first part of §402 is clear; if a food contains a poisonous or deleterious substance it cannot be used as a food—a fairly broad standard. The second part of the section "…but in case the substance is not an added…the quantity of such substance does not ordinarily render it injurious to health…" requires an explanation. This clause simply means that although toxic substances may be present in foods, the food is not adulterated if the amount present in the food is not ordinarily injurious to health. For example, tomatine in tomatoes, psoralens in celery or glycoalkaloids in potatoes are normally

present in concentrations that are not harmful; however, in the event these amounts are increased (through such processes as breeding, mishandling during harvesting, storage or transportation) and become harmful, these foods are then considered to be adulterated. This second and narrower part of the statute is followed up in §406 of the FFDCA [8]:

> §406 Any poisonous or deleterious substance added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice shall be deemed to be unsafe for purposes of the application of clause (2) (A) of section 402(a); but when such substance is so required or cannot be so avoided, the Secretary shall promulgate regulations limiting the quantity therein or thereon to such extent as he finds necessary for the protection of public health, and any quantity exceeding the limits so fixed shall also be deemed to be unsafe for purposes of the application of clause (2) (A) of section 402(a).

§406 then, allows the FDA to establish tolerances for these unavoidable contaminants, that is, a food may contain a toxin (such as mercury), if the presence of that toxin is (a) unavoidable and (b) under the level tolerated, the food is not considered to be unsafe. Because establishing a "tolerance" requires an extensive rule-making process, the FDA has adopted the use of "action levels", which are non-binding guidelines [9]. For food ingredients (e.g., additives), potentially harmful constituents or contaminants are addressed by limiting the amount present in the specifications; higher than allowed amounts render the ingredient and the food to which it has been added, adulterated.

A few potential foods are banned outright by regulation such as the slaughter of companion animals (cats, dogs and horses) for food, offal and colostrum or those foods whose preparation is regulated by guidelines other than current good manufacturing practices (e.g., pufferfish preparation). Some naturally sourced substances (while present in some foods) are banned for addition to food for reasons of safety and include safrole, calamus and coumarin (a full list of which may be seen in 21 Code of Federal Regulations (CFR) 189). Other foods which may contain toxic substances, such as prussic acid in peach leaves, β-thujone in wormwood, saxitoxin in seafood, *etc.*, are controlled by regulation through the use of tolerances, or more correctly, specifications for the product that limit the amount of toxin that may be present. For those foods or ingredients with potential for harm, but not addressed by a specific regulation, action level, *etc.*, the reference in the FFDCA to substances "unfit for food" and flowing from that provision, Sections 402 and 406 of the FFDCA, apply. That is, the lack of a specific action taken by the FDA (or any regulatory agency), for a potentially harmful substance is not a license to market that substance.

3. Factors Driving the Acceptance of Certain Foods

Beyond the basic requirements of nutritional or hedonic value, the concept of exactly what constitutes food is largely culturally based; that is, the consumption of pork, shellfish, eel, "rocky mountain oysters", cracklings, chitlin's (chitterlings), brain, monkey, guinea pig, dog, snake, insects and arachnids, *etc.*, may be prohibited by religious practices or a matter of personal taste and, in the case of brains (or neural tissue) at least from cattle, has recently become no longer acceptable. Interestingly, there are no fruits or vegetables on any theocratic forbidden list.

There are some personal prohibitions that are genetically driven, but may not be perceived as a "toxicity" concern. For example, a genetic variant has been described for cilantro, which is perceived

by some people as having an unpleasant soapy taste or rank smell [10]. Another, better known variant is the ability to taste phenylthiourea (also known as phenylthiocarbamide, PTU or PTC) [11]. The ability to taste and smell certain substances may be key to evolutionary survival, as while the alkaloids of many potentially poisonous plants confer a bitter flavor, Goff and Klee have indicated that certain flavors and odors may also provide sensory cues for nutritional value of some plants [12]. For example, the characteristic odor profile of tomato (e.g., "tomato", "green", or "grassy") are derived from *cis*-3-hexenal, *cis*-3-hexenol and *trans*-hexenal along with visual cues, to promote repeated consumption of an enjoyable food. In the context of promoting consumption of a specific food anosmia (lack of odor perception) or "specific anosmia" (which may be genetically based), will put the individual at a competitive disadvantage in food selection. Persistent or total anosmia also represents a clear safety hazard as the individual could not detect the tell-tale signs of decay or putrefaction of unfit foods.

There are some food prohibitions that are medically driven, as the result of genetics or autoimmune disease, as shown in Table 1.

Table 1. Medically driven food prohibitions (compiled from NORD [13]).

Disease/Syndrome	Causative Food	Cause	Comment
Disaccharide intolerance	Sucrose, dextrins	Autosomal recessive trait characterized by the deficiency or absence of enzymes sucrase and isomaltase in the intestine.	Attacks characterized by bloating and diarrhea.
Favism	Broadbean (*Vicia fava*)	X-linked recessive trait resulting in low amounts of glucose-P-dehydrogenase. Several subtypes known.	Hemolytic anemia may result from consumption of offending foods.
Galactosemia	Galactose and lactose (dairy products)	Autosomal recessive trait with low levels of any one of three enzymes directly responsible for galactose metabolism.	High levels of galactose in the blood results in hepatomegaly, cirrhosis, and renal failure. Infant mortality is ~75%.
Gluten intolerance	Wheat, barley, gluten containing foods	Autoimmune disease	Sensitivity to storage protein (gliadin) in some grains.
Lactose intolerance	Dairy products	Inborn error of metabolism—low or no lactase enzyme in the intestine.	Lactase is required to cleave lactose (a disaccharide of galactose and glucose). Bloating and diarrhea may develop.
Ornithine transcarbamylase deficiency	Dietary nitrogen (primarily meat)	X-linked recessive disorder resulting in low production of hepatic ornithine transcarbamylase interrupting the urea cycle and leading to accumulation of ammonia.	Although usually first seen in neonates, there may be an adult onset. Citrullinemia is another genetic disease affecting the urea cycle.
Phenylketonuria (PKU disease)	Phenylalanine in foods	Autosomal recessive trait characterized by inadequate hepatic phenylalanine hydroxylase.	Leads to accumulation of phenylpyruvate which may accumulate in the brain and lead to seizures, mental retardation, *etc.* Products containing phenylalaine must be labeled.

Table 1. *Cont.*

Refractory sprue	Wheat, barley and rye	Autoimmune disorder triggered by gliadin, a gluten storage protein.	Unlike common celiac sprue, adherence to a gluten-free diet may not cause symptoms to abate.
Trimethylaminuria	Fish	Autosomal recessive resulting in low production of flavin containing monoxygenase enzyme 3 (FMO3).	Fish odor syndrome. Failure to breakdown trimethylamine, a build of which results in a fish odor.
Very long chain Acyl CoA dehydrogenase deficiency (LCAD)	Very long chain fatty acids	Autosomal recessive trait resulting from a mutation in the HADHA gene.	Prevents mitochondrial metabolism of very long chain fatty acids.

Other medically driven prohibitions include food allergies, the most common of which are to milk, egg, fish, crustacean shellfish, tree nuts, wheat, peanuts and soybeans which account for 90% of all food allergies in the US. The *Food Allergen Labeling and Consumer Protection Act of 2004* (FALCPA), effective January 1, 2006, requires labeling of any product containing these ingredients or a protein derived from one of these offending foods or incidental additives or flavors derived therefrom. Exceptions are limited to any highly refined oil derived from a major food allergen (e.g., peanut or soybean oil) or any food ingredient exempt from labeling under a *petition* or *notification process* specified in the law [14].

There are also a number of food-drug interactions, the consumption of one interfering with the metabolism of the other, which may result in an enhanced or abated effect of the drug (Table 2).

Table 2. Food drug interactions (used with permission from Kotsonis and Burdock [15]).

Enzyme or Transporter	**Food**	**Drug**
CYP1A2	Caffeine, theophylline, grapefruit juice (naringen and furanocourmarins bergmottin and dihydroxybergamotin), grape juice, cruciferous vegetables, apiaceous vegetables, cooked meat	Clozapine, fluvoxamine, imipramine
CYP2E1	Watercress and possibly other isothiocyanate-containing cruciferous vegetables; polyunsaturated fatty acids (corn oil, menhaden oil)	Ethanol, halothane, enflurane
CYP3A4	Grapefruit, orange juice, red wine, possibly other polyphenol-containing substances, St. Johns wort, garlic	Ketoconazole, cyclosporine, erythromycin, protease inhibitors, HMG-CoA reductase inhibitors Acetaminophen, oxazepam, morphine, ibuprofen Digoxin, cyclosporine, parvastatin
UGT and GST	Brussels sprouts, cabbage, watercress, broccoli	
P-glycopeptide and OATP	Vegetables, fruit juice, St. Johns wort	

UGT: uridine diphosphae glycuronosyltransferases; GST: glutathione-*S*-transferases; OATP: organic anion transporting polypeptides.

4. Toxin Incorporation during Growth, Storage or Processing

4.1. Environmental contaminants

4.1.1. Selenium in grain

Selenium (Se) enters the food chain via plant and microorganism conversion of inorganic selenium to organically bound forms [16]. Selenium toxicity (*i.e.*, selenosis), caused by excessive selenium intake, has occurred on a large scale in seleniferous regions in China as the result of increased consumption of selenium-containing foods (approximate daily intake of 3–6.5 mg Se/day) [17]. The most common symptoms of selenosis are loss of hair, deformity, and loss of nails. Other reported symptoms include increased blood selenium levels, diarrhea, fatigue, a garlic-like odor of the breath and bodily secretions, irritability, peripheral neuropathy, and skin lesions [18]. Selenium intake levels that cause selenosis have not yet been well defined. Studies in China suggest that approximately 3–5 mg/day (0.05–0.08 mg/kg/day) will cause selenosis. Residents of seleniferous regions in South Dakota who consumed approximately 700 μg selenium/day (0.01 mg/kg/day) showed no symptoms of selenosis. The EPA has proposed an oral reference dose (RfD) of 0.005 mg/kg bw/day, or 350 μg/day [19].

4.1.2. Methyl mercury in seafood

Exposure to elemental mercury is relatively rare, although was once an occupational disease of hat manufacturers as elemental mercury was used for the curing of animal pelts. Inhalation of the mercury fumes led to mental deterioration and subsequently named “mad hatter syndrome” [20].

Of interest to food toxicology, is the methyl derivative, methyl mercury, formed by bacterial action in an aquatic environment from anthropogenic and natural sources of elemental mercury. Anthropogenic sources include burning of coal (which contains mercury), chloralkali process and other sources of elemental mercury into aquatic environments. In the case of Minamata, Japan, there was a direct discharge of methyl mercury into the environment. Methyl mercury exposure may cause neurological paresthesias, ataxia, dysarthria, hearing defects and death. Developmental delays have been documented in children borne of mothers exposed to methyl mercury [21]. Other than direct exposure to methyl mercury, exposure usually comes about as the result of methyl mercury becoming incorporated into the food chain, moving up as each predator consumes the smaller and less fortunate animal. Near the peak of the food chain, methyl mercury becomes concentrated in fish including, bonito (*Sarda* spp.), halibut (*Hippoglossus* spp.), mackerel (*Scomberomorus* spp.), marlin (*Makaira* spp.), shark (all species), swordfish (*Xiphias gladius*), and bluefin tuna (*Thunnus* spp.). The selection of these species was based on historical data on levels of methyl mercury found in fish consumed in the U.S. The selection was also based on an FDA action level of 1.0 ppm in the edible portion of fish [22]. However, the allowable level of mercury depends on whether the mercury was “added”; that is, did the presence of mercury arise from an anthropogenic source (*i.e.*, was the fish caught in an area known for mercury discharge), or was not added and the result of mercury naturally present in the environment [23].

4.2. Naturally formed substances

4.2.1. β-Thujone

Thujone, a monoterpene ketone, is the primary constituent of essential oils derived from a variety of plants, including sage (*Salvia officinalis*), clary (*Salvia sclarea*), tansy (*Tanacetum vulgare*), wormwood (*Artemisia* spp. and white cedar (*Thuja occidentalis* L.) [24]. Essential oils from these plants are used in herbal medicines, as flavorings in alcoholic drinks and fragrances throughout the world. Thujone is potentially toxic and the presence of alpha- or beta-thujone in food and beverages is regulated by law in several countries. In the US, thujone as an isolated substance is banned as an ingredient to be added to food and many of the natural thujone-containing plant oils (e.g., wormwood, white cedar, oak moss (*Evernia prunastri*) and tansy) are used as flavorings in food under the condition that the finished food is thujone-free [25]. Absinthe (made from wormwood) contains significant levels of thujone and is available in Spain, Denmark and Portugal. Wormwood itself is a popular flavoring for vodka in Sweden, while vermouth, chartreuse, and Benedictine all contain small levels of thujone [26]. Sage oil is used to provide the characteristic flavor in sausages, meats, condiments and sauces, and contains approximately 20–30% thujone (alpha- and beta-) [27,28]. Both alpha- and beta-thujone act as noncompetitive blockers of the gamma-aminobutyric acid (GABA)-gated chloride channel [29]. The essential oils of sage, hyssop (*Hyssopus officinalis* L.), and cedar all contain thujone and have been cited to have caused central nervous system effects characterized by tonic-clonic or solely clonic convulsions [30]. Thujone is believed to be the toxic agent in absinthism, a syndrome produced by the chronic use of absinthe, made from the essence of wormwood. The syndrome is characterized by addiction, hyperexcitability and hallucinations. The debilitating illnesses suffered by Vincent Van Gogh and Henri de Toulouse-Lautrec have been linked to absinthism, while the toxicity of thujone was a major factor in banning absinthe in the early 1900s [31]. A published case report detailed a male subject that drank about 10 mL of essential oil of wormwood (believing it was absinthe) and became agitated, incoherent and disoriented, subsequently developing renal failure [32]. The no observable effect limit (NOEL) for convulsions in subchronic toxicity studies in female rats was 5 mg/kg bw/day [24]. Detoxification of thujone is thought to occur via CYP450-dependent oxidation and subsequent glucuronidation and excretion [33]. The FDA limits exposure to β-thujone from *Artemisia* spp., when used as a natural flavoring substance or natural substance used in conjunction with flavors (21 CFR 182.20).

4.2.2. Prussic acid in cherry, apple and peach pits

Prussic acid (also known as hydrocyanic acid, hydrogen cyanide, or cyanide) is formed when cyanogenic glycosides found in leaves, cherry, apple and peach pits, oak moss and other plant tissues are damaged and come into contact with *beta*-glycosidase or emulsion enzymes. The enzymes release the cyanide from the glycoside, and the cyanide prevents the body's cells from utilizing oxygen, resulting in cellular necrosis and tissue damage. The mucous membranes and blood are bright red as they are oxygenated, but the cells in the tissues cannot utilize the oxygen. Clinical signs of prussic acid poisoning include rapid breathing, trembling, incoordination and in extreme cases, respiratory and/or cardiac arrest [34]. Many fruit trees contain prussic acid glycosides in the leaves and seeds, but only

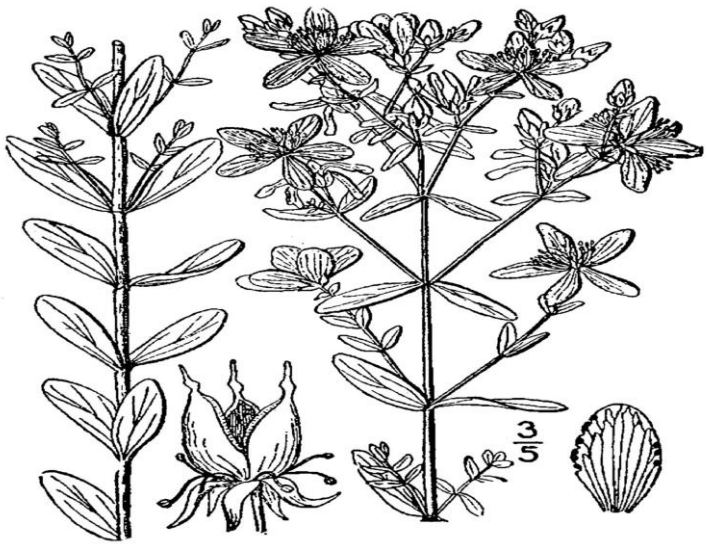
negligible levels are present in the fleshy parts of the fruit [35]. In the west African tropics, cassava is consumed as a dietary staple and inappropriate handling of the cassava prior to processing and consumption can result in a chronic form of cyanide poisoning termed "tropical ataxic neuropathy", the result of demyelinization of the optic, auditory, and peripheral nerve tracts [36].

Prussic acid as found in flavoring ingredients is limited to 25 ppm in cherry pits (*Prunus avium* L. or *P. cerasus* L.), cherry laurel leaves (*Prunus laurocerasus* L.), elder tree leaves (*Sambucus nigra* L.), and peach leaves (*Prunus persica* (L.) Batsch) (21 CFR 172.510); although the extract of bitter almond (*Prunus amygdalus* Batsch, *Prunus armeniaca* L., or *Prunus persica* (L.) Batsch) must be prussic acid free (21 CFR 182.20). There are no FDA regulations or guidelines restricting the presence of prussic acid in apple seed (*Malus* spp.), probably because extracts of these seeds have no economic value as flavor ingredients.

4.2.3. Hypericin in St. John's wort

St. John's wort (*Hypericum perforatum*; Figure 1) is an herbal thought to alleviate symptoms of depression, and standardized extracts of St. John's wort are consumed typically in tablet or capsule form. The major active antidepressive constituents in St. John's wort are thought to be hyperforin and hypericin [37,38]. The mechanism of action is not fully understood, but may involve inhibition of serotonin (5-HT) reuptake, similar to conventional antidepressive drugs. In this manner, hyperforin and hypericin taken in conjunction with other serotonin reuptake inhibitors may contribute to *serotonin syndrome*, a potentially life-threatening elevation of serotonin in the central nervous system. Hyperforin is also known to induce cytochrome P450 enzymes CYP3A4 and CYP2C9, which can lead to increased metabolism of certain drugs and decreased clinical response [39].

Figure 1. St. John's wort (*Hypericum perforatum*) [40].



In large doses, St. John's wort is poisonous to grazing animals, with published cases of livestock poisoning characterized by general restlessness and skin irritation, hindlimb weakness, panting, confusion, depression and in some instances, mania and hyperactivity resulting in the animal running in circles until exhausted [41]. In humans, consumption of St. John's wort may result in

photosensitization, and at high continuous doses, some liver damage may occur [39]. The FDA limits exposure to St. Johns wort (*Hypericum perforatum*), including the leaves, flowers, and caulis, by mandating that only hypericin-free alcohol distillate form may be used and then, only in alcoholic beverages (21 CFR 172.510).

4.2.4. Goitrogens (glucosinolates) in *Brassica* spp.

Certain raw foods have been found to contain substances that suppress the function of the thyroid gland by interfering with the uptake of iodine, an essential nutrient in growth, cognitive function, and hormonal balance. A lack of functional iodine is known to result in cognitive deficiencies (e.g., Cretinism). The decrease in iodine uptake causes the thyroid gland to enlarge, forming a goiter. Foods that have been identified as goitrogenic include spinach, cassava, peanuts, soybeans, strawberries, sweet potatoes, peaches, pears, and vegetables in the *Brassica* genus, which include broccoli, brussels sprouts, cabbage, canola, cauliflower, mustard greens, radishes, and rapeseed [42]. Goiter has also been attributed to the consumption of large quantities of uncooked kale or cabbage.

High temperatures (*i.e.*, cooking) inactivate the goitrogenic substances, collectively termed glucosinolates. Cassava (*Manihot esculenta*) is an essential dietary source of energy in the tropics, but contains high levels of linamarin, a glucosinolate. Cassava must be properly processed-dried, soaked in water or baked to effectively reduce the linamarin content [43]. Glucosinolates are sulfur-containing substances that are metabolized in the body by thioglucosidase to form thiocyanate, isothiocyanate, nitriles and sulfur. Under certain conditions the isothiocyanates undergo cyclization to form goitrins, increasing their potent goitrogenic activity. The oils from rapeseed (*Brassica napus*) must be analyzed for potential goitrins to circumvent potential goitrogenic activity when consuming these oils [44]. No FDA regulations were located for permissible concentrations of glucosinolates in human food. Glucosinolates (calculated as epi-progoitrin) and goitrin are limited to not more than 4% and 0.1% (respectively) of the seed meal of *Crambe abyssinica* (Crambe meal) obtained after the removal of the oil and used as an animal feed ingredient (21 CFR 573.310).

4.2.5. Erucic acid in rape

Rape (*Brassica napus* L. or *Brassica campestris* L.) is an annual herb of the mustard family native to Europe and is grown in the United States because it produces oil-rich seeds for cooking oil [45]. Rapeseed oil had been used for hundreds of years as oil for lamps and more recently as machine oil lubricant. Widespread use of rapeseed oil as a food ingredient was not considered until the late 1940s and 50s. However, early studies found that feeding high levels of rapeseed oil to rats significantly increased cholesterol levels in the adrenal glands and lipidosis in the cardiac tissue [46,47]. This effect was also noted in chickens, ducks and turkeys fed high levels of rapeseed oil, resulting in growth retardation, mortality, and a thickening of the epicardium and increased fibrous tissue in different areas of the myocardium [48]. Erucic acid was identified as the causative agent of these effects of rapeseed oil. Erucic acid is a long-chain fatty acid with one unsaturated carbon-carbon bond (C22:1). High levels of erucic acid have been liked to fatty deposit formation in heart muscle in animals [49]. Erucic acid is poorly oxidized by the mitochondrial β-oxidation system, especially by the myocardial cells, which results in an accumulation of erucic acid, producing myocardial lipidosis which has been

reported to reduce the contractile force of the heart [50]. Although myocardial lipidosis due to erucic acid consumption has not been confirmed in humans, animal feeding studies confirmed the formation of myocardial lipidosis in a variety of animal species in a dose-dependent manner, which has been the standard assessment by government agencies of potential adverse effects in humans. Canola oil is obtained from Canola (Canadian oil, low acid), a rapeseed variety that was conventionally bred in the late 1970s in Canada to contain reduced levels of erucic acid and glucosinolates [51,52]. The FDA limits the amount of erucic acid in Canola oil to no more than 2% of the component fatty acids (21 CFR 184.1555).

4.2.6. Furocoumarins

Furocoumarins represent a family of natural food constituents with phototoxic and photomutagenic properties. They are found mainly in plants belonging to the *Rutaceae* (e.g., citrus fruits) and *Umbelliferae* (e.g., parsnip, parsley, celery, carrots) families. Furocoumarins are produced in response to stress, to aid plants in defense against viruses, bacteria, fungi, insects and animals, and are regarded as natural pesticides [53]. Concentrations may also increase after exposure to UV radiation, changes in temperature, prolonged storage, or treatment with hypochlorite or copper sulfate (Chaudhary et al., as cited in Wagstaff 1991 [53], p. 270 and Beier *et al*., as cited in Ashwood-Smith [54], p. 916).

The three most active furocoumarins in producing photodermatitis are psoralen, 5-methoxypsoralen (5-MOP, bergapten), and 8-methoxypsoralen (8-MOP, xanthotoxin or methoxsalen) [55]. In the presence of near UV light (320–380 nm), these three linear furocoumarins can form adducts with DNA and DNA-crosslinks. The consequences of these photoadditions to cells are cell death, mutations and chromosome aberrations [54]. In the presence of ultraviolet A radiation, 5-MOP and 8-MOP produce skin tumors in experimental animals. At a chronic dose of 37.5 mg/kg bw/day in the diet, 8-MOP produces increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland in rats [56]. Cases of skin cancer have been reported in patients treated with 8-MOP and long-wave ultraviolet light for treatment of psoriasis or mycosis fungoides [57,58]. IARC has classified 5-MOP and 8-MOP plus ultraviolet radiation in group A (probably carcinogenic in humans) and in group 1 (carcinogenic to humans), respectively [57,59].

Citrus fruits, especially grapefruit, produce a variety of chemicals in their peels that may have adverse interactions with drugs. Typically, citrus fruit juice is produced utilizing the whole fruit, including the peel. One chemical found in the peel is bergamottin (also known as bergamot), a natural furanocoumarin that is known to inhibit some isoforms of the cytochrome P450 enzyme (CYP) 3A4 [60]. Inhibition of this enzyme prevents oxidative metabolism of certain drugs, resulting in an elevated concentration of a drug in the bloodstream [61]. Bergamot and other chemicals in citrus (e.g., lime, grapefruit, orange, lemon) oils [62] are also phototoxic, causing significant toxicity to the skin when exposed to sunlight [63]. 5-Methoxypsoralen, the most phototoxic constituent of bergamot oil, showed mutagenic activity in bacterial assays and clastogenic effects in mammalian cells in culture when exposed to UV light [64].

Celery reportedly contains 100 ppb psoralens (100 micrograms/kg) and parsnips as much as 40 ppm (40 mg/kg) [65]. The estimated dietary intake of furocoumarins for people eating furocoumarin-containing foods (est. 80% of the population) is 1.31 mg/day [53], which is approximately 0.022 mg/kg bw/day

for a 60 kg human. This is approximately 1000-fold lower than the 13-week dietary no observable adverse effect level (NOAEL) for liver toxicity in the rat (25 mg 8-MOP/kg bw/day) and 1700-fold lower than the dietary dose that has been shown to induce cancer in rats (37.5 mg/kg). Therefore, the risk of developing liver toxicity or cancer due to ingestion of psoralens in the diet is low.

In humans, the phototoxic threshold dose of furocoumarin mixtures after dietary exposure is of the order of 10 mg 8-MOP plus 10 mg 5-MOP, which is equivalent to about 15 mg 8-MOP per person. This phototoxic threshold dose is not reached by the consumption of celery roots and other conventional vegetables under normal dietary habits, which result in intake of approximately 2–8 mg furocoumarins per person [66]. Therefore, ordinarily dietary exposure to psoralens is not considered to be a significant risk for development of photodermatitis, albeit the margin of safety is low [65]. There are no FDA regulations or guidelines specific to the presence of furocoumarins in food.

4.2.7. Amylase inhibitors

Naturally occurring inhibitors of α-amylase are found in aqueous extracts of wheat, rye and kidney beans. The physiological role of α-amylase inhibitors in plants is not well understood, but may protect them against insect infestation. In mammals, some amylase inhibitors have been shown to attenuate the normal increase in blood glucose that occurs after ingestion of starch. However, since α-amylase inhibitors have been shown to be inactivated by gastric acid, pepsin or pancreatic proteinases, their potential as "starch blockers" is limited [67]. α-Amylase inhibitors were once added to foods as "starch blockers" to limit carbohydrate absorption for the purpose of weight loss; however, the FDA later determined that at least this use of α-amylase inhibitors was as drug, and they were consequently taken off the market [68].

α-Amylase inhibitor protein is a major allergen (referred to as Asp o 2) that has been implicated in the development of occupational toxicity known as "baker's asthma disease" [69]. Although α-amylase inhibitor protein is naturally found in wheat flour, it is also found in flour in which α-amylase from *Aspergillus oryzae* has been added to enhance carbohydrate fermentation by yeast [70]. Consequently, α-amylase inhibitor protein can be potentially found in baked products that are derived from sources other than wheat. Cases of food allergy have been reported in people ingesting bread containing α-amylase inhibitor protein. Symptoms of allergy include sneezing, rhinorrhea, oropharyngeal itching, hoarseness, cough and dyspnea [71].

High α-amylase inhibitor activity against human salivary α-amylase has been found in wheat flour (590 units/g), whole wheat flour (351 units/g) and whole rye flour (186 units/g). Bread baking reduces the activity by 80–100%, depending on type. The activity in uncooked spaghetti (248 units/g) is reduced more than 98% by 15 minutes of boiling. Boiling of red beans for 1.5 hours reduces activity to undetectable levels [71]. However, α-amylase has been shown to retain some allergenic activity when heated to 200 °C (Baur *et al.*, as cited in Phadia AB 2010 [72], p. 2).

4.2.8. Lectins in legumes

Lectins are a group of glycoproteins that are present in high levels in legumes (e.g., black beans, soybeans, lima beans, kidney beans and lentils) and grain products [73,74]. Lectins can reversibly bind to carbohydrates without altering their covalent structure [73]. The ability of lectins to bind to and

agglutinate red blood cells is well known and used for blood typing—hence the lectins are commonly called hemagglutinins. Lectins also can bind avidly to mucosal cells and interfere with nutrient absorption from the intestine [75]. Because the ability of the lectins to cause intestinal malabsorption is dependent on the presence of enteric bacteria, it has been hypothesized that lectins may also produce toxicity by facilitating bacterial growth in the GI tract [76].

Lectins isolated from black beans can produce growth retardation when fed to rats at 0.5% of the diet, and lectin from kidney beans causes death within two weeks when fed to rats at 0.5% of the diet. Soybean lectin produces growth retardation when fed to rats at 1% of the diet. The castor bean lectin ricin (one of the most toxic natural substances known) is notorious for causing deaths of children, and has been used as an instrument of bioterrorism [75].

Phytohaemagglutinin (PHA) is a lectin found in significant quantities (as much as 2.4–5% of total protein) in legumes such as red or white kidney beans, green beans and fava beans. PHA has a number of different properties, including the ability to induce mitosis, affect membrane transport and permeability to proteins, and agglutinate red blood cells. Rats fed a diet containing 6% PHA exhibit weight loss, associated with malabsorption of lipid, nitrogen and vitamin B12 [76]. PHA from red kidney beans inhibits sodium and chloride absorption in the rabbit ileum, indicating that PHA can affect electrolyte transport in the gut [77]. Symptoms of toxicity to PHA in humans such as nausea, vomiting, or diarrhea occur within three hours of ingestion. Recovery generally occurs within four or five hours of onset [78].

There are no FDA regulations or guidelines restricting the presence of lectins in food, but the FDA does provide recommended cooking practices prior to consuming legumes. Concentrations of PHA (and other lectins) are higher in uncooked than cooked beans. A raw, red kidney bean can contain up to 70,000 hemagluttinating units (hau). Most lectins are reduced by moist, but not dry heat. Therefore, steaming or boiling causes a significant reduction in concentrations of lectins in beans. Boiling for at least ten minutes has been shown to reduce hau in beans by 200-fold. Because cooking temperatures under 176 ° F do not destroy lectin, use of slow cooking and/or a crockpot is not advised for cooking beans [79].

4.2.9. Anti-thiamine compounds

Substances that act on the availability of vitamins are commonly referred to as antivitamins. These include materials that can cause a deficiency of vitamins by competing with vitamins in various metabolic reactions as the result of similar chemical structure or destroying or decreasing the effects of a vitamin by modifying the molecular conformation or by forming a complex [67].

Thiaminase cleaves thiamine (vitamin B1) at the methylene linkage, rendering it biologically inactive. Activity of thiaminase requires a cosubstrate—usually an amine or sulfhydryl-containing protein such as proline or cysteine. Thiaminase is found in fish, crab, clams and in some fruits and vegetables such as blueberries, black currants, red beets, Brussels sprouts and red cabbage [67].

Thiamine is an essential vitamin involved in energy production. Thiamine deficiency is associated with impaired pyruvate utilization, resulting in a shortage of cellular ATP. In humans, thiamine deficiency may lead to weakness and weight loss. Severe thiamine deficiency produces “beri-beri”, a disease characterized by anorexia, cardiac enlargement, and muscular weakness leading to ataxia [80].

Cooking destroys thiaminases in fish and other sources. There are no FDA regulations or guidelines specific to the presence of thiaminase in food.

4.2.10. Pyrrolizidine alkaloids

Pyrrolizidine alkaloids (PAs) are found in some plants of the Apocyanacae, Asteraceae, Boraginaceae, Compositae (*Senecionae* and *Eupatoriae*), Fabaceae, Leguminosae (*Crotalaria*), Rannuculaceae and Scrophulariaceae families. Herbs such as comfrey root and leaf (*Symphytum* spp.) (Figure 2), coltsfoot leaf and flower (*Tussilago farfara*) and borage leaf (*Borago officinale*), and several species of *Eupatorium* typically contain high levels of PAs. Humans are exposed to PAs through the accidental contamination of foodstuffs and intentional ingestion of PA-containing vegetables and herbal medicines. Serious incidences of illness have been reported in people consuming cereal grains that are contaminated with the seeds of PA-containing plants [81]. PAs are also present in milk from cows and goats and in honey [82].

Figure 2. Comfrey (*Symphytum officianale* L.) [83].

The pyrrolizidine structure is based on two fused, five-membered rings that share a bridgehead nitrogen atom, forming a tertiary alkaloid. The rings contain a hydroxymethylene group at the C-1 position and a hydroxyl group at the C-7 position, forming a necine base. Several PAs that contain unsaturated necine rings are hepatotoxic, mutagenic, teratogenic and/or carcinogenic. Toxicity is thought to be due to enzymatic conversion of PAs to pyrroles, which act as alkylating agents [67]. Pyrroles formed in the liver can travel to the lungs, causing thickening of the pulmonary vasculature and pulmonary hypertension [82].

The sale of comfrey products for internal use has been banned in the United States and Canada [82]. However, comfrey tea is still widely available. It is estimated that consumers of comfrey tea could be ingesting up to 5 mg of PAs per day (Speijers and Egmond, as cited in Deshpande 2002b [81], p. 368), or 0.083 mg/kg bw/day. The range of toxic doses in humans is thought to be 0.1–10 mg/kg per day [84], although the World Health Organization has reported a case of veno-occlusive disease in a subject ingesting 0.015 mg PAs/kg of body weight per day from comfrey.

4.2.11. Rhubarb and oxalic acid

Oxalic acid (oxalate) is generally found in rhubarb (0.2–1.3%), tea (0.3–2.0%), spinach (0.3–1.3%), parsley (1.7%) and purslane (1.3%), but may also be found in asparagus, broccoli, Brussels sprouts, collards, lettuce, celery, cabbage, cauliflower, turnips, beets, peas, coffee, cocoa, beans, potatoes, berries, and carrots [67,73,85].

Oxalic acid is an organic acid that can bind calcium and other minerals, making them insoluble and decreasing their bioavailability. Ingestion of foods containing high concentrations of oxalates may cause decreased bone growth, kidney stones, renal toxicity, vomiting, diarrhea, convulsions, coma and impaired blood clotting [73]. The significant role oxalate plays in kidney stone development is exemplified by the fact that approximately 65% of kidney stones consist of calcium oxalate [86].

Using the oral LD_{50} value of 375 mg/kg in rats, it has been estimated that ingestion of approximately 22 g of oxalic acid could be lethal to a 59 kg human [85]. Because approximately 4.5 kg of rhubarb leaves would have to be ingested in order to achieve a lethal dose, it has been hypothesized that documented cases of fatal rhubarb poisoning in humans were due to consumption of some other substance than oxalic acid [67].

Because cooking does not remove oxalate, and mineral complexes with oxalate are insoluble in water, oxalates are somewhat difficult to remove from foods. Therefore, diets rich in oxalate-containing foods should be supplemented with minerals such as calcium or potassium to prevent deficiencies. Limits on oxalic acid have been cited in ferric ammonium ferrocyanide and ferric ferrocyanide when used as color additives (21 CFR 73.1298 and 21 CFR 73.1299) with oxalic acid or its salts at not more than 0.1% of the colorant.

4.2.12. Zucchini and cucurbitacins

Members of the *Cucurbitacea* family (zucchini, cucumbers, pumpkins, squash, melons and gourds) produce cucurbitacins (oxygenated tetracyclic terpenes) that act as movement arresters and compulsive feeding stimulants for Diabriticine beetles (corn rootworms and cucumber beetles). Cucurbitacins are among the most bitter compounds known, and in nanogram quantities they deter most non-Diabrotic herbivores [87].

Because cucurbitacins act as feeding stimulants, they are added to insecticidal baits to increase efficacy [88]. Therefore, dietary exposure to cucurbitacins could occur through ingesting plants that normally contain them or by ingesting plants to which cucurbitacin-containing pesticides have been applied.

Under normal circumstances, cucubitacins are produced at low enough concentrations that are not perceived as being bitter by humans. In response to stresses such as high temperatures, drought, low soil fertility and low soil pH, concentrations in fruits such as cucumbers may increase and cause the fruits to have a bitter taste [89]. Occasional cases of stomach cramps and diarrhea have occurred in people ingesting bitter zucchini. Twenty–two cases of human poisoning from ingestion of as little as 3 grams of bitter zucchini were reported in Australia from 1981 to 1982, and in Alabama and California in 1984. The cultivar implicated in the Australia poisonings was “Blackjack” [90]. There are no FDA regulations or guidelines specific to the presence of cucurbitacins in food.

4.2.13. Coumarins (tonka bean, woodruff, clover)

Coumarin (2H-1-benzopyran-2-one) is found in herb teas made from tonka beans (*Dipteryx odorata*), melilot (*Melilotus officinalis* or *Melilotus arvensis*) and woodruff (*Asperula odorata*), the flavoring oil of bergamot (from *Citrus bergamia*) and the spice cassia (*Cinnamomum cassia*; sometimes sold as cinnamon) [91]. Coumarin is liberated from the glycoside melilotoside (an ether of glucose bonded with an ester bond to coumarin) on drying coumarin-containing herb material.

Molds present in spoiled sweet (Melilotus) clover and other hay products can metabolize coumarin to dicoumarol, which is similar in structure to vitamin K [92]. Vitamin K is necessary to activate prothrombin, which is converted to the blood clotting substance thrombin. By inhibiting vitamin K, dicoumarol promotes bleeding. Concentrations of dicoumarol in fodder >10 ppm have been responsible for fatalities by hemorrhaging in cattle [91].

The addition of coumarin to food in the United States was banned in 1954, based on reports of hepatoxicity in rats. However, because a number of foods contain coumarin, humans ingest approximately 0.02 mg coumarin/kg bw/day. The chronic administration of high doses of coumarin causes liver tumors in the rat and liver and lung tumors in the mouse. Overall, available data indicate that coumarin is not genotoxic. It is thought that the carcinogenicity of coumarin is caused by metabolism to toxic epoxides. Because doses of coumarin that cause toxicity and carcinogenicity in the lung and liver of experimental animals are more than 100 times the maximum human intake, exposure to coumarin from food poses no health risk to humans [93].

The addition of coumarin is prohibited in 21 CFR 189.130. The regulation notes that coumarin is found in tonka beans and extract of tonka beans, among other natural sources, and is also synthesized. It has been used as a flavoring compound, therefore addressing not just natural products (which would include buffalo grass or sweetgrass (*Hierochloe odorata*) used in flavoring vodka and other natural sources (see above)), as well as synthesized coumarin. Further, according to the regulation, "(b) Food containing any added coumarin as such or as a constituent of tonka beans or tonka extract is deemed to be adulterated under the act, based upon an order published in the Federal Register of March 5, 1954 (19 Federal Register 1239)." An analytical method for detection of coumarin in foods is specified in 21 CFR 189.130.

4.2.14. Phytates and phytic acid

Phytic acid (also referred to as phytate) is found in bran and germ of many plant seeds and in grains, legumes and nuts. Phytic acid is a simple sugar (myo-inositol) containing six phosphate sidechains, and as such, is a dietary source of phosphorus and an effective chelator of divalent cations such as zinc, copper, iron, magnesium and calcium [67,94]. Studies indicate that phytate-mineral complexes are insoluble in the intestinal tract, reducing mineral bioavailability [73]. Phytate also has been shown to inhibit digestive enzymes such as trypsin, pepsin, α-amylase and ß-glucosidase. Therefore, ingestion of foods containing high amounts of phytate could theoretically cause mineral deficiencies or decreased protein and starch digestibility. Vegetarians that consume large amounts of tofu and bean curd are particularly at risk of mineral deficiencies due to phytate consumption.

Because phytate-rich foods are digested at a slower rate and produce lower blood glucose responses than foods that do not contain phytate, it has been hypothesized that phytate could have a therapeutic role in management of diabetes [67]. It also may have utility as an antioxidant [95]. However, because the beneficial effects of phytate are outweighed by its ability to cause essential mineral deficiencies, consumption of a diet containing high amounts of phytate is not recommended. Food manufacturers are developing methods to reduce phytate in foods, such as addition of the microbial phytase, which releases phosphates from the inositol backbone of phytate [96].

Phytate is fairly heat stable, but can be removed by soaking or fermentation [67]. The soybean has one of the highest phytate levels of any grain or legume, and requires a long period of fermentation for reduction [94]. In people who consume large amounts of soy products, mineral deficiencies can be prevented by consumption of meat or dairy products or use of supplemental vitamins. There are no FDA regulations or guidelines restricting the presence of phytates in food.

4.2.15. Hypoglycin in Ackee

Ackee (*Blighia sapida*; Figure 3) is the national fruit of Jamaica and is also found in other Caribbean nations, Central America, South American and southern Florida [97]. Consumers of the unripe fruit sometimes suffer from "Jamaican vomiting sickness syndrome" allegedly caused by the alkaloids hypoglycin A (HGA) and B. Levels of HGA in the opened, ripe fruit are undetectable, making opened fruit safe for consumption [98].

The hypoglycin toxin (L-methylenecyclopropylalanine) inactivates several flavoprotein acyl-CoA dehydrogenases, causing disturbances of the oxidation of fatty acids and amino acids [99]. This leads to a secondary inhibition of gluconeogenesis which can precipitate an extreme, dangerous drop in blood-glucose levels (hypoglycemia) that can be fatal. Symptoms of poisoning from unripe ackee fruit occur within 6 to 48 hours of ingestion and include drowsiness, repeated vomiting, thirst, delirium, fever or loose bowels. Exhaustion of the muscular and nervous systems, collapse, coma, and death may ensue [100,101].

Figure 3. Unripe Ackee Fruit (left panel) and ripe Ackee Fruit (right panel) [100].

Dietary exposure to hypoglycin in Jamaicans ranges from 1.21–89.28 micrograms/gram ackee [102]. Ingestion of one 100 gram fruit could therefore result in a dose of approximately 300 micrograms/kg bw in a 30 kg child. This dose is approximately one-fifth of the maximum tolerated dose of HGA in male and female rats of 1500 micrograms/kg bw/day [103], indicating that normal use levels of ackee do not have a large margin of safety.

The importation of canned ackee fruit into the United States is restricted to certain manufacturers to insure that only properly ripened ackees are used for canning [104], and the FDA routinely analyzes incoming shipments of ackee for hypoglycin levels that could be a health concern, having issued a recall of canned ackee fruit for this very reason in 2005. If hypoglycin poisoning is expected, glucose, fluids and electrolytes should be administered. Antiemetics may be used to control vomiting and benzodiazepines to control seizures. Endotracheal intubation should be performed in people exhibiting seizures or coma [97].

4.2.16. Safrole

Safrole (1-allyl-3,4-methylenedioxybenzene) is found in aromatic oils of nutmeg (*Myristica fragrans*), cinnamon (*Cinnamomum verum*) and camphor (*Cinnamomum camphora*) and is a major constituent of oil of sassafras (*Sassafras albidum*) [105]. Prior to being banned as a food additive in the United States in 1960, safrole was commonly used to flavor root beer and other foods. Most commercial "sassafras teas" and root beers are now artificially flavored as a result of the FDA ban (21 CFR 189.180).

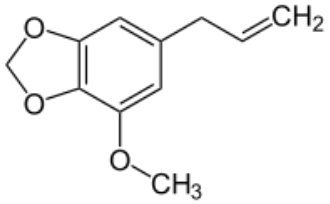
At a concentration of 1% in the diet, safrole produces weight loss, testicular atrophy, bone marrow depletion and malignant liver tumors in rats [106]. Based on sufficient evidence of carcinogenicity in experimental animals, safrole is reasonably anticipated to be a human carcinogen [107]. The mechanism of carcinogenicity is thought to involve cytochrome P450 catalyzed hydroxylation of safrole to 1'-hydroxysafrole, and its subsequent metabolism to highly reactive electrophiles that bind to DNA [108].

Despite the FDA ban, sassafras is still a popular ingredient in herb teas and preparations [73]. The hazardous dose of sassafras oil for humans (which typically contains 80% safrole) is considered to be 0.66 mg/kg [109]. This may be exceeded by ingesting sassafras tea, which has been estimated by Segelman and Bisset (as cited in Burfield 2009 [109], p. 3) to give a dose of 3 mg/kg for a 60 kg individual.

4.2.17. Myristicin

Myristicin (Figure 4) is a naturally occurring insecticide and acaracide that is found in nutmeg and mace (*Myristica* spp.) at concentrations of 1.3% and 2.7%, respectively [110]. It is also present in black pepper, carrot, celery parsley and dill [67]. It is estimated that the average total intake of myristicin from dietary sources is "in the order of a few mg per person per day" [110].

Figure 4. Structure of myristicin.



Myristicin is a weak inhibitor of monoamine oxidase, and is structurally related to mescaline. At a dose level of 6–7 mg/kg bw, it may cause psychotropic effects in man, such as increased alertness, and

a feeling of irresponsibility, freedom and euphoria. Unpleasant symptoms, such as nausea, tremor, tachycardia, anxiety and fear have also been reported in humans ingesting this dose. Although the metabolism of myristicin resembles that of safrole, there is no evidence to suggest that myristicin is carcinogenic [110]. There are no FDA regulations or guidelines specific to the presence of myristicin in food.

At the concentrations normally present in spices or food, the likelihood of toxicity arising from myristicin is low. However, ingestion of greater than 5 grams of nutmeg (corresponding to 1–2 mg/kg bw myristicin) has produced toxicological symptoms in humans that are similar to alcohol intoxication. Because the myristicin content of nutmeg is approximately 1–3%, it is likely that components of nutmeg in addition to myristicin contribute to nutmeg toxicity [110].

4.2.18. Tomatine in tomatoes

The leaves, stems and unripe fruit of the tomato plant contain α-tomatine, a steroidal alkaloid containing D-xylose, D-galactose, and two molecules of D-glucose. Tomatine is toxic to a number of different fungi, thereby acting as a natural fungicide. It has been hypothesized that the toxic effects of tomatine on fungi are due to the ability of tomatine to complex with membrane sterols, causing membrane disruption [111].

Currently, there is no evidence to suggest that tomatine is a substance of concern. There are no reports of acute toxicity in humans due to ingestion of green tomatoes and there are no FDA regulations or guidelines specific to the presence of tomatine in food. Ingestion of a rare variety of ripe tomato (*Lycopersicon esculentum* var. cerasiforme) that contains up to 5 mg tomatine/g of dry weight has no adverse effects on natives who commonly ingest them [112].

Concentrations of tomatine decrease as tomatoes ripen, and ripe fruit contains approximately 36 mg per a 100 gram tomato [73]. Microwaving or frying does not reduce content of tomatine, and delayed-ripening varieties of tomatoes contain similar concentrations of tomatine as other tomatoes [113]. At this time, there is no evidence to suggest that a diet high in green tomatoes would be injurious to human health. Tomatine forms strong, insoluble complexes with cholesterol *in vitro*, and has been shown to lower plasma LDL cholesterol in hamsters [114], suggesting that it may have beneficial effects on blood lipids of humans.

4.2.19. Japanese star anise

Chinese star anise (*Illicium verum*) is a common source of anethole, a popular flavoring ingredient. On the other hand, Japanese star anise (*Illicium anisatum*) is scientifically recognized as highly poisonous and not fit for human consumption. Japanese star anise contains the potent neurotoxins anisatin and neoanisatin, as well as the neurotoxic sesquiterpene lactone veranisatins that are normally found in other kinds of star anise, including Chinese star anise [115].

Brewed “teas” containing star anise have been associated with illnesses affecting about 40 individuals, including approximately 15 infants. The illnesses ranged from serious neurological effects, such as seizures, to vomiting, jitteriness and rapid eye movement. Due to the potential for adulteration, on September 10, 2003, the FDA issued an advisory to the public not to consume “teas”

brewed from star anise, until the FDA is able to differentiate between the Japanese star anise and Chinese star anise, which does not contain anisatin [116].

4.3. Substances formed as the result of product abuse

4.3.1. Glycoalkaloids (solanine and chaconine) in potatoes

The glycoalkaloids α-solanine and α-chaconine are natural pesticides that are produced in potatoes. α-Solanine is also found in eggplant, apples, bell peppers, cherries, sugar beets and tomatoes [74,117]. The only difference between α-solanine and α-chaconine is the sugars in the trisaccharide potion of the molecule, *i.e.*, glucose with two rhamnoses for α-solanine and a glucose, galactose and a rhamnose for α-chaconine [118].

Depending on variety and storage conditions, concentrations of α-chaconine and α-solanine in potato tubers vary between 0.5–635 ppm (0.0005–0.64 mg/g potato) and 5–125,100 molecule ppm (0.005–25.1 mg/g potato), respectively (Beckstrom-Sternberg, as cited in Tice 1998 [117], p. 9). Although glycoalkaloids are found throughout the potato tuber, the greatest concentrations are in the sprouts, peels and sun-greened areas [74]. The FDA considers the maximum acceptable glycoalkaloid content to be 20–25 mg/100 g fresh potato weight (or 200–250 ppm) (Crocco, as cited in FDA 2008 [119], p.1). Under current FDA regulations, 20 milligrams of solanine per 100 grams (a small potato) can render it unfit to eat.

Synthesis of α-chaconine and α-solanine is stimulated by light, mechanical injury, aging and potato beetle infestation [117,120]. Exposure of potatoes to light in the field or marketplace can lead to glycoalkaloid concentrations that are unsafe for human consumption. Concentrations of solanine in green or blighted potatoes have been shown to increase by seven fold [73].

The symptoms of acute toxicity to α-solanine and α-chaconine are due to their ability to act as inhibitors of acetylcholinesterase and disruptors of cell membranes. Glycoalkaloid doses of 1 to 5 mg/kg have been shown to be acutely toxic to humans, and doses of 3 to 6 mg/kg have resulted in death [117]. Symptoms of glycoalkaloid toxicity in humans include drowsiness, itchiness in the neck region, increased sensitivity (hyperesthesia), labored breathing and gastrointestinal symptoms (abdominal pain, nausea, vomiting and diarrhea) [74].

α-Solanine and α-chaconine are not mutagenic or only weakly mutagenic *in vitro*, are not genotoxic *in vivo*, and are embryotoxic and teratogenic to experimental animals. Teratogenic effects in mammals include central nervous system abnormalities (e.g., exencephaly, cranial bleb, encephalocele, and anophthalmia), mild hydronephrosis, hydroureter, and irregular or fused ribs. Although one human case study reported a correlation between the severity of potato late-blight and the incidence of spina bifida, no other studies in humans have found a correlation between the consumption of potatoes and birth defects [117]. There is no evidence that α-solanine and α-chaconine are carcinogenic in animals or humans.

In 1993, the National Institute of Environmental Health Sciences determined that the average consumption of glycoalkaloids from potatoes was 12.75 mg glycoalkaloids/person/day (0.18 mg/kg bw based on a bw of 70 kg) [117], which is approximately one-fifth of the lowest dose that has been shown to produce acute toxicity in humans (1 mg/kg bw).

4.3.2. Furocoumarin in parsnips

Ceska *et al*. reported that older 'spoiled' and diseased parsnips freely available in grocery stores may contain furocoumarin concentrations 2500% higher than fresh parsnips [121]. Microbial infection of parsnip roots can result in a dramatic increase in furocoumarin levels. Furocoumarin concentrations (the sum of five furocoumarins: angelicin, isopimpinellin, 5-MOP, 8-MOP and psoralen) in freshly harvested parsnips are generally lower than 2.5 mg/kg and do not increase after storage at −18°C for up to 50 days. In contrast, storage of whole parsnips (but not cubes or homogenate) at 4 °C resulted in a marked biphasic increase of furocoumarin concentrations (to approximately 40 mg/kg) after seven or 38 days of storage. A dramatic increase in furocoumarin concentrations (up to 566 mg/kg) was observed when whole parsnips were kept at room temperature over 53 days, resulting in a visible microbial (mold) infection [122].

In celery, infection with fungal pathogens has been shown to produce timethylpsoralen (which is absent from plants that are not infected) and increased concentrations of 8-MOP. The resulting "pink rot" has caused repeated outbreaks of photophytodermatitis in commercial celery handlers [55]. Fungal infection also has been shown to stimulate a 155-fold increase in furocoumarin production by carrots (Ceska *et al*., as cited in Wagstaff 1991 [53], p. 268). There are no FDA regulations or guidelines specific to the presence of furocoumarins in food.

4.4. Substances formed as the result of processing

4.4.1. Heterocyclic aromatic amines

There are two major classes of heterocyclic aromatic amines (HAAs). Pyrolytic HAAs are formed from the pyrolysis of amino acids or proteins at high temperature and aminoimidazoarenes (AIAs) are formed from creatine, free amino acids and monosaccharides, via the Maillard reaction. HAAs are present in many protein-rich foods of animal origin including cooked meat, fish, poultry and gravies and sauces derived from pan residues and scrapings of cooked meats. The formation and yield of HAAs are dependent on cooking temperature and time (concentrations increase with higher temperatures and longer cooking times), cooking technique and equipment (concentrations of HAAs in meat are generally higher after grilling and panfrying than broiling or roasting), and the ability of HAA precursors to migrate to the surface [123].

The AIAs 2-amino-3-methylimidazo-[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) and 2-amino-3,8,dimethylimidazo[4,5-f]quinoxaline (MeIQx) are among the most potent mutagens ever tested in the Ames assay. The pyroltic AIA 2-amino-1-methyl-6-phenylimidazol(4,5-b)pyridine (PhIP) and the HAAs 2-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 2-amino-4-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), 2-amino-9H-pyrido[2,3-b]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC) are also mutagenic. PhIP accounts for 75% of the mass of genotoxic material that has been attributed to HAAs in fried ground beef. Therefore, the potential for genotoxicity due to PhIP may be higher than that of more genotoxic HAAs in meat consumers [123].

Several HAAs are carcinogenic in rodents after long-term dietary administration. The doses required to induce tumors at a 50% rate (TD_{50}) vary for each HAA, and range from 0.1 to 64.6 mg/kg bw/day [123]. Four HAAs (IQ, MeIQ, MeIQx and PhIP) are "reasonably anticipated to be

human carcinogens" [124]. Due to the fact that exposure to HAAs in cooked meats is highly variable (concentrations in cooked meat may range from <1 to 500 ng/g), it has been estimated that the risk of developing cancer from exposure to HAAs in food is anywhere from 50 in one million to one in a thousand [123]. Currently, no tolerable upper limit of exposure to HAAs has been established.

4.4.2. Polycyclic aromatic hydrocarbons

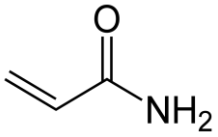
Polycyclic aromatic hydrocarbons (PAHs) are known carcinogens that are formed from the incomplete combustion of fossil fuels such as wood, coal and oil. PAHs can enter the food chain from environmental contamination or from food processing. Foods containing the highest concentrations of PAHs include cooked or smoked meat or fish, smoked or cured cheese, tea and roasted coffee. Grilling or broiling of meat, fish or other foods over intense heat or direct contact with flames promotes production of PAHs. In general, concentrations of PAHs in meat are highest after charcoal grilling, followed by smoking, roasting and steaming. Concentrations of PAHs in smoked foods are influenced by temperature, type of wood, oxygen concentration and type of smoker. Concentrations of PAHs in tea dried over burning wood, oil or coal are generally higher than in tea dried over air, and coffee beans that are roasted over a direct fire contain higher concentrations than beans that do not come in contact with flames [125].

The European Commission's (EC) Scientific Committee on Food and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has concluded that thirteen different PAHs are genotoxic and carcinogenic benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. Three of the four PAHs that have been tested for carcinogenicity in rats after oral exposure (benz[a]anthracene, benzo[a]pyrene and dibenz[a,h]anthracene) are carcinogenic. The estimated high and safe levels of intake of the benchmark PAH benzo[a]pyrene are 0.01 and 100 μg benzo[a]pyrene/kg bw/day, respectively, indicating that the estimated intake of PAHs in food is 10,000-fold lower than the level that is expected to cause toxicity in humans [126]. Currently, no tolerable upper limit of exposure to PAHs has been established by the FDA.

4.4.3. Acrylamide

Acrylamide (Figure 5) is found in a number of starch-based foods that are fried or baked at temperatures greater than 120 ℃ (248 °F), including bread, bakery products, breakfast cereal, and potato products (e.g., chips, french fries) [127]. It also is found in cocoa-based products and coffee. Acrylamide is formed via a Maillard reaction, a reaction between the carbonyl group of a reducing sugar and the nucleophilic group of an amino acid. Although a number of carbohydrates can be used as the source of the carbonyl group, the amino acid required for the formation of acrylamide is asparagine.

Figure 5. Structure of acrylamide.



Acrylamide is mutagenic and has been shown to be a neurotoxicant, reproductive toxicant and carcinogen in experimental animals and is classified by IARC as a probable human carcinogen. The main metabolite, glycidamide (an epoxide) is thought to be responsible for genotoxicity [127]. In humans, the only toxicological effect that has been linked to acrylamide is neurotoxicity in individuals occupationally exposed to high levels. Epidemiological studies have failed to show an increased risk of cancer from either occupational or dietary exposure to acrylamide and reproductive toxicity has not been reported in humans exposed to acrylamide [128]. Acrylamide is a unique substance that exemplifies the concept that the structure of the substance greatly influences the toxicity, as acrylamide is an animal feed ingredient (thickener and suspending agent) only when a part of a long-chain polymer having a minimum molecular weight of 3 million and a viscosity range of 3,000 to 6,000 centipoises at 77 ° F. The residual acrylamide cannot be more than 0.05% (21 CFR 573.120).

In 2005, JECFA estimated that average and high intake consumers ingest 1 or 4 μg/kg bw/day acrylamide from food, respectively. Using a NOAEL for neurotoxicity of 200 μg/kg bw/day in animals, margins of safety of 200 and 50 for the average and high intake groups were derived, respectively. Utilizing a benchmark dose of 0.3 mg/kg bw/day and a NOAEL of 2 mg/kg bw/day for development of mammary tumors or reproductive in rats (respectively), higher margins of safety were calculated for carcinogenicity (300 and 75, respectively) and reproductive toxicity (200 and 50, respectively) [128].

Exposure to acrylamide can be reduced by avoiding deep-fried foods, soaking potato slices before cooking, cooking french fries at lower temperatures and to a lighter color, and toasting bread to a lighter color [127].

4.4.4. Chloropropanols

Chloropropanols are formed in hydrolyzed vegetable proteins (HVP) produced by hydrochloric acid (HCl) hydrolysis of proteinaceous by-products from edible oil extraction, such as soybean meal, rapeseed meal and maize gluten [129,130]. The chloropropanol most commonly found in food is 3-MCPD (3-monochloropropane-1,2-diol), although others may also be present, including 2-MCPD (2-monochloropropane-1,3-diol), 1,3-DCP (1,3-dichloro-2-propanol), and 2,3-DCP (2,3-dichloro-1-propanol) [130]. The two most widely studied chloropropanols are 3-MCPD and 1,3-DCP. It is thought that 3-MCPD is formed as a result of a reaction between a source of chlorine (chlorinated water or sodium chloride) in a food or a food contact material and a lipid. Two basic pathways have been proposed: thermally driven and enzyme-catalyzed (generally lipase) reactions. Direct precursors are thought to be glycerol and chloride. Recent work has also suggested glycidol (2,3-epoxy-1-propanol) as a precursor. 1,3-DCP is thought to arise from 3-MCPD.

High concentrations of 3-MCPD have been found in acid hydrolyzed HVP (acid-HVP), and soy or oyster sauce produced using an acid hydrolysis process. Other foods that may contain 3-MCPD are

cereal, toasted bread, coffee, cheese, licorice, baked goods, processed garlic, liquid smokes, malts, cured or smoked meat or fish or foods containing acid-HVP as a savory ingredient (soups, prepared meals, savory snacks, gravy mixes and stick cubes [129–132]. Foods containing 1,3-DCP include raw meat and soy sauce produced using an acid hydrolysis process [129].

In rats and mice, 3-MCPD is toxic to the kidney, producing renal tubule hyperplasia. It is also carcinogenic in rats when given in high doses over prolonged periods. Although 3-MCPD is genotoxic *in vitro*, it is not *in vivo*. The UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) has concluded that 3-MCPD is unlikely to present a carcinogenic risk to man, provided the exposure is 1000 times lower than the no observed effect level (NOEL) of 1.1 mg/kg bw/day for tumorigenicity. JECFA set a tolerable daily intake (TDI) of 2 μg 3-MCPD/kg of body weight in 2001 and a maximum allowable content of free 3-MCPD in liquid condiments at 0.4 mg/kg (400 μg/kg) in 2008 [130]. Assuming 400 μg/kg 3-MCPD is present in soy sauce, a 60 kg human would have to ingest 300 g of soy sauce (approximately two-thirds of a 444 mL bottle) per day to achieve the TDI. The FDA has provided a policy statement stating that acid-H[V]P or Asian sauces that contain 3-MCPD at levels greater than 1 ppm are not Generally Recognized As Safe (GRAS); therefore, these ingredients are unapproved food additives [133].

1,3-DCP is hepatotoxic, genotoxic and induces a variety of different types of tumors in rats. Therefore, 1,3-DCP is considered to be a potential carcinogen in humans. In 1993, FAO/WHO and JECFA concluded in that 1,3-DCP is an undesirable contaminant in food and that levels should be reduced to as low as "technologically achievable" [131].

4.4.5. Furan

Furan (Figure 6) is a by-product of high-energy and thermal treatment of carbohydrate. Meat and vegetable containing foods that are heat processed in cans and jars (such as soups, pastas, sauces, gravy and baby food) and brewed coffee, typically contain the highest concentrations. Concentrations of furan present in food and coffee range from undetectable to approximately 175 μg/kg [134]. Coffee powders may contain up to 5000 μg/kg on a dry weight basis. Although the mechanism of formation of furan in food is not completely understood, it can be synthesized from vitamin C, amino acids, reducing sugars, organic acids, carotenes and polyunsaturated fatty acids in the presence of heat [135].

Figure 6. Structure of Furan.

Furan is mutagenic and clastogenic in a number of *in vitro* mammalian cell assays, causes damage to chromosomes in mice, and is carcinogenic in both rats and mice after oral administration [134,136–138]. Furan is classified by IARC as possibly carcinogenic to humans [134].

In the United States and Europe, exposure to furan from food is estimated to be a maximum of 1.00 and 1.75 μg/kg bw/day, respectively [134]. The upper estimate of consumption is approximately

300 and 1000-fold lower than the NOAELs for cytotoxicity and hepatocarcinogenicity of 500 and 2000 μg/kg bw in female B6C3F1 mice, determined by Moser *et al.* [136].

Mitigation of furan in foods is difficult because the mechanism for its formation in food is unclear. Due to the fact that furan is volatile, it is thought that concentrations can be reduced by heating food in open containers or leaving ready-to-eat foods open to air after preparation. However, the effectiveness of this strategy in reducing exposure to furan has yet to be demonstrated [135]. Currently, there are no FDA regulations specific to the level of furan in food.

4.4.6. Trans fatty acids

Trans fatty acids (also known as trans fat) are the sum of all unsaturated fatty acids that contain one or more isolated double bonds in a trans configuration. Trans fatty acids more closely resemble saturated fatty acids than cis unsaturated fatty acids because their trans configuration makes them rigid. Trans fatty acids in the diet originate from two sources. The first is from bacterial hydrogenation in the forestomach of ruminants, which produces trans fatty acids that are found in beef and mutton fat, milk and butter. Trans fatty acids are also produced from the hydrogenation of liquid oils (mainly of vegetable origin). This produces solid fats and partially hydrogenated oils such as margarines, spreads, shortenings and frying oil, which are more stable than liquid oils [139].

Biochemically, trans-fatty acids act similarly to saturated fatty acids, raising low density lipoprotein (LDL) cholesterol and decreasing high-density lipoprotein (HDL) cholesterol levels [139]. High intakes of trans fatty acids have been associated with an increased risk of coronary heart disease (CHD) independent of other risk factors in large epidemiological studies [140]. A tolerable upper limit of trans fatty acids has not been set because any incremental increase in the intake of trans fatty acids increases the risk of coronary heart disease [141].

In the US, the main sources of intake of trans fatty acids are baked goods (28%), fried foods (25%), margarine, spreads and shortenings (25%), savory snacks (10%), milk and butter (9%) [139]. In 1996, processed foods and oils accounted for 80% of the trans fat in the diet [141]. In 1999, the FDA estimated that the average daily intake of trans fat in the United States is about 5.8 grams or 2.6% of calories per day [142]. It has been hypothesized that replacing 2% energy from trans fatty acids with 2% energy from oleic acid would reduce mean plasma LDL cholesterol concentration by 0.08 mmol/L, and increase plasma HDL concentration by 0.08 mmol/L. These changes could reduce the incidence of CHD by 5–15% [139].

Due to increased efforts by food manufacturers to reduce or eliminate the use of partially hydrogenated vegetable fat in food production, it is estimated that trans fatty acid content of processed foods has decreased over the last decade [143].

4.4.7. Nitrosamines formed during drying, curing and preserving

Nitrosamines are formed from the interaction of nitrites or other nitrosating agents with amines in food (or *in vivo*), under acidic conditions. Nitrites may be directly added to food or can be formed from bacterial reduction of nitrate. Nitrites and nitrates may occur naturally in water or foods such as leafy vegetables due to the use of fertilizer, or may be added to foods to prevent growth of *Clostridium botulinum*, or to add color or flavor [144].

Nitrosamines have been found in a variety of different foods such as cheese, soybean oil, canned fruit, meat products, cured or smoked meats, fish and fish products, spices used for meat curing, and beer and other alcoholic beverages [145]. Beer, meat products and fish are considered the main sources of exposure. Drying, kilning, salting, smoking or curing promotes formation of nitrosamines [146].

The nitrosamines most frequently found in food are nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosopiperidine (NPIP), and *N*-nitrosothiazolidine (NTHZ) [146]. NDMA, NPYR, NPIP are reasonably anticipated to be human carcinogens based on evidence of carcinogenicity in experimental animals [145,147,148]. Evidence from case-control studies supports an association between nitrosamine intake with gastric cancer, but not esophageal cancer in humans [149].

Levels of nitrosamines have been declining during the past three decades, concurrent with a lowering of the nitrite used in food, use of inhibitors such as ascorbic acid and use of lower operating temperatures and indirect heating during food processing. Based on an estimated exposure level of 3.3–5.0 ng/kg bw/day, the and the benchmark lower limit of 60 μg/kg bw/day, a margin of error associated with a low level of concern (12,000–18,2000) has been derived for NDMA, the most common nitrosamine in food [146].

Although current FDA regulations do not limit nitrosamine levels in foods, the FDA has provided an action level of 10 ppb for individual nitrosamines in both consumer and hospital rubber baby bottle nipples, while the FDA limits the approval of nitrites in curing mixes to the FDA-regulated food additive process (21 CFR 170.60), with the approval of sodium nitrite as a food additive (food preservative) (21 CFR 172.175). The USDA monitors finished meat products to insure that nitrite is not present in amounts exceeding 200 ppm (9 CFR 424.21).

4.4.8. Biogenic amines

Biogenic amines are normally formed in humans by normal cellular metabolism. In food, biogenic amines are mainly formed from microbial decarboxylation of amino acids. They are commonly found in fermented meat, beverages and dairy products, sauerkraut, and spoiled fish. The main biogenic amines in food are histamine, tyramine cadaverine, putrescine, spermidine and spermine. The two biogenic amines that have been associated with acute toxicity are histamine and tyramine. Putresine, spermine, sperimidine and cadaverine are not toxic in and of themselves, but may react with nitrite or nitrate to form nitrosamines (see Section 4.4.7 above) [150].

Scombrotoxicosis is a common seafood-borne disease associated with the consumption of toxic levels of histamine in spoiled scombroid fish such as tuna (*Thunnus* spp.), mackerel (*Scomber* spp.), saury (*Cololabis* saira) and bonito (*Sarda* spp.). Red wine may also contain relatively high levels of histamine. Symptoms of histamine intoxication from food are similar to allergies to other substances and include sneezing, nose congestion, breathing difficulties and urticaria [150].

Consumption of tyramine may precipitate migraine headache or a hypertensive crisis. The most serious case reports of tyramine toxicity have occurred in people consuming aged cheese. Because monoamine oxidase inhibitor (MAOI) drugs inhibit metabolism of amines, people taking these drugs may be particularly susceptible to tyramine toxicity. Whereas 200–800 mg of dietary tyramine induces only a mild rise in blood pressure in unmedicated adults, 10–25 mg may produce a serious adverse

event in those taking MAOI drugs. Other potentiating factors for tyramine toxicity include alcohol consumption, gastrointestinal distress and exposure to other amines [150].

Efforts taken by food manufacturers to reduce biogenic amine concentrations in fermented foods include using amine-negative starter cultures, adding probiotic bacterial strains alone or in combination with starter cultures, high pressure processing or low-dose gamma radiation [150]. FDA guidelines specify 50 mg/100 g as the toxic concentration of histamine in scombroid fish and the agency has published guidance on how to control levels [151].

5. Substances Passed from Animals to Humans

5.1. Toxins in seafood

5.1.1. Toxins involving algae

Consumption of seafood contaminated with algal toxins results in five different syndromes, paralytic, neurotoxic, amnesic, or diarrhetic shellfish poisoning and ciguatera fish poisoning [152].

5.1.1.1. Paralytic shellfish poisoning

Paralytic shellfish poisoning (PSP) is caused by the consumption of molluscan shellfish contaminated with heterocyclic guanidines called saxitoxins. Currently, over 21 known saxitoxins are produced by dinoflagellate species from three genera: *Alexandrium*, *Gymnodium* and *Pyrodinium*. Toxicity is caused by binding of saxitoxins to voltage-dependent sodium channels, which blocks neuronal activity. The primary site of action in humans is the peripheral nervous system. Symptoms of toxicity include tingling and numbness of the perioral area and extremities, loss of motor control, drowsiness, and incoherence. Ingestion of 1–4 mg saxitoxin has resulted in death from respiratory paralysis [152].

Outbreaks of PSP have occurred worldwide, due to the fact that saxitoxin-producing species of dinoflagellates can live in either temperate or tropical waters. Saxitoxins are not inactivated by cooking, and must be mitigated at their source to prevent ingestion. PSP is prevented by large-scale, proactive monitoring programs and rapid closures of harvest in areas containing dinoflagellate algal blooms [153]. In the United States, the permissible level of saxitoxin equivalents in shellfish is 80 micrograms/100 grams [154].

5.1.1.2. Neurotoxic shellfish poisoning

The dinoflagellate *Karenia brevis* produces brevetoxins that are lethal to fish, but not to mollusks such as oysters, clams and mussels. Consequently, they can accumulate in healthy-appearing mollusks to concentrations that are toxic to humans who ingest them. *Karenia brevis* brevitoxins cause the syndrome known as neurotoxic shellfish poisoning (NSP), which affects sodium transport in the autonomic nervous system and causes inhibition of neuromuscular transmission in skeletal muscle.

NSP is usually a relatively mild illness and should not be confused with the more serious condition of PSP. NSP symptoms usually occur within three hours of ingesting contaminated shellfish and may include abdominal pain, nausea and vomiting, vertigo, malaise, generalized muscle weakness, ataxia,

incoordination, chills, headache, myalgia, a reversal of hot/cold temperature sensation and progressive parasthesias. Dilated pupils, bradycardia and convulsions may occur in cases of severe poisoning [155]. Unlike PSP, no deaths have been reported from NSP [152].

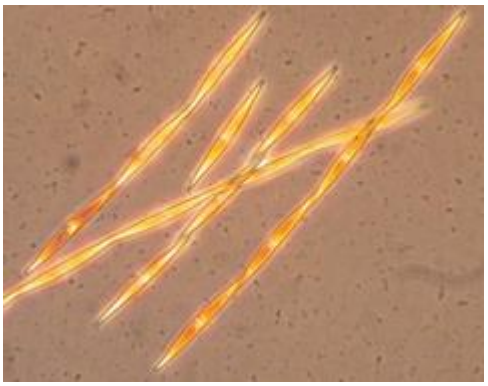
K. brevis is the organism that is usually responsible for the red tides in the Gulf of Mexico and along the southern Atlantic coast of North America. Blooms along the west coast of Florida occur regularly [156]. Biotoxin control plans that are implemented during period of red tide are generally effective in preventing NSP, but have not eliminated NSP entirely.

The FDA has established an action level of 0.8 ppm (20 mouse units/100 g) brevetoxin-2 equivalents [154].

5.1.1.3. Amnesic shellfish poisoning (Domoic acid)

Amnesic shellfish poisoning (ASP) is caused by domoic acid produced by diatoms of the genus *Pseudo-nitzchia* (Figure 7), which are consumed by mussels, scallops, clams and crabs. Domoic acid is a water-soluble, tricarboxylic amino acid that is a structural analog of the neurotransmitter glutamate and is a glutamate receptor agonist. Persistent activation of the kainite glutamate receptor causes an increase in intracellular calcium, which can cause neuronal cell death and lesions of the brain where glutamineric pathways are concentrated. Areas of the brain involved in learning and memory processing are particularly susceptible [152]. The symptoms of ASP are gastroenteritis, dizziness, disorientation, lethargy, seizures and loss of short term memory. Respiratory difficulty, coma and death may ensue [153]. Human toxicity has occurred after ingestion of 1–5 mg/kg domoic acid [152].

Figure 7. *Pseudo-nitzchia* [157].



In 1987, approximately 100 people became ill and died in Prince Edward Island, Canada, after eating contaminated mussels. In 1991, domoic acid poisoning caused the deaths of numerous pelicans and cormorants in Monterey Bay that ingested sardines and anchovies. Domoic acid also was responsible for a massive sea lion kill in Monterey Bay in 1998 [158]. *Pseudo-nitzchia* and domoic acid are now closely monitored throughout the world [159]. The FDA has established an action level of 20 ppm for domoic acid, except in the viscera of Dungeness crab, where 30 ppm is permitted [154]. Regulatory guidance has been effective in preventing ASP in humans, since no human outbreaks of ASP have occurred since 1987.

5.1.1.4. Diarrhetic shellfish poisoning

Diarrhetic shellfish poisoning (DSP) is caused by the production of okadaic acid and dinophysistoxins in the dinoflagellates *Dinophysis fortii* or *Prorocentrum lima*, which are consumed by mollusks. Okadaic acid and dinophysistoxins are inhibitors of serine/threonine phosphatases, critical components of signaling cascades that regulate a number of cellular processes involved in metabolism, ion balance, neurotransmission and cell cycle regulation [152].

Compared to other types of shellfish poisoning, symptoms of DSP are relatively mild, and generally consist of diarrhea, abdominal cramps, nausea, chills or vomiting within 30 minutes to a few hours after consumption of DSP toxins. Symptoms generally resolve within 2–3 days, with or without medical treatment [153]. Diarrhea is most likely due to the hyperphosphorylation of proteins (including ion channels) in the intestinal epithelia, resulting in impaired water balance and fluid loss. The long term consequences of low level exposure to DSP toxins may be more serious, as they have been shown to be tumor promoters [152]. The FDA has established an action level of 0.2 ppm okadaic acid plus 35-methyl okadaic acid (DXT 1) [154].

5.1.1.5. Ciguatera poisoning

Ciguatera fish poisoning (CFP) is caused by the dinoflagellate *Gambierdiscus toxicus*, which grows on filamentous macroalgae associated with coral reefs. The lipophilic precursors to ciguatoxin are biotransformed to ciguatoxins in herbivorous fish and invertebrates that consume the macroalgae, and bioaccumulate in large carnivorous fishes associated with coral reefs. High ciguatoxin concentrations may be found in barracuda, snapper, grouper and jacks [152].

Ciguatoxins are structurally related to the brevetoxins and compete with brevetoxin for binding to the same site on the voltage-dependent sodium channel. However, because ciguatoxin has a higher binding affinity for the site than brevetoxin, the toxic potency of ciguatoxin is higher than that of brevetoxin. The threshold level for toxicity in humans is estimated to be 0.5 ng/g [152].

CFP is estimated to affect over 50,000 people worldwide each year. The symptoms of CFP generally include gastrointestinal disturbances (nausea, vomiting and diarrhea) within 2–6 hours, followed by neurologic symptoms such as numbness of the perioral area and extremities, a reversal of hot/cold temperature sensation, muscle and joint aches, headache, itching, tachycardia, hypertension, blurred vision and paralysis. In rare cases, CFP is fatal [152].

Inasmuch as ciguatoxin is produced by organisms that live beneath the surface and is not routinely monitored for concentration in seafood, the only way to prevent consumption is to completely abstain from ingesting tropical reef fish, as the occurrence of toxic fish is sporadic, and not all fish of a given species or from a given locality will be toxic [153]. Currently, there are no FDA regulations limiting levels of ciguatoxins in fish, although a recent publication suggests an advisory level of 0.1 ppb pacific ciguatoxin equivalent (P-CTX-1) toxicity values in fish from the tropical Atlantic, Gulf of Mexico, Caribbean, and 0.01 ppb P-CTX-1 equivalent toxicity in fish from Pacific regions [160].

5.1.2. Toxins not involving algae

5.1.2.1. Gempylotoxin

There are naturally occurring toxins in some species that do not involve marine algae. Escolar (*Lepidocybium flavobrunneum*, Figure 8), and Oilfish or Cocco (*Ruvettus pretiosus*), a marine fish of the snake mackerel family, are sometimes sold under the category of "butterfish", and contain a strong purgative oil, that when consumed can cause diarrhea known as Gempylid Fish Poisoning, Gempylotoxism or Keriorrhea [161]. The toxin consists of wax esters (C32, C34, C36 and C38 fatty acid esters), the primary component of which is $C_{34}H_{66}O_2$ [162]; these constitute a substantive portion of the lipid present in these fish (14–25% by weight). Escolar oil contains >90% wax esters [163]. Ingestion of fish containing wax esters in large amounts, coupled with their indigestibility and low melting point, results in diarrhea [164]. No tolerances have been established, and the FDA recommends avoidance of these fish [161].

Figure 8. Juvenile Oilfish (*Ruvettus* pretiosus) [165].

5.1.2.2. Tetramine in whelks

Tetramine is a toxin found in the salivary glands of *Buccinum*, *Busycon* or *Neptunia* spp., a type of whelk or sea snail that is distributed in temperate and tropic waters and has long been a food source for humans. Whelk are associated with a heat-stable neurotoxin, tetramine, which upon ingestion produces, among other symptoms, eyeball pain, headache, dizziness, abdominal pain, ataxia, tingling in the fingers, nausea and diarrhea [166,167]. Power *et al*. report that the highest concentration of tetramine is in the salivary gland (up to 6530 μg/g), but varies according to season [168]. Reid *et al.* reported levels of 37.5 μg tetramine/g of salivary gland tissue [166]. Because the whelk is a predator of bivalves, it is assumed the toxin is used for food procurement [168]. Although the FDA recommends removal of the salivary gland to avoid possible intoxication [154], tetramine is present in other tissues, albeit at lesser concentrations [169].

5.1.2.3. Trimethylamine oxide

The meat of the Greenland shark (*Somniosus microcephalus*) and the related member of the dogfish family, the pacific sleeper shark (*Somniosus pacificus*), is known to be poisonous to both man and dogs. The causative agent is trimethylamine oxide, which breaks down to trimethylamine in the gut, probably by enteric bacteria. The result is absorption of trimethylamine, which acts as a neurotoxin,

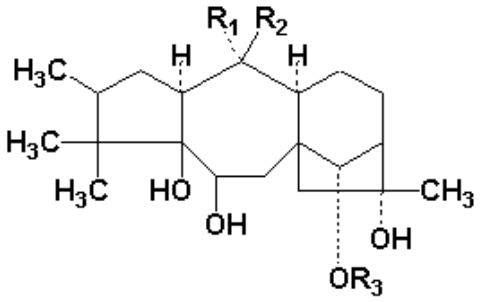
producing ataxia in both man and dogs. However, the flesh may be consumed if boiled several times with changes of water, or as the Inuit prepares it, by burying it in the ground and allowing the meat to go through several freezing and thawing cycles [170–172].

5.2. Toxins from animal, non-seafood sources passed on to humans

5.2.1. Grayanotoxins in honey and direct contact with food

Rhododendrons and azaleas (*Rhododendron* spp.), oleander (*Nerium oleander* or *Nerium indicum*), mountain laurel (*Kalmia latifolia*) and sheep laurel (*Kalmia angustifolia*), all produce grayanotoxins (Figure 9) whose action is to bind to sodium channels in muscle, including the heart. Although not all rhododendrons produce grayanotoxins (also known as oleander toxin, andromedotoxin, acetylandromedol or rhodotoxin), several species growing in the US are known to produce grayanotoxins and include *Rhododendron occidentale*, *Rhododendron macrophyllum* and *Rhododendron albiflorum*, all in the western US. Grayanotoxin is also found in the eastern US, within the botanical family Ericaceae, to which rhododendrons belong and are probably the most important sources of the toxin [173].

Figure 9. Grayanotoxins [173].



GRAY	R_1	R_2	R_1R_2	R_3
GRAY1	OH	CH_3	-	H
GRAY2	-	-	$=CH_2$	H
GRAY3	OH	CH_3	-	Ac

Grayanotoxin consists of a series of cardiac glycosides: thevetin, convallarin, steroidal, helleborein, ouabain, and digitoxin. At first, sympathetic nerves are paralyzed; the cardiotoxin stimulates the heart muscles similar to the action of digitalis, and gastric distress ensues. Symptoms start out as nausea, vomiting, abdominal pain and diarrhea; followed by tremor, drowsiness and ataxia. In severe cases, ectopic beats occur which may be followed by ventricular tachycardia and fibrillation. The origin of toxicity may be honey (made from the nectar of the flowers), milk from a cow having eaten the foliage and meat (e.g., hot dogs) roasted on oleander sticks [15,174]. The pooling of large quantities of grayanotoxin-containing honey or milk during commercial processing typically dilutes grayanotoxin to nontoxic levels. There are no FDA regulations specific to grayanotoxin levels in foods.

5.2.2. Tremetol contamination of milk from white snakeroot

"Milk sickness" also known as "puking fever", "sick stomach", "the slows" and "the trembles", was a mysterious scourge of the Midwest United States in the 18th and 19th centuries. Thousands of people have been reported as dying, including Abraham Lincoln's mother, Nancy Hanks Lincoln. In humans, milk sickness is characterized by loss of appetite, listlessness, weakness, vague pains, muscle stiffness, vomiting, abdominal discomfort, constipation, foul breath and finally, coma. For many years the origin of milk sickness was unknown, because there was nothing comparable in Europe (origin of most of the pioneers) and the outbreaks were sporadic. It was not recognized until the late 19th and early 20th century, that white snakeroot (*Ageratina altissima* née *Eupatorium rugosum*) and rayless goldenrod (*Bigelowia* spp., *Haplopappus heterophyllus* and *Isocoma pluriflora*) when eaten by cattle, was the source. The sporadic nature of outbreaks became clear when it was realized that cattle would consume these plants in over-grazed pasture or in years of drought; additionally, the toxin levels in plants can vary considerably, making identification of the source of poisonings difficult. Tremetol or tremetone is the toxic agent and consists of a mixture of sterols and derivatives of methyl ketone benzofuran. The three major benzofuran ketones are tremetone, dehydrotremetone and 3-oxyangeloyl-tremetone [173–177]. Currently, there is no USDA guidance specific to tremetol levels in dairy products.

6. Conclusions

Given the state of the science, the pressure on the food supply and the development of new products, the FDA has performed admirably in protecting the consumer from exposure to toxins in food with its judicious use of warning labels, action levels, tolerances, specifications, prohibitions and the ability conferred by Congress to declare substances "unsafe" or "unfit for food." However, the FDA cannot protect consumers absolutely from exposure to toxins normally present in foods. At normal levels of food consumption, there is little potential for toxicity from natural food toxins. Nevertheless, there is always the possibility of an idiosyncratic response or undetected contamination.

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From: acvimcardio <acvimcardio@vin.com>
To: (b) (6)
Subject: dilated cardiomyopathy and kangaroo and lentil diets [(b) (6)]
Date: Wed, Jan 17, 2018 10:51 am

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Folder: **Myocardial Disease**
Discussion: **dilated cardiomyopathy and kangaroo and lentil diets**

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New Messages (2)

12. Posted by (b) (6) on 01-16-18 07:50 ET

201C
Original Message
plasma taurine changes very quickly with diet so may not reflect very accurately blood taurine status
201D

This is definitely true in cats. We documented this in the late 80's. Fasting 24 hours can bring plasma taurine down below normal range, but not where we generally see in truly deficient cats. Still hard to interpret if fasted.

You also need to worry about false elevation from damaged WBCs and platelets that have high taurine concentrations.

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snap frozen whole blood is better though a pain to collect and transport.
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As long as it's sterile, whole blood taurine should be pretty stable even if not frozen. We didn't note big degradation impacts of not freezing. Freezing and transporting likely is best still.

201C

Original Message
Equally blood taurine levels may not reflect cardiac muscle taurine 201D

These correlate pretty well in a steady state. There is a lag during depletion meaning that if you feed cats (I don't have data in dogs), a taurine depleting diet, the half-life of taurine in plasma is very short, whole blood relatively long and of skeletal muscle is longer. Cardiac muscle likely parallels skeletal, but we couldn't get as many data points since we could not sample cardiac muscle (by endomyocardial biopsy) as frequently as we sampled skeletal muscle.

>> (b) (6) <<

(b) (6)

13. Posted by (b) (6) **on 01-17-18 10:45 ET**

Hi All - I have been working with a few other cardiologists to collect a group of golden retrievers with taurine-deficiency and DCM. We have a total of 24 unrelated golden retrievers and the vast majority of these were eating diets that are labeled as grain free and use a large amount of peas or lentils in the ingredients.

Our nutritionists at UCD have been involved and analyzed some of the diets but certainly not all. We have reported the findings to the FDA. We are working on the (b) (4) . We are also interested in the trend that many of the designer diets include higher quality meats with relative little organ meat or byproducts. Perhaps this switch is also important as there is a fair amount of taurine to be found in the lower quality organ meats / byproducts that these designer diets try to avoid.

We have seen a fair number of other breeds as well but have not included them in our data gathering. A few of our cases were in CHF and have resolved with supplementation. Almost all have improved on supplementation. We have initial screening data and 3-4month follow-up on each of these cases now and I have a student working on writing this up. Many dogs had pretty low whole blood taurine levels, but a few had "low normal" values yet still responded favorable to supplementation.

Would love to chat with anyone interested.

(b) (6)

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Complete Discussions (13)

1. Created by (b) (6) **on 01-10-18 02:15 ET**

Hi,

I am currently seeing my fourth patient in a year with dilated cardiomyopathy that eats a

kangaroo and lentil diet. The first two were related Labrador retrievers (aunt and niece) in the same household. The second was a Cocker spaniel. Today's patient is a mixed breed 55 pound brindle lab mix. The first Labrador had a normal whole blood taurine, and we did not test the second housemate since she presented at a later date and we knew that her housemate fed the same diet was not taurine deficient. The Cocker Spaniel was taurine deficient based on a low whole blood taurine. The first Labrador is a year out from her initial heart rate episode, and still alive. We have changed diet, but not until her housemate was also found to have DCM (this patient was euthanized when she had a quick recurrence of CHF), just a few months ago, and an echo recently did not show significant change from baseline. We did look up carnitine levels, and supposedly kangaroo diets have high carnitine levels. I had just tacked the odd diet up to coincidence suspecting that the Labs may have had a heritable form of DCM, and the Cocker taurine-deficient DCM. Now, though, with number four, I thought I would extend the question to the group to see if anyone else has had any cases of DCM in dogs on a kangaroo diet.

Thanks for any input!

(b) (6)

2. Posted by (b) (6) on 01-10-18 06:03 ET

Hi (b)

(b) (4)

Hopefully we will have more information soon.
Take care
(b) (6)

(b) (6), DVM, DACVIM (Cardiology)

3. Posted by (b) (6) on 01-11-18 11:38 ET

Hi,
When I was a resident I saw two American cockers with DCM that were being fed a kangaroo and lentil diet - they were taurine deficient and responded to diet change and supplementation. Both dogs belonged to the same owner but were unrelated. We consulted the nutrition team at UC Davis to see whether they thought there might be a problem with the diet - however, they thought it was more likely to be breed related, which seemed reasonable at the time.

I'm really intrigued by your cases though - were they all eating the same brand of food, or were they kangaroo and lentil diets from a variety of manufacturers?

Let me know if more information would help,

(b) (6)

4. Posted by (b) (6) on 01-12-18 09:54 ET

I have seen 3 dogs from one household on a lentil based diet with DCM. Mixed breeds. One died. Other 2 recovered with diet change and taurine supplementation.

Blood taurine levels were extremely low in all dogs. Owner was into vegan diets but fed nothing but lentils and a supplement for more than a year.

(b) (6)

5. Posted by (b) (6) on 01-12-18 11:32 ET

What good timing!

I too have had several atypical DCM breeds over the past few months, but the most recent one was this week - a 2 kg 5yo Pomeranian who is on Zignature Kangaroo diet and has been eating this for the past several years. I was highly suspicious for taurine deficiency based off the unusual breed, but it's interesting to know that others are seeing this as well.

(b) (6)

6. Posted by (b) (6) on 01-12-18 01:39 ET

We have also seen a few dogs recently. They were a 2 yo Boston eating kangaroo and lentil wit a normal blood taurine, an 8 month old husky mix eating a grain free diet, and an 8 month old GSD mix also eating grain free (no blood taurines on either of the latter two).

(b) (6)

7. Posted by (b) (6) on 01-15-18 11:14 ET

Hi all,
I have three Cocker Spaniels, same family, all Taurine-Responsive systolic dysfunction receiving Zignature's Kangaroo & Lentil diet. One was in severe left sided congestive heart failure, hospitalized for 4 days with severely decreased systolic function and severe mitral regurgitation. Three months later, (on Taurine, pimobendan, furosemide and ACE inhibitor), his systolic function had resolved and his mitral regurgitation was significantly decreased (to trivial). Nine months out he has normal systolic function and persistently trivial mitral regurgitation (the previously noted severe MR is attributed to mitral valvular annular dilation and not primary valvular regurgitation). The other two have normal systolic function with Taurine supplementation, observed at six months following Taurine supplementation.
(b) (6)
(b) (6).com [mailto (b) (6)]
(b) (6)

8. Posted by (b) (6) on 01-15-18 04:23 ET

Good afternoon,
I have had two dogs with DCM that were eating lentil and rice based diets. One was a mixed breed shepherd type dog that was eating a vegan diet (owner was vegan). the dog was not taurine deficient or responsive and died about six months after diagnosis. The second, a pit bull, developed after eating a lentil/rice diet for 12 weeks as part of a food allergy trial. The following three months the dog ate a few different proteins but went back to lentils/rice in between. This dog is low taurine and we are hoping will respond to supplementation. I am wondering if there is something inherently negative about the lentils and amino acid

processing...kind of a scary situation.
Best,
(b) (6)

9. Posted by (b) (6) on 01-15-18 10:36 ET

This compilation of reports related to a few food types is impressive, especially those demonstrating resolution after taurine supplementation. Someone needs to compile these and publish them.

Has anyone contacted the manufacturers of the foods? Response from them?

Has anyone analyzed the taurine content of the foods? I'm happy to arrange and pay for that if samples of the foods these dogs were eating are available.

>>(b) (6)<<

(b) (6)

10. Posted by (b) (6) on 01-16-18 03:11 ET

Hi All

Thankfully Kangaroo and lentils do not seem to have arrived en masse in the UK yet - I would have thought parasitic disease from kangaroos may also be a potential issue depending on how it has been prepared and stored as there is a reasonable prevalence of Toxoplasma - so just a thought on whether this could be related to cardiac disease.
Not sure how people are measuring taurine but plasma taurine changes very quickly with diet so may not reflect very accurately blood taurine status – snap frozen whole blood is better though a pain to collect and transport. Equally blood taurine levels may not reflect cardiac muscle taurine .....

(b)

11. Posted by (b) (6) on 01-16-18 04:56 ET

Paul has forwarded an excellent idea. My understanding is that kangaroo has low levels of taurine and methionine and lentils are low in sulfur containing amino acids methionine and cysteine. The metabolism of these amino acids are complexly intertwined.

The dogs I saw were on lentils and begged oh supplement possibly before more taurine was added to the supplement. Certainly, one would not expect kangaroo meat to provide sufficient taurine to eliminate the risk of deficiency. Sounds like a good opportunity for a nutrition-oriented cardiologist or group to sort this out.

Until that time, consider supplementation of taurine and also possibly methionine.

(b) (6)

12. Posted by (b) (6) on 01-16-18 07:50 ET

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(b) (4)

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Would love to chat with anyone interested.

(b) (6)

800.700.4636 | VINGRAM@vin.com | 530.756.4881 | Fax: 530.756.6035
777 West Covell Blvd, Davis, CA 95616

Sample Submission Form

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/aal/aal.html

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Vet/Tech Contact: Account # (b) (6) / Contact: (b) (6) Date: 9-5-17
Company Name: (b) (6)
Address: (b) (6)
(b) (6)
Email: (b) (6)
Tel: (b) (6) **Fax** (b) (6)

Billing Contact: (b) (6) **TAX ID:**
Email: (b) (6) **Tel:** (b) (6)

Patient Name: (b) (6)
Species:
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☑ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☑ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 10 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

(b) (6)

(b) (6)

Patient Information

Patient:	(b) (6)	Age: 6 years	Referring Veterinarian: (b) (6)
Patient Number	(b) (6)	Weight:(kg) 25.10	Cardiologist: (b) (6)
Breed:	lab mix	Sex: F	Client Number: (b) (6)
Exam Date:	(b) (6) 08:21	BSA: 0.87	

History: (b) (6) was presented for evaluation of cough, labored breathing, multiple episodes of collapse, cardiomegaly, and suspected congestive heart failure. (b) (6) was initially seen by her rDVM on December 30th for cough. She was initially treated with a humidifier, hydrocodone, and a decrease in length of walks and did not improve. On January 4th she had a collapse episode during which she circled, fell over, and flopped for about a minute. She has a second less severe episode on January 5th. Labwork at this date showed mild elevation of ALT and AST with mild increase in CK. T4 was low normal. She has continued to be short of breath and tires easily. She chased a squirrel a couple of days ago and stood with a wide based stance afterward. She is on year-round heartworm, flea and tick preventative and was last tested negative for heartworm 9/9/17. She eats California Natural Kangaroo and Red Lentil dry food with vegetables and 2 tbsp of canned pumpkin. She is on Apoquel 8mg twice daily and sublingual immunotherapy. The hydrocodone was discontinued yesterday.

Physical Examination: T 102.7 P 208 R 150. Grade 3/6 left apical systolic murmur and gallop. Regular tachycardia. Quiet heart sounds. Localized fine crackles left cranial hilar region, dry cough. Poor femoral pulses. Unremarkable abdominal palpation. mm pale pink, normal refill. Hydration OK. Normal PLNs.

Diagnostic Tests:

(b) (6)
BP - 152mmHg 4 cm cuff LF
Echo - see below. Sinus tachycardia on ECG.
Taurine level (whole blood): pending, will call with results.
ECG - HR 189bpm, sinus tachycardia. Wide and tall P waves (0.06s, 0.5mV) consistent with atrial enlargement, tall R waves (3.7mV) consistent with LVE, normal PR (0.12s) and QT (0.2s) intervals.

(b) (6)
Thoracic radiographs: Mild decrease in severity of cardiomegaly (as compared to rDVM films from 1/9/18). Resolving cardiogenic edema.

Hospitalization:
An IV catheter was placed and (b) (6) was hospitalized in ICU with continuous ECG monitoring. She was started on IV Lasix (50 mg IV q8h) and pimobendan (5 mg in AM and 10 mg in PM). She did well overnight with an improvement in respiratory rate/effort. (b) (6) had occasional short paroxysms of "slow" ventricular tachycardia (160-270 bpm) that were noted noted to persist beyond ~7 pm.

(b) (6) was started on enalapril (10 mg q12h) the following morning. She continued to do well with a normal appetite and improved respiratory rate.

Echocardiographic Report

2D ECHO

LA Systolic Diameter LX	6.5 cm

DOPPLER

AV Peak Velocity	70.8 cm/s	PV Peak Gradient	1.3 mmHg
AV Peak Gradient	2 mmHg	TR Peak Velocity	255 cm/s
MR Peak Velocity	396 cm/s	TR Peak Gradient	26.1 mmHg
PV Peak Velocity	56.2 cm/s		

M-MODE

LV Diastolic Diameter MM	7.4 cm	LVPW Diastolic Thickness MM	0.92 cm
LV Systolic Diameter MM	6.5 cm	LVPW Systolic Thickness MM	0.96 cm
LV Fractional Shortening MM	12 %	LVPW Percent Thickening MM	0.048
LV Diastolic Volume Cube	398 cm^3	IVS to PW Ratio MM	1
LV Systolic Volume Cube	271 cm^3	LV Mass MM	324 g
LV Ejection Fraction Cube	0.32	LV Mass Normalized MM	374 g/m^2
IVS Diastolic Thickness MM	0.96 cm	RV Diastolic Diameter MM	0.86 cm
IVS Systolic Thickness MM	0.89 cm	LA Systolic Diameter MM	4.8 cm
IVS Percent Thickening MM	0.069	Aortic Root Diameter MM	2.1 cm

Left Ventricle: Severe dilation (normalized LVIDd 2.85) with severe myocardial dysfunction (normalized LVIDs 2.34). Increased sphericity.

Left Atrium: Severe dilation.

Right Ventricle: Mild dilation with subjective decrease in contractility.

Right Atrium: Mild dilation.

Mitral Valve: Normal valve morphology. 4+ central mitral regurgitation.

Aortic Valve: Normal.

Tricuspid Valve: Mildly thickened valve leaflets. 1+ tricuspid regurgitation. Normal regurgitant velocities.

Pulmonic Valve: Mildly thickened valve leaflets. Mild pulmonic insufficiency.

Aorta: Normal

Pericardium: Normal

Diagnosis

Dilated cardiomyopathy - This is a disease characterized by weakening of the heart muscle and dilation of the heart chambers. As the disease progresses, it can lead to congestive heart failure (fluid in the lungs causing shortness of breath and cough). Abnormal heart rhythms are common and can result in sudden death. Most commonly this is an inherited disease, though it can occur secondary to a deficiency in an amino acid called taurine.

Left sided congestive heart failure

Recommendations

Give all medications as directed:

Furosemide (Lasix, Salix) 40 mg tablets- Give 1 1/2 tablets by mouth every 12 hours. DUE: This evening.
This is a diuretic (water pill), that prevents the body from retaining excessive sodium and water. It will cause your pet to drink and urinate more frequently. It is important that fresh water is always available.

Benazepril 10 mg tablets- Give 1 tablet by mouth every 12 hours. DUE: ~9:00 PM
This medication is a strong drug that dilates blood vessels, permitting the heart to pump blood more efficiently. It can lower blood pressure (hypotension) and cause changes in kidney function and electrolyte values. If your pet develops weakness or depression, decrease the drug dose by ½ and call. A kidney panel and blood pressure should be reevaluated 7-10 days after beginning this medication.

Spironolactone (Aldactone) 50 mg tablets- Give 1 tablet by mouth every 24 hours. START: Tonight or tomorrow morning.
This is a diuretic (water pill) that also blocks a hormone that can injure the heart muscle. It works well in combination with the furosemide and enalapril.

Pimobendan (Vetmedin) 10 mg tablets- Give 1/2 of a tablet by mouth in the morning and 1 tablet in the evening. Give at 12 hour intervals. DUE: ~6:00 PM (1 tablet)
This is a drug that is approved for the treatment of congestive heart failure secondary to dilated cardiomyopathy or chronic valve disease (endocardiosis). Studies have shown improved quality of life and increase survival time when this drug is added to other standard cardiac medications. In our experience, side effects are uncommon, but it is important that you advise us if you feel your pet is having any potential adverse effects from this medication. The reported potential side effects listed for this medication are increased heart rate, vomiting, diarrhea, inappetence, uneasiness, incoordination, convulsions, increased drinking and increase urinating.

One thing that can be very helpful for home monitoring is checking sleeping or resting respiratory rates. A recent study showed that even pets with severe heart disease rarely have resting respiratory rates greater than 30 breaths per minute unless they are starting to decompensate for that disease. Elevated respiratory rates at home may be even more sensitive than chest radiographs at picking up early decompensation. Count your pet's respiratory rate when he/she is at rest or sleeping (not within 20 minutes of being active). If his/her respiratory rate is greater than 30 breaths per minute, recheck again in a couple of hours. If persistently elevated above this level, call.

With advanced heart disease, our biggest dietary concerns are adequate calorie content and low sodium content. We aim for less than 80mg sodium per 100 kilocalories (kcal) in patients that have developed congestive heart failure. We do not advise protein restriction unless there is concurrent kidney disease (i.e. kidney diets are not advised unless there is concurrent kidney disease). Please refer to our diet handouts with a list of currently adequate diets and treats, though this list is not exclusive. If you wish to feed a diet that is not on these lists, you will need to call the manufacturer of the diet to obtain a sodium content.

As we discussed, we have had three other cases of severe DCM where the dogs have been eating a kangaroo and lentil diet. There is no data that has shown an association with this diet and DCM but we are concerned there may be a connection there and are looking into it at this time. For this reason, we would consider changing (b) (6) diet.

We sent (b) (6) home with a few cans of Hill's Science Diet Canine Maintenance canned food. This food has an appropriate level of sodium for dogs in congestive heart failure and is available at most pet stores. Lamb should be avoided as a protein source but any other protein is appropriate (with the exception of kangaroo).

The very best diet for dogs with DCM/heart failure is probably Hill's Science Diet Prescription j/d. This food has a good source of taurine, carnitine and fatty acids. However, this diet is rather costly.

We have submitted a taurine level and will call you with the results when they are available.

Exercise is also a concern in advanced heart disease. While cage rest is ideal with active heart failure, some exercise is permissible in asymptomatic disease. However, vigorous or extended exercise should be avoided.

***As long as (b) (6) does well at home we would like to re-evaluate her in 7-10 days. At this time we will recheck her kidney values/electrolytes and blood pressure as well as repeat chest x-rays.

(b) (6)

(Electronically Signed)

Final Date:

Like us on Facebook!

(b) (6)

Notes to our clients

-Please bring all medications to your pet's scheduled appointments.

-We require a 48 hour notice for all refills. When you call to request a refill, please leave the pharmacy phone number or clearly indicate if you plan on picking up the medication at our facility. PRESCRIPTION REFILLS ARE NOT AVAILABLE AFTER (b) (6) (b) (6) REGULAR BUSINESS HOURS (Evenings, Fridays, holidays and weekends).

-Check out WWW.GOODRX.COM and enter your local zip code to search for the best prices on your medications at your local pharmacies.

-If an emergency arises with your pet, (b) (6) is a 24 hour facility.

From:	Conway, Charlotte
To:	Edwards, David
Subject:	FW: DCM investigation - Pet Food Institute
Date:	Monday, June 24, 2019 12:38:31 PM

FYI

From: Ask CVM <AskCVM@fda.hhs.gov>
Date: June 24, 2019 at 10:20:06 AM CDT
To: Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>, Norris, Anne <Anne.Norris@fda.hhs.gov>, Steinberg, Nadine <Nadine.Steinberg@fda.hhs.gov>, DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>, Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>, Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>
Subject: FW: DCM investigation - Pet Food Institute

From: Susan Thixton <susan@truthaboutpetfood.com>
Sent: Monday, June 24, 2019 9:37 AM
To: Ask CVM <AskCVM@fda.hhs.gov>
Subject: DCM investigation - Pet Food Institute

We were alerted that the FDA has provided the Pet Food Institute details of a soon to be released update of the Agency's investigation of pet food related DCM. It is more than concerning that FDA shared details of their investigation with industry well in advance of sharing those details with pet owners.

Pet owners – not industry – are the ones suffering the most from this nutritional failure of pet food. Pet owners are reporting to us that veterinarians are telling clients "*they are literally seeing thousands of new cases* (of DCM) *everyday*" and veterinarians are pushing pet owners to provide their pets ONLY a grain-based pet food made by Purina, Mars, or Hill's. Needless to say, the focus of this situation is completely out of control.

The focus should be on the actual problem – a nutritional failure of 'Complete and Balanced' pet foods. We ask the FDA to keep their focus on the Complete and Balanced nutritional failure and to update pet owners in the same timely manner as they do industry.

Susan Thixton

--
Susan Thixton
Pet Food Consumer Advocate
TruthaboutPetFood.com
AssociationforTruthinPetFood.com

Compendium

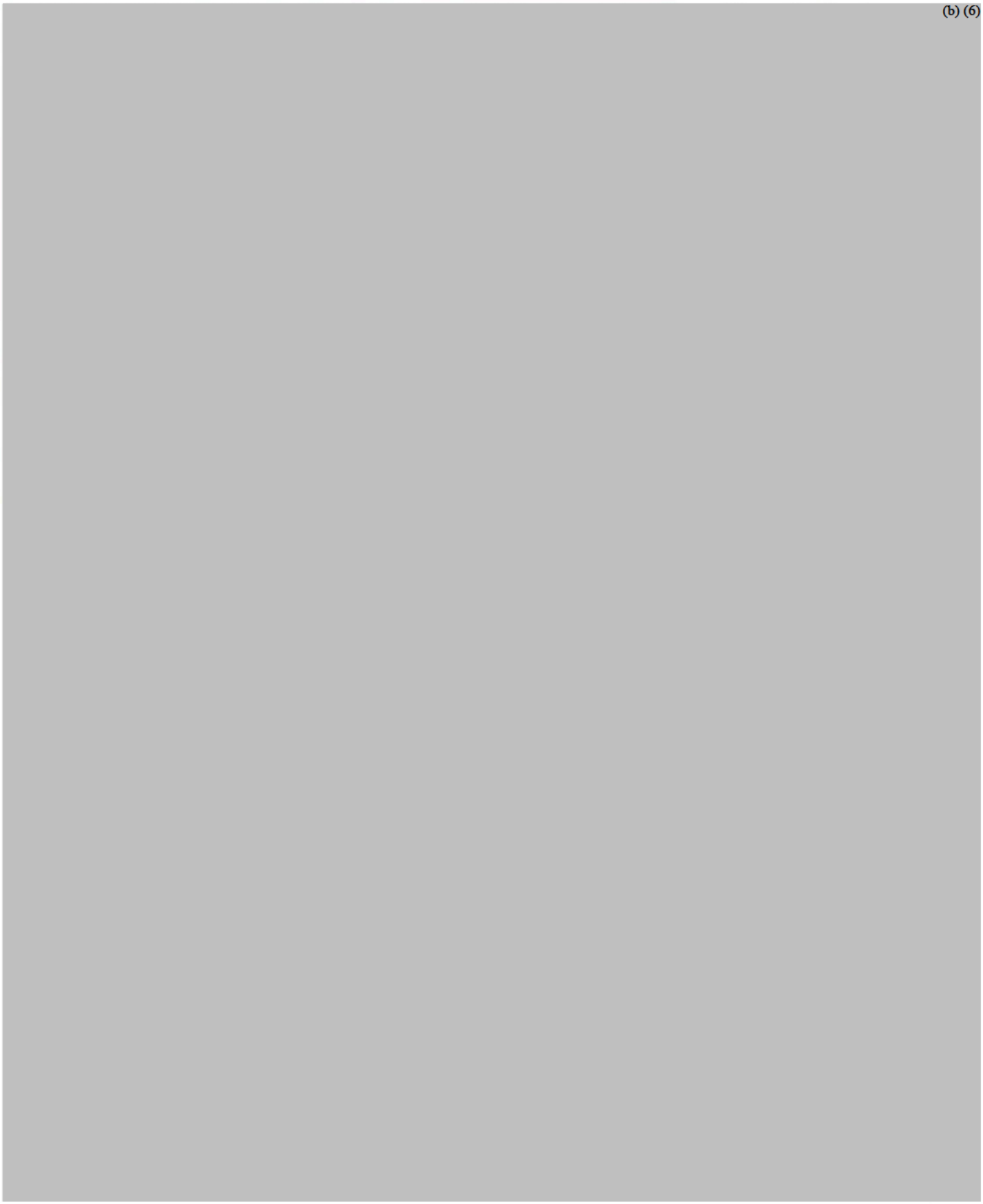
Cardiovascular Effects of Thyroid Disease

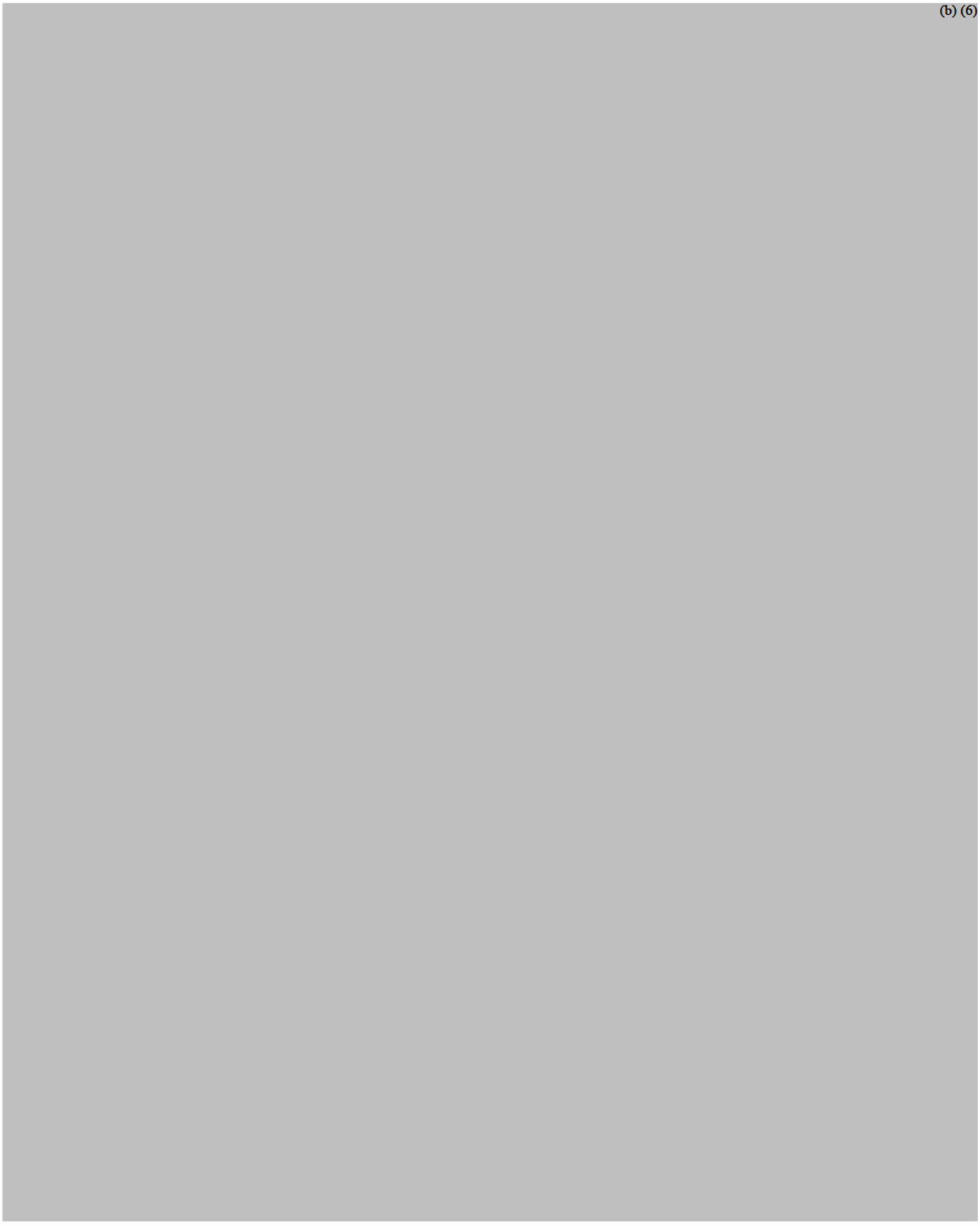
Jodi K. Sangster, DVM
David L. Panciera, DVM, MS, DACVIM (Small Animal Internal Medicine)
Jonathan A. Abbott, DVM, DACVIM (Cardiology)
Virginia Tech
Blacksburg, Virginia

Abstract: *Thyroid hormones have many effects on cardiovascular function, and deficiency or excess of thyroid hormones can result in cardiac dysfunction. Abnormalities of the cardiovascular system are often identified during examination of hyperthyroid and hypothyroid patients. This article addresses the effects of thyroid hormones on the cardiovascular system and the clinical relevance of the cardiovascular response to thyroid dysfunction. In addition, treatment recommendations are presented.*

(b) (4)

(b) (4)





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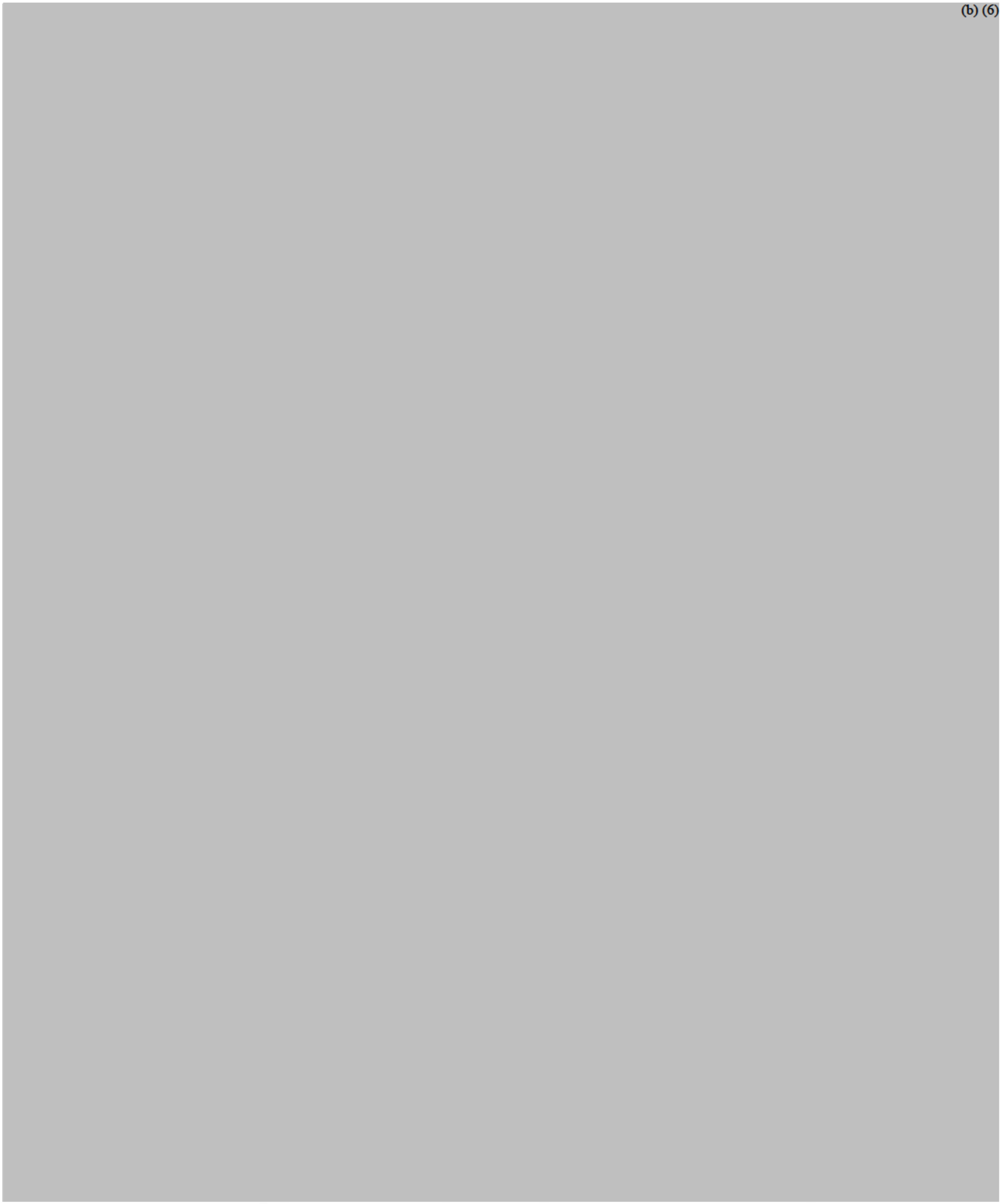

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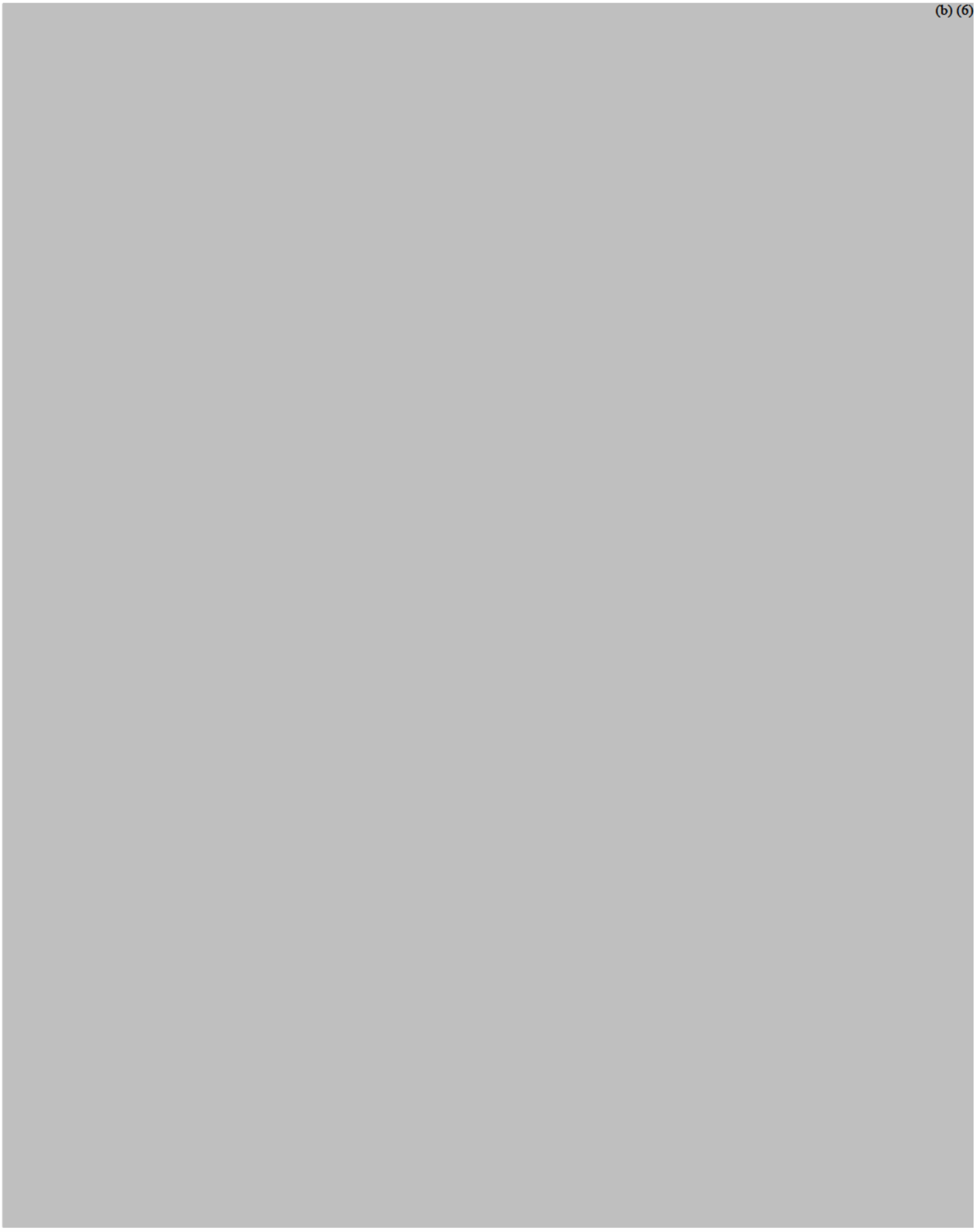

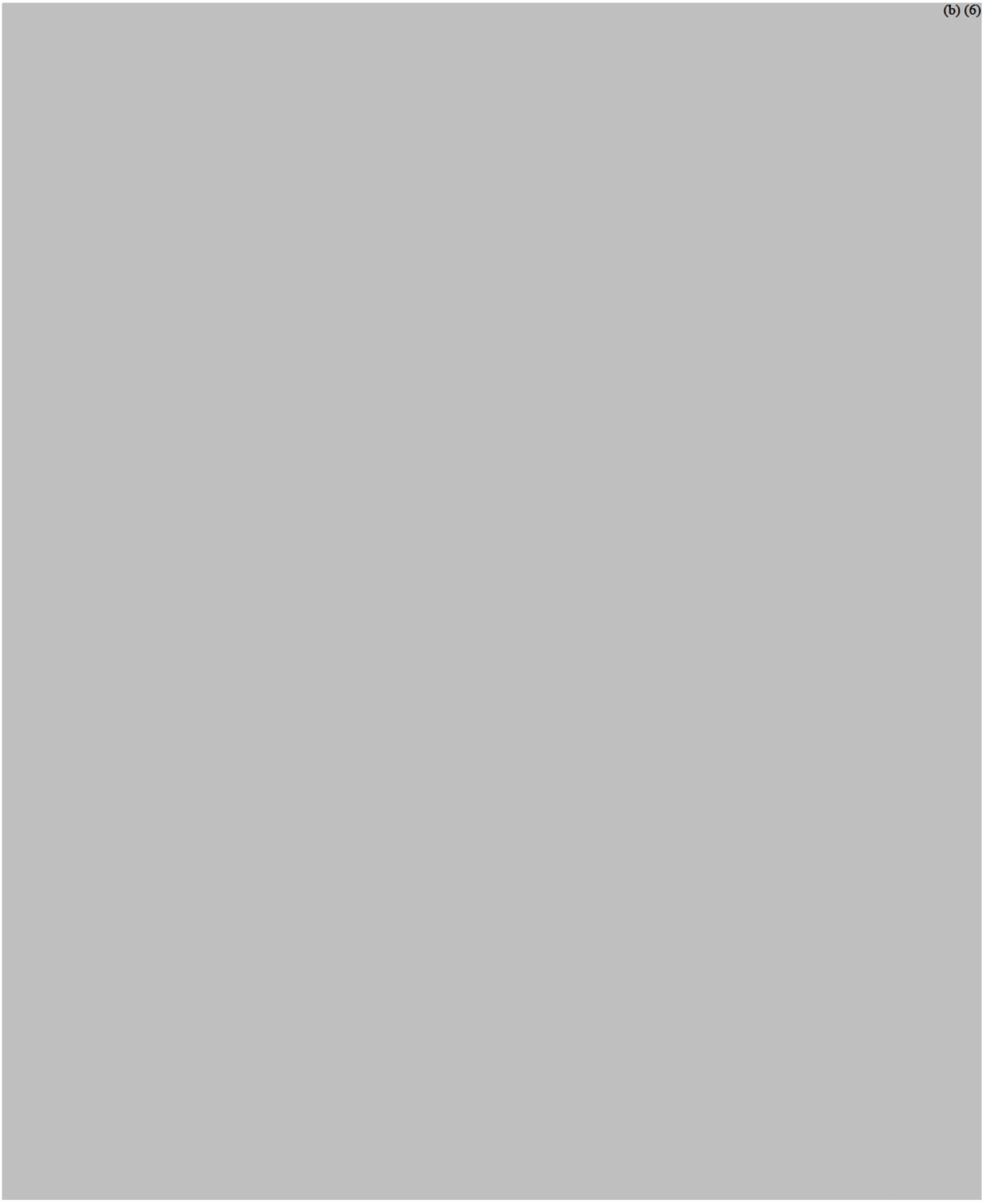

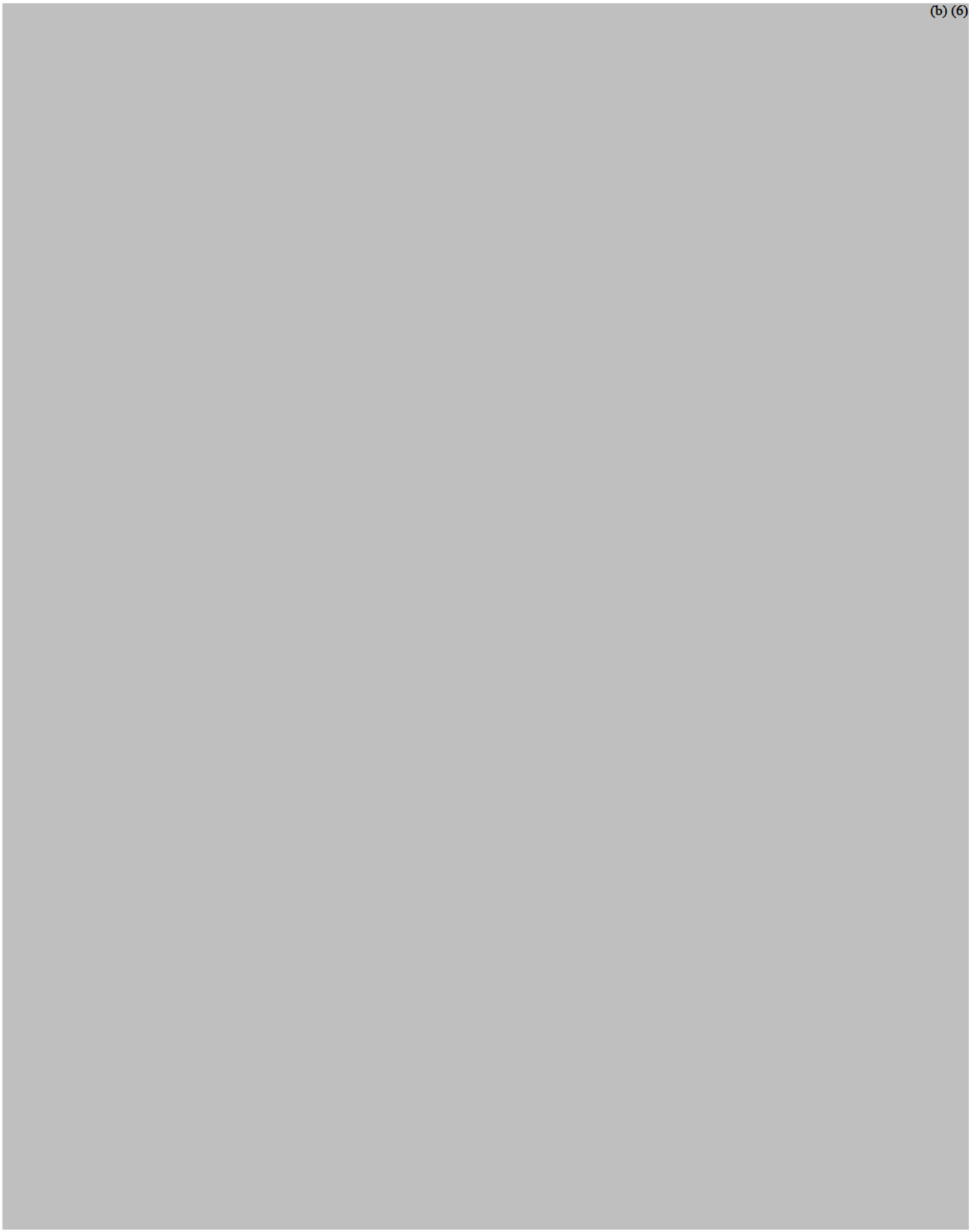

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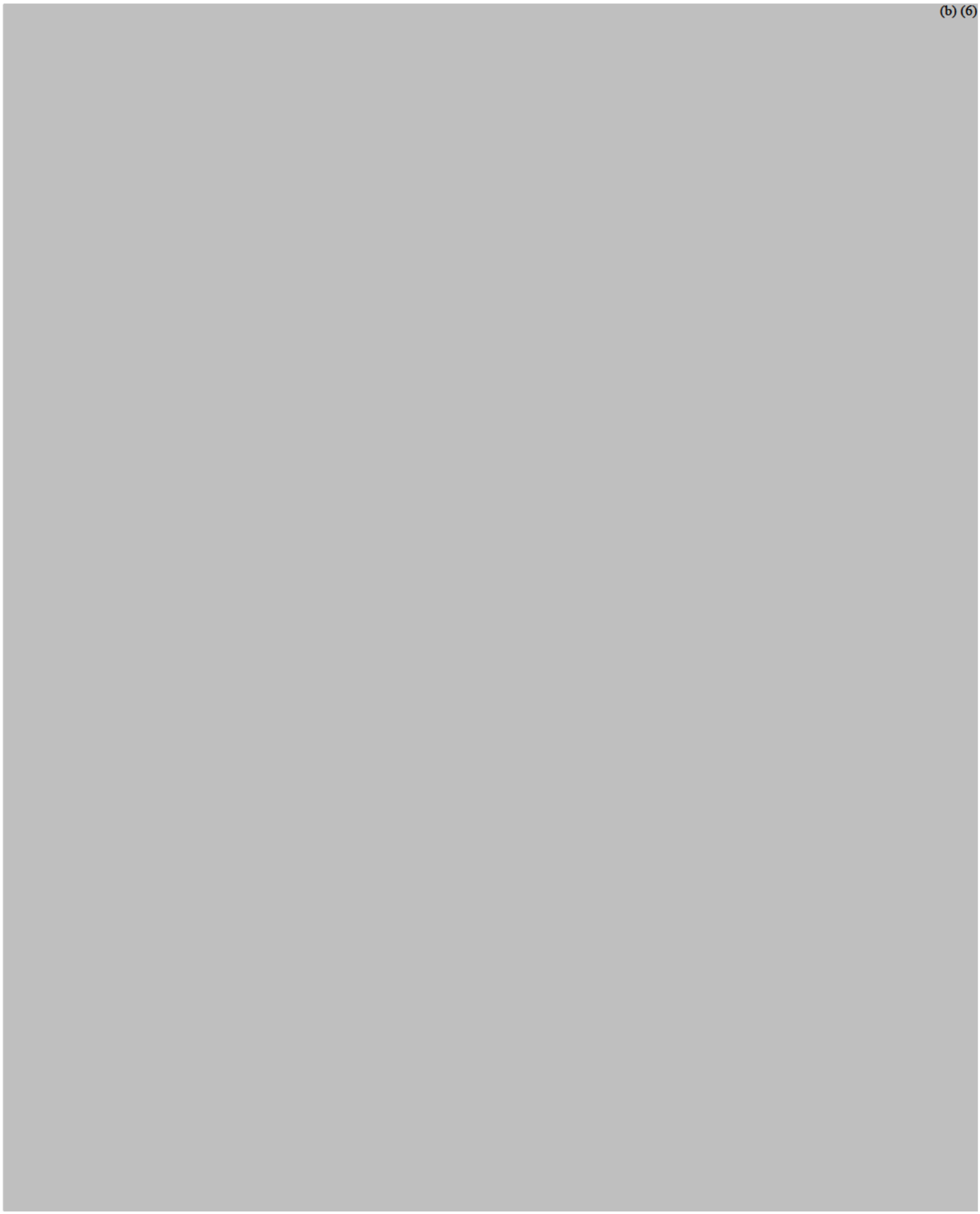

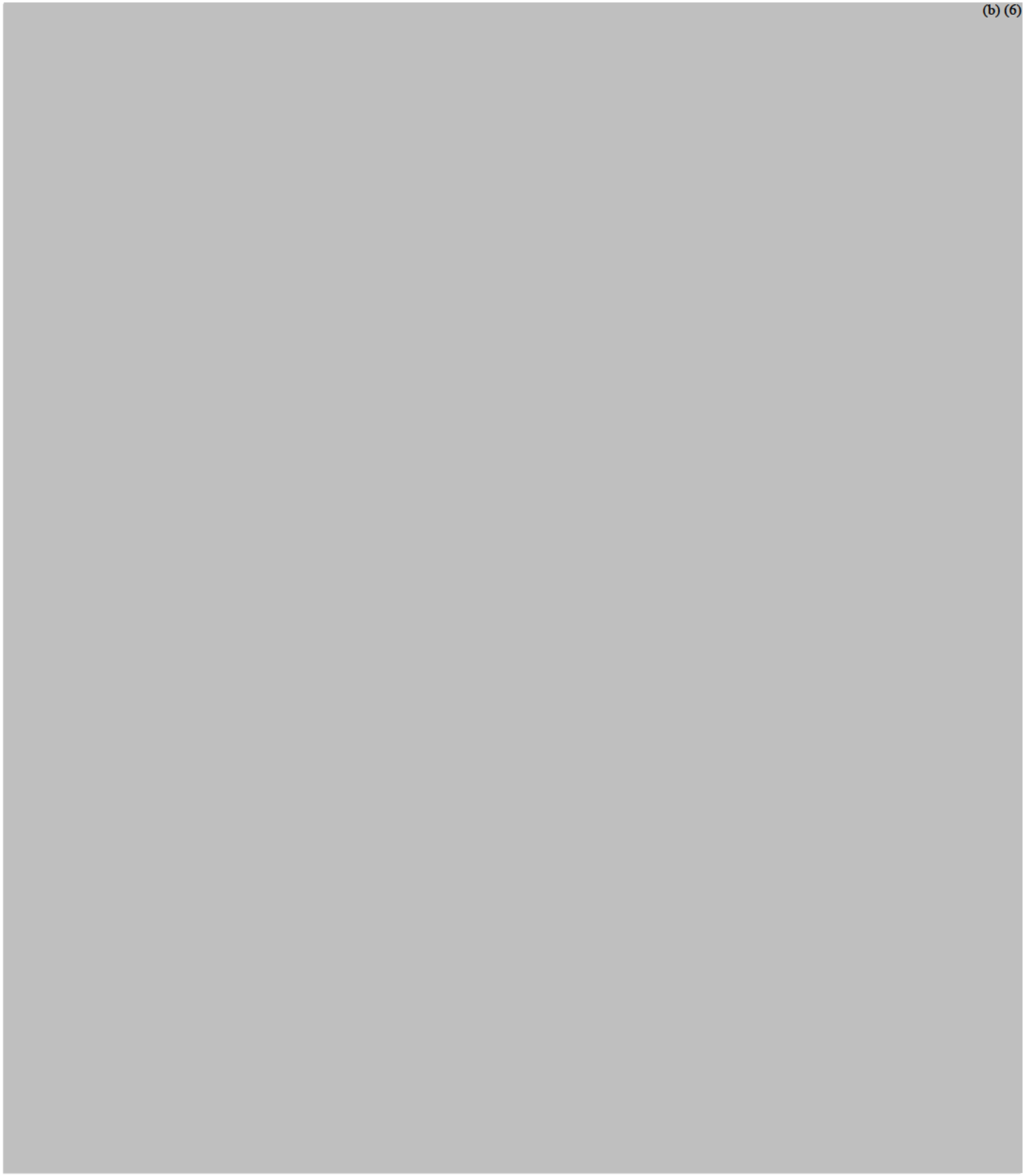

Vet-LIRN Case Summary Document

Vet-LIRN Case Number:	800.218
EON/CC #:	EON-323515-323519
Vet-LIRN Initiation Date:	7/13/2017
MedRec: Requested:	7/12/2017
MedRec: Received:	7/13/2017
MedRec: Significant finding:	Cardiogenic heart failure clinically but less supported histologically
Vet-LIRN Tests (planned):	(b) (4), (b) (5)
Vet-LIRN Test Results:	TAMU: • Fumonisin-Negative (b) (4) • Taurine, Carnitine-*pending*
Result Interpretation:	
IF NFA, justification:	

COMPLAINT Narrative:

(b) (6) housemate, (b) (6) (separate report submitted) was diagnosed with DCM and CHF 2/17 and was euthanized after aggressive treatment of CHF. At that time (b) (6) had 2 syncopal events closely related to each other. His appetite for dog food declined but he would eat it if tempted with treats mixed in. He was presented (b) (6) for more syncopal events and was similarly diagnosed with severe DCM and CHF. He was able to be successfully treated however and is clinically doing well on CHF medications as of 7/10/17. A re-review of the myocardial histopathology for (b) (6) housemate ((b) (6)) was requested at this time because of the unusual diagnosis of DCM in a small breed dog living in the same house as another dog similarly diagnosed a few months ago. This re-review by one of our pathologists showed myofiber vacuoles reminiscent of the changes seen in doxorubicin toxicity. Since the dog had not received doxorubicin, the pathologist recommended culturing the food for Streptomyces peucetius - the bacterium which produces doxorubicin. He also recommended testing for Fusarium spp. a fungus which produces Fumonisin B1, a toxin that produces heart failure in pigs. Like (b) (6) (unrelated, younger miniature schnauzer), (b) (6) had been fed Caifornia Naturals Adult - both kangaroo with lentils and venison with lentils along with Milo's kitchen treats. We have samples of these foods from 6/17 but not the original bags from when he was presented 2/17. These samples were provided at the time (b) (6) also presented with severe DCM and CHF. Like (b) (6), (b) (6) had extensive infectious disease testing which was negative and nutritional amino acid deficiencies were ruled out. Because of this, their unrelated lineages (although the same breed, they were from different lines), different ages but similar time of presentation, we are considering common environmental factors which could precipitate DCM, including food contamination or toxin exposure. We have plasma, serum, urine and myocardial tissue samples (latter only for (b) (6)) stored at -80 Celsius in addition to food and treat samples.

3 week history of cough treated unsuccessfully with doxycycline and prednisone. 3 day history of inappetence and vomiting prior to presentation to (b) (6) emergency service for dyspnea. Radiographs showed severe pulmonary edema and echocardiogram showed severe Dilated Cardiomyopathy. There was an initial response to diuretic therapy however, he declined and was placed on the ventilator for respiratory support and continued CHF treatment. Attempts to wean off the ventilator were unsuccessful and aquaphoresis was performed. He continued to decline despite aggressive therapy and was euthanized. Infectious disease testing was negative and taurine and carnitine analysis showed adequate levels. Necropsy initially did not reveal a cause for DCM and supported alveolar injury (possibly ventilator related). A rereview of the myocardial histopathology by one of our pathologist showed myofiber vacuoles reminiscent of the changes seen in doxorubicin toxicity. Since the dog had not received doxorubicin, the pathologist recommended culturing the food for Streptomyces peucetius - the bacterium which produces doxorubicin. He also recommended testing for Fusarium spp. a fungus which produces Fumonisin B1, a toxin that produces heart failure in pigs. (b) (6) had been fed Caifornia Naturals Adult - both kangaroo with lentils and venison with lentils along with Milo's kitchen treats. We have samples of these foods from 6/17 but not the original bags from when he was presented 2/17. These samples were provided at the time his housemate, (b) (6) (unrelated, older miniature schnauzer) also presented with severe DCM and CHF. I will enter this dog as a separate affected patient. Both dogs had extensive infectious disease testing which was negative and nutritional amino acid deficiencies were ruled out. Because of this, their unrelated lineages (although the same breed, they were from different lines), different ages but similar time of presentation ((b) (6) had clinical signs at the time (b) (6) was treated, but didn't present with CHF for several months), we are considering common environmental factors which could precipitate DCM, including food contamination or toxin exposure.

Signalment:

- (b) (6): 7 yr MC Miniature Schnauzer
- (b) (6): 2 yr MC Miniature Schnauzer-deceased

Signs: syncopal episodes, dyspnea, cough, heart failure

Food: Alternated feedings between:

- California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils Recipe Dog Food
- “” Kangaroo and Lentils

Vet-LIRN PLAN OF ACTION:

-
- (b) (5)

Medical Record Review:

(b) (6):

Presenting complaint (b) (6) **:** dyspnea, cough of 3 week duration-wheezing type more frequent at night → rDVM, treated w/ prednisone and doxycycline for kennel cough → (b) (6) inappetance, vomiting → (b) (6) dyspneic and recheck, hospitalized and treated for pneumonia, regurgitated → (b) (6) treated as outpatient, (b) (6) as syring feeding, dog regurgitated and had marked dyspnea → ER → refer to NCSU → (b) (6) put on mechanical ventilator → (b) (6) euthanized

PE (b) (6) **:** P 160 bpm, R 64 rpm, pale pink mm, Gr I-II/VI left apical systolic murmur, femoral pulse hypokinetic but synchronous, jugular venous distention

Labwork: (b) (6) **Labs:** unremarkable (unclear what was done)

(b) (6) **Big 4:** Glu 135, Azo 15-20

- (b) (6) vBGA: Lact 2.4, rest wnl
- Chem: P 6.2, K 4.9, Na 140, TP 4.2
- Chem: BG 225, BUN 29, P 11.7, K 3.3, Cl 95, Na 144
- Chem: BG 136, P 4.6, CK 13,621, K 4.3, Na 151, Cl 109, AST 577
- Chem: BG 165, BUN 37, P 8.1, ALT 147, AST 1006, CK 35,930, Na 135, K 3.8, Cl 90

(b) (6) **CBC:** WBC 9.4, NP 7.9, Band .18, Plt 157

- (b) (6): WBC 9.9, NP 8, Band .7
- : WBC 6.8, TP 6.9, NP 4.2, Ban .54, Toxic NP-mild, Plt 97

(b) (6) **BP sys:** 90

(b) (6) **UA post Lasix:** 1.011

Cardiac troponin 0.79

BAP GM-pending

Vector borne panel: pending

Taurine/Carnitine: pending

(b) (6) **Coag:** PT 9.1, PTT 14, Dimer 189, Fib 539, INR 1.09

Urine Creat: 27.9

Urine Na: pending

ECG: suspected atrial tachycardia

Rads (b) (6): concern for aspiration pneumonia

- (b) (6): cardiomegaly, severe diffuse mixed interstitial to alveolar pattern most severe caudo-dorsally, hepatomegaly, dec abdominal serosal contrast
- (b) (6): severe generalized cardiomegaly with biventricular heart failure, improved vs rDVM rads
- (b) (6): worsening cardiogenic pulmonary edema, cannot exclude lung induced injury +/- pneumonia
- (b) (6): post ultrafiltration, improved cardiogenic edema, hypovolemia, residual interstitial to patchy alveolar
- (b) (6): improved CHF with possible concern for bronchopneumonia, suspected hiatal hernia
- (b) (6): markedly progressive alveolar pattern with significantly worse cardiogenic edema

tFAST (b) (6)**:** severe cardiomegaly with ventricular hypocontractility

Echo (b) (6)**:** dcm vs. myocarditis vs pacing induce vs. other (severely dilated & hypocontractile left & right ventricles, severely dilated left and right atria)

Necropsy: Lung-severe diffuse alveolar injury with marked fibrin deposition (hyaline) and marked alveolar histiocytosis and multifocal type II pneumocyte hyperplasia; mod to marked diffuse pulmonary edema; mild cardiomegaly with mild mitral valve endocardiosis and mild left ventricular hypertrophy and left atrial dilation; thorax with mild pleural effusion; Suspect primary non-cardiogenic etiology but if clinical cardiac dysfunction then functional cardiac abnormalities cannot be ruled out

Prior MHx: coffee brown urine including clumping after strenuous activity when it is hot outside and resolves with 24-36 hours; also Crystalluria

(b) (6)

Presented (b) (6) **:** episodes of collapse, first occurred mid February, fall 6 seconds without losing consciousness → immediately return to normal →2 weeks later again collapse, then on → 6/3 post 2 hour hike collapsed again; panting more than usual; good appetite for treats but reluctant to eat food since February; → recheck (b) (6), doing better, no collapsing episodes except a stumbling moment when excited, respiratory rate normal, diet changed to Hill's

(b) (6) **PE**: P 130 bpm, R pant, mild increased breath sounds in all lung fields

-(b) (6): T 99.7F, P 136 bpm, R 36 rpm, equivocal mild dehydration<5%, Gr II/VI left apical systolic murmur

Labs: (b) (6) **Big 4:** BG 64 (recheck 79), BUN 15-26
BP-sys: 130 mmHg
-7/10: 110 mmHg
ECG: left ventricular enlargement suggested
UA: 1.019
Taurine & Carnitine: normal (no values)
Vector borne panel (PCR and IFA): normal
BAP GM
Troponin 1
T4
Toxoplasma/Neospora
Chagas
Complete AA: no significant abnormalities, consulting with UC Davis

-7/10 **Renal Panel:** K 4.0

(b) (6) **Rads**: left sided congestive heart failure

-7/10: moderate left sided cardiomegaly without heart failure, moderate hepatomegaly

(b) (6) **Echo:** mitral valve endocardiosis with left atrial enlargement and heart failure, decreased left ventricular systolic function, suspected DCM

Thoughts: possible fumonisin induced cardiomyopathy, vs. other non-food related toxic compound (lily of valley, digitalis, ionophores, sicklepod, gossypol, white snake root), ethyl alcohol, foxglove, buttercups

7/18/2017
JJ-DR agrees fumonisin worth testing and suggests asking about fish/sardines/anchovies being fed to dogs in February (re: domoic acid toxicity which can appear doxorubicin-like).

Will ask vet re: fish/sardines/anchovies and also how much bag of food weighs. Emailed TAMU requesting fumonisin testing.

7/19/2017
JJ-TAMU can do the testing at (b) (6). Vet sent product weight and dimensions. Vet confirms, neither dog received anchovies, sardines, or seafood in February or Chronically.

7/22/2017
JJ-Vet did not receive a return shipping label. Will make PO for shipping to Vet-LIRN.

7/28/2017
JJ-Vet sent info-she's had 2 other cases of DCM with dogs eating the California Naturals diet. Info below:

> *I don't know if there is anything to this but I have treated 2 other dogs in the last 2 weeks with DCM and CHF that are being fed California natural food (one kangaroo and lentil, and we are trying to find out about the other one which is in our ER now). One is mixed breed and we'll recommend testing for taurine deficiency. The other was a golden and taurine was a bit low but not super low so although we supplemented, I wasn't totally convinced it was the cause. Unfortunately she died a week later.*

I don't have a sense for how widely fed this diet is but I don't see it on the top selling lists I can find by google, so seeing 4 DCM dogs recently eating this particular food is interesting to say the least. I thought this might be of interest to you as you start to look at the California natural food sample from the Ramsammy dogs.

Vet also sent journal article about Acrolein chronic oral ingestion causing idiopathic DCM in rats.

Found (b) (6) can test for Acrolein in pet food.

Sent info to group, and DR thinks testing for taurine, carnitine, and acrolein (if not too expensive) worth it. I can send an email to (b) (6) to request testing cost/method info/shipping info/amount needed for testing.

8/3/2017
JJ-Prepared pet food on 8/2 and lab submission forms. Make boxes today and send for testing.

JG – Shipped samples to (b) (4)

8/4/2017
JJ-(b) (6) responded. Acrolein testing would require method development and be quite costly. Will not pursue at this time.

8/7/2017
JJ- (b) (4) sent final results. Fumonisin negative. Will send all product test results to vet when received.

8/9/2017
JJ-DR suggested-maybe vet at (b) (6) has funds to do acrolein.

8/22/2017
JJ-Received (b) (4) results for Taurine and Carnitine. Filed. Vet asked about results- Send Fumonisin results and taurine/carnitine in progress until we interpret. Plan to send those after interpretation finalized. Asked vet which dry food we were sent.

Flavor	Moisture Max (label)	Ingredient lists:	Label claims	Nutrient Analysis (web)
Kangaroo	10%			
(b) (5)				

1. Taurine 231 mg/100g food → 0.23% As Is basis → assume max 10% moisture (label) for either food:
   Kangaroo → 0.26% (on DMB calculated using label moisture max)
   (b) (5)

Interpretation: No AAFCO minimum for Taurine in dogs. (b) (5) . If the food is either flavor, it is compliant for minimums of cat taurine levels.

2. L-Carnitine 69,900 ppb = 69 ppm = 0.0069% As Is basis → assume max 10% moisture (label):
   Kangaroo → 0.0077% (on DMB calculated using label moisture max)
   (b) (5)

Interpretation: No carnitine minimum for dogs or cats. Unclear whether or not this is low, normal, or high.

Final Conclusion: The cause of the two dogs' DCM is unclear. The bloodwork for these dogs showed normal taurine and carnitine levels. Based on the dogs' blood taurine/carnitine levels and the dry dog food test results, it is unlikely that Fumonisin, taurine, or carnitine caused the dogs' illness.

Vet responded-flavor is Kangaroo. Let her know acrolein not available as a test unless she wants to pursue method development with (b) (6) .

DR agrees-sent final interpretation to vet. NFA.

10/16/2017
JJ-From: Garland T. Overview of Gossypol Poisoning. Merck Veterinary Manual. Found at:
http://www.merckvetmanual.com/toxicology/gossypol-poisoning/overview-of-gossypol-poisoning

"Differential diagnoses (for gossypol) include poisonings by cardiotoxic ionophoric antibiotics (eg, monensin, lasalocid, salinomycin, narasin) and ammonia, nutritional or metabolic disorders (eg, selenium, vitamin E, or copper deficiency), infectious diseases, noninfectious diseases (eg, pulmonary adenomatosis, emphysema), mycotoxicoses caused by Fusarium-contaminated grain, and toxicoses caused by plants with cardiotoxic and other effects. Cardiotoxic plants (see Poisonous Plants), which may cause confusing or similar clinical signs and postmortem lesions, include English yew (Taxus baccata), Japanese yew (T cuspidata), laurel (Kalmia spp), azalea (Rhododendron spp), oleander (Nerium oleander), yellow oleander or yellow-be-still tree (Thevetia peruviana), purple foxglove (Digitalis purpurea), lily-of-the-valley (Convallaria majalis), dogbane (Apocynum spp), coffee senna (Senna occidentalis), bracken fern (Pteridium aquilinum), white snakeroot (Eupatorium rugosum), death camas (Zygadenus spp), lantana (Lantana camara), monkshood (Aconitum napellum), and milkweed (Asclepias spp)."

14768

Sample Submission Form

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/labs/aal/index.cfm

Vet/Tech Contact: (b) (6)
Company Name:
Address: (b) (6)

Email: (b) (6)
Tel: **Fax:** (b) (6)

Billing Contact: Finance Dept. **TAX ID:** (b) (6)
Email: (b) (6) **Tel:** ext

Patient Name: (b) (6)
Species: Feline
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☒ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☐ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 196 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
6/6/2016	C	(b) (6)	RECEPTION ACTIONS NOTE Sympathy card sent-AG
6/6/2016	C	(b) (6)	MEDICAL COMMENTS 6/6/2016 11:15 FDA complaint submitted: Pet Food Safety Report, ID 53897, was successfully submitted on 6/6/2016 11:15:17 AM EST to the FDA, and it was issued an Individual Case Safety Report Number (ICSR) of 1053335.
(b) (6)	C	(b) (6)	MEDICAL COMMENTS (b) (6) 16:26 (b) (6) ReplyReply AllForwardActions To: (b) (6) Sent Items (b) (6) Hi (b) (6) , Sorry for the delay in getting back with you, I needed to get permission from the owner's before providing you with their contact information. Below is the their information as well as the names of the individual cats. The cat, (b) (6) , with dilated cardiomyopathy was euthanized yesterday. Owners: (b) (6) and (b) (6) Cats: -(b) (6) : (b) (6) Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016, euthanized on (b) (6) 5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016 -(b) (6) : 9yr male neutered domestic long hair: 196 nmol/ml -(b) (6) : 8y female spayed domestic short hair: 368 nmol/ml -(b) (6) : 9yr male neutered domestic long hair: 124 nmol/ml -(b) (6) : 9yr male neutered domestic long hair: 536 nmol/ml Please let me know if you have any other questions.

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Sincerely,

(b) (6)

(b) (6) @merrickpetcare.com]Reply All
(b) (6) 12:56 PM
Thank You for providing me this information (b) (6). Could you provide us the pet parents information as well. We would like to reach out to the pet parent as well and speak with her. Thanks.

(b) (6) C (b) (6) COMMUNICATIONS WITH CLIENT
(b) (6) 15:55
SWO - expressed my condolences. asked for permission to provide contact info to company and the FDA - owner consented. Discussed what to expect when talking to company. Owner thankful for call.

(b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

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Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

TO: (b) (6)
FAX #: (b) (6)
FROM: (b) (6)
DATE: (b) (6)

RE:
Client: (b) (6)
Patient: (b) (6) / ID: (b)(6)
Breed: Shorthair, Domestic Sex: Spayed Female
Age: 12 Yrs. 5 Mos.

~EUTHANASIA NOTIFICATION~

Dear (b) (6):

This letter is to inform you that your patient, (b) (6) was visited by (b) (6) *At Home* house call service today for end-of-life care.

If you have any questions, please feel free to contact me at the location noted above.

Thank you,

(b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	C	(b) (6)	MEDICAL COMMENTS - Closed Jun 04/2016 (b) (6) 18:03 Seen on emergency today, embolic event secondary to DCM. Discussed necropsy and advised that nutritionist and cardiologist agreed that prior test results were sufficient; necropsy would not reveal anything not already documented. Owner had already admin 0.2ml buprenex sublingually, requested I admin the remaining 0.3ml dispensed today which I did. They then spent time privately with the patient prior to euthanasia. Flushed cephalic catheter in right front leg; patent. Admin 20mg (2ml) expired propofol IV, apneic and unresponsive Admin 975mg (2.5ml) beuthanasia IV, 3 exhalation spasms followed Confirmed deceased by prolonged thoracic auscultation Removed IVC, placed (b) (6) in coffin, nested in owner's blanket
(b) (6)	D	(b) (6)	Pleural Effusion Final
(b) (6)	D		Feline Arterial Thromboembolic Disease Final
(b) (6)	R	(b) (6)	Referral Letter - Cardio Resident Eval and labs - FINAL (b) (6) - REF fxd

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6)

TO: (b) (6)
FAX #: (b) (6)
FROM: (b) (6)
DATE: (b) (6)
RE:
Client: (b) (6)
Patient: (b) (6)
Breed: Shorthair, Domestic
Age: 12 Yrs. 5 Mos. **Sex:** Spayed Female
Current Weight: 5.3 kilograms as of 5/25/2016

Thank you for referring (b) (6). The following is a case summary.

Date of evaluation: (b) (6)

Date of previous cardiac evaluation: Wednesday, May 25, 2016

CHIEF COMPLAINT: heavy breathing, dragging RH limb

HISTORY: (b) (6) previously doing well. Appetite had improved, eating 2/3 can max cal per day. Normal breathing. Owner noted acute onset of dragging RH limb this morning and heavy breathing. No interest in food this morning. Brought in to ER immediately. Received 9mg lasix IV total and 0.075mg buprenohpine IV on presentation.
Previous hx: Diagnosed with DCM (b) (6) 6. Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.
Taurine level confirmed taurine deficiency

PHYSICAL EXAM: The patient was quiet, alert and responsive. No murmur on auscultation. The patient was tachypnic with moderate increased effort, RR 48. Normal BV sounds, no crackles on auscultation. Unable to bear weight on RH limb, dragging. Femoral pulses were fair in left hind, absent in left hind limb. Right paw pads cold to the touch. Heart rate was 160 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals (b) (6) 16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Cursory Ultrasound: small volume pleural effusion. No pericardial effusion. Large thrombus in LV.
Brief Echo 5/25/16: moderate volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle unchanged from previous.
Brief Echo 5/15/16: small volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle.
ECHOCARDIOGRAM (b) (6)/2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm
Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), taurine deficiency, moderate left atrial enlargement, pleural effusion, hx azotemia, LV thrombus, FATE (partial)

THORACOCENTESIS 4/10/16: a total of 180ml of yellow fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed scant effusion remained in the patient.
THORACOCENTESIS (b)**/16**: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.
ER thoracocentesis 5/8/16: 25ml yellow tinged fluid from the right side.

SUMMARY: (b) (6) clinical signs are due to a partial aortic thromboembolus and recurrent left sided congestive heart failure. Thrombus previously noted in LV is still present. There is a very guarded prognosis of recovery with ATE. Discussed with owners, if they wants to go forward with treatment recommend hospitalization to manage CHF and pain. Discussed risk of reperfusion injury, main concern hyperkalemia which can be fatal. At home care will consist primarily of pain medication and nursing care. She may or may not gain function back to hind limb, and this could take up to 1-2 months to see improvement if any. Owner elected to take home to spend a few hours to spend time and have (b) (6) come for euthanasia this evening. IVC left in place (right cephalic). Sent home with additional dose of buprenorphine to keep her comfortable until euthanasia. Owners understand she may pass on her own. Gave owners my condolences.

MEDICATIONS:
Buprenorphine 0.3mg/ml- give entire contents of syringe (0.5ml) at 3pm today for pain control

Thank you for the courtesy of this interesting referral. Please feel free to contact me

with any questions or comments.

Sincerely,

(b) (6)

(b) (6)
Sent electronically - no signature required

(b) (6)

Client ID: (b) (6) Patient ID: (b) (6) Patient Name: (b) (6)

Pain Condition Scores

Have you noticed the “PCS” aka Pain Condition Score in our medical records? As part of our AAHA specialty accreditation, we evaluate the level of pain or discomfort for each and every patient we see. You will see this reflected in the physical exam recordings as a "PCS”. We follow the guidelines created and published by Colorado State University. If you have questions about interpreting pain or treatment options, or you need to schedule a pain management consult, please speak with our Anesthesiology & Pain Management Department by calling (b) (6) .

Clinical Studies

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

We are recruiting for a variety of clinical studies across multiple specialties and are looking for eligible patients. For a complete list, please visit our website at and click on Veterinary Professionals and then Current Clinical Studies.

Avian & Exotics

(b) (6) is happy to re-introduce our Avian & Exotics Department and welcome (b) (6) to our team! We offer advanced diagnostics and treatment methods to complement the care provided by referring veterinarians. In addition to referral services, we provide primary care and medical boarding. Our facility maintains a dedicated exotics ward and a 24-hour monitored critical care unit. The Avian & Exotics Department accepts appointments 5 days a week (Tuesday-Saturday) and our emergency service receives patients, including exotic species, 24 hours a day. You can contact (b) (6) at (b) (6)

(b) (6) C (b) (6)

EMERGENCY PHYSICAL EXAM - Closed Jun 04/2016
(b) (6)

Chief Complaint: Respiratory distress

History: (b) (6) presented for STAT evaluation of respiratory distress. Owner noticed progressive tachypnea this AM and difficulty using right hindlimb. She did not want to eat this AM so she did not receive her AM medication. She is currently under the care of our Cardiology Service for Dilated Cardiomyopathy (DCM) (suspect secondary to taurine deficiency), moderate left atrial enlargement, pleural effusion, azotemia, and LV thrombus.

Other Medical Problems: None

Medications/Supplements: Pimobendan, Lasix, taurine supplementation, appetite stimulant

Environment: indoors only, several other cats

Vaccination Status: UTD

Current Diet (Type): Tempting to eat
- Frequency:

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Date	Type	Staff	History

- Amount:

Physical Examination:

S(ubjective): BAR/distressed, hydration WNL, BCS 7/9, pain score: 1/4

O(bjective):
Weight: 5.3 kilograms
TPR: T: 94.8 HR: 188, RR/RE: 60/rapid/shallow
EENT: clear AU/OU, no nasal discharge, normal cervical palpation, mm pink, moist/CRT<2 sec
INTEG: Hair coat ok
PLN: WNL
CV: NSR, no murmur ausculted, left femoral pulse moderate/synchronous, right very difficult to feel to absent
RESP: tachypneic, sl. dull ventrally, no crackles/wheezes
GI: soft, nonpainful, no masses
UG: FS, NSF
M/S: laterally recumbent, a
Neuro: alert/appropriate, cranial nerves intact, no placing deficits or spinal/neck pain

Problems/Differential Diagnoses:
Respiratory distress, decreased motor/absent femoral pulse RHL

Diagnostics:
None performed

Assessment:
12yo FS DSH
- absent to faint femoral pulse RHL, decreased motor, hypothermia, hx: DCM with LV thrombus- r/o saddle thrombus vs. other
- respiratory distress, mild amount pleural effusion on TFAST- r/o secondary to CHF secondary to DCM (suspect taurine deficiency)
- hx: Dilated Cardiomyopathy (DCM) (suspect secondary to taurine deficiency), moderate left atrial enlargement, pleural effusion, azotemia, and LV thrombus.

Treatment:
Placed in oxygen. IVC placed. 4mg Lasix IV, followed by additional 5mg IV. 0.015mg/kg Buprenorphine IV. Improved rr/re with above.

Plan/Recommendations:
Discussed PE at length with owner. Concerned for partial vs. full saddle thrombus RHL secondary to LV thrombus we know she has. Discussed options- point to

Patient History Report

Client: (b) (6)
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(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			severity of underlying disease- ATH, repeat Echo, supportive care, vs. euthanasia. Owner elected to continue supportive care until they could speak with (b) (6), considering euthanasia. Elected RED code, transferred to cardiology.
(b) (6)	P	(b) (6)	0.50 mg of Buprenex (Buprenorphine) 0.3mg/mL MG C3 (MOBHL0) Rx #: 2579780 0 Of 0 Refills Give the entire contents of the syringe (0.5ml) under the tongue at 3pm.
(b) (6)	C	(b) (6)	CARDIAC EVALUTION - CLOSED 06/04/2016 - Cardiac Evaluation

Date of evaluation: (b) (6)

Date of previous cardiac evaluation: Wednesday, May 25, 2016

CHIEF COMPLAINT: heavy breathing, dragging RH limb

HISTORY: (b) (6) previously doing well. Appetite had improved, eating 2/3 can max cal per day. Normal breathing. Owner noted acute onset of dragging RH limb this morning and heavy breathing. No interest in food this morning. Brought in to ER immediately. Received 9mg lasix IV total and 0.075mg buprenohpine IV on presentation.
Previous hx: Diagnosed with DCM (b) (6) /16. Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.
Taurine level confirmed taurine deficiency

PHYSICAL EXAM: The patient was quiet, alert and responsive. No murmur on auscultation. The patient was tachypnic with moderate increased effort, RR 48. Normal BV sounds, no crackles on auscultation. Unable to bear weight on RH limb, dragging. Femoral pulses were fair in left hind, absent in left hind limb. Right paw pads cold to the touch. Heart rate was 160 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) (b) (6) **16:** Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Cursory Ultrasound: small volume pleural effusion. No pericardial effusion. Large thrombus in LV.
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ECHOCARDIOGRAM (b) (6) /2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm

Patient History Report

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Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

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THORACOCENTESIS (b) (6) **/16**: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.
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SUMMARY: (b) (6) clinical signs are due to a partial aortic thromboembolus and recurrent left sided congestive heart failure. Thrombus previously noted in LV is still present. There is a very guarded prognosis of recovery with ATE. Discussed with owners, if they wants to go forward with treatment recommend hospitalization to manage CHF and pain. Discussed risk of reperfusion injury, main concern hyperkalemia which can be fatal. At home care will consist primarily of pain medication and nursing care. She may or may not gain function back to hind limb, and this could take up to 1-2 months to see improvement if any. Owner elected to take home to spend a few hours to spend time and have (b) (6) come for euthanasia this evening. IVC left in place (right cephalic). Sent home with additional dose of buprenorphine to keep her comfortable until euthanasia. Owners understand she may pass on her own. Gave owners my condolences.

MEDICATIONS:
Buprenorphine 0.3mg/ml- give entire contents of syringe (0.5ml) at 3pm today for pain control

(b) (6) CK (b) (6) STAT
Reason for Visit: Emergency
Date Patient Checked Out: (b) (6) Practice TF

(b) (6) TC (b) (6) MEDICAL COMMENTS - TENTATIVE
(b) (6) 10:40
SW (b) (6) at Merrick - updated company that we have documented taurine deficiency in 2 other cats in house hold. The quality assurance team indicated that the level of taurine in the lot # I gave them was sufficient - discussed that this likely takes 3-6 month to develop and likely to be related to earlier lot and they need to

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

investigate further. Asked if the level they gave me was from them retesting the food after I called or from an earlier test - not sure. Asked for information from the taurine tests - told I can send summary of lab results. Also indicated that I am reporting this to the FDA. She give new info to her manager and quality assurance. Told them I expect them to follow up with me. Below email sent to Merrick:

Taurine Levels
(b) (6)
To:
(b) (6) @merrickpetcare.com
Hi (b) (6) ,

Thank you for your help with these cases. Here is the summary of the lab results:

12yr female spayed domestic short hair diagnosed and clinical for dilated cardiomyopathy
(b) (6) /2016 Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016

5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016
-9yr male neutered domestic long hair: 196 nmol/ml
-8y female spayed domestic short hair: 368 nmol/ml
-9yr male neutered domestic long hair: 124 nmol/ml
-9yr male neutered domestic long hair: 536 nmol/ml

Please let me know if you have any other questions.

Sincerely,

(b) (6)

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Patient History Report

Client: (b) (6)
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Address: (b) (6)
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Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE (b) (6) 08:42 O Imom- was previously doing well. Eating ~2/3 can of max cal per day, sRR 6-7breaths/15sec. Now, this morning, dragging RH limb and breathing heavier. SWO- recommended to come in as soon as possible since (b) (6) breathing heavy. Unfortunately, cardiology will be in surgery this morning. Should go through emergency and I will consult.
(b) (6)	B	(b) (6)	.08 mg of Buprenex (Buprenorphine) 0.3mg/mL MG C3 (MOBHL0) by (b) (6)
	B		1.00 Specialty/Referral Exam Level 3 (REF03) by (b) (6)
	B		Echo Guided Thoracocentesis Group (EGT) by (b) (6)
	B		1.00 EGT Procedure (USSC50) by (b) (6)
	B		1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
	B		1.00 Cared for by (b) (6) (b) (6)) by (b) (6)
	B		.50 mg of Buprenex (Buprenorphine) 0.3mg/mL MG C3 (MOBHL0) by (b) (6)
	B		At Home Euthanasia Group (HCEUTH) by (b) (6)
	B		1.00 At Home Euthanasia Service (HC08) by (b) (6)
	B		1.00 At Home Burial (HC10) by (b) (6)
	B		100.00 mg of Telazol 100mg/mL inj per mg (C3-N) 2103 (MLTZL1) by (b) (6)
	B		10.00 mg of Acepromazine 10mg/mL Inj per mg (MLA2L1) by (b) (6)
	B		1.00 mg of Butorphanol 10mg/mL Inj per mg (C4) (MOB2L10) by (b) (6)
	B		100.00 mg of Beuthanasia Soln 390 mg/mL (C-3N) / MG (MOB2L1) by (b) (6)
	B		1.00 IV Catheter Placement (CATH) by (b) (6)
	B		1.00 each of Tx Catheter IV 22g x 1" Surflo (BLUE) (H113) by (b) (6)
	B		1.00 each of Tx IV Ext T Set Hospira 1265028 (H027) by (b) (6)
	B		At Home Euthanasia Group (HCEUTH) by (b) (6)
	B		1.00 At Home Euthanasia Service (HC08) by (b) (6)
	B		1.00 At Home Burial (HC10) by (b) (6)
	B		-100.00 mg of Telazol 100mg/mL inj per mg (C3-N) 2103 (MLTZL1) by (b) (6)
	B		-10.00 mg of Acepromazine 10mg/mL Inj per mg (MLA2L1) by (b) (6)
	B		-1.00 mg of Butorphanol 10mg/mL Inj per mg (C4) (MOB2L10) by (b) (6)
	B		875.00 mg of Beuthanasia Soln 390 mg/mL (C-3N) / MG (MOB2L1) by (b) (6)
	B		1.00 Cared for by (b) (6) (b) (6)) by (b) (6)
	B		1.00 each of Tx Injection Cap/Plug Termo 007110 (H118) by (b) (6)
	B		Oxygen Therapy (Caged)/Hour (Group) (O2CAGE) by (b) (6)
	B		1.00 O2 Therapy Per Hour (T044) by (b) (6)
	B		1.00 Oxygen-related Patient Care / Hour (O2CARE) by (b) (6)
	B		1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
	B		4.00 mg of Furosemide (Lasix) 5% Injection per mg (MMF2L5) by (b) (6)
	B		5.00 mg of Furosemide (Lasix) 5% Injection per mg (MMF2L5) by (b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
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(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	B	(b) (6)	1.00 Cared for by (b) (6) ((b) (6) by (b) (6)
(b) (6)	B		1.00 Emergency Exam Level 4 (EE04) by (b) (6)
5/29/2016	P		7.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) Rx #: 2578487 0 Of 6 Refills Feed up to 1 can daily.
5/29/2016	TC		COMMUNICATIONS WITH CLIENT - TENTATIVE 5/29/2016 13:52 SWO- (b) (6) doing ok. sRR7breaths/15 sec. Ate ~3/4 can of the Iams max cal last night. Had normal BM yesterday. Hind limbs are very weak, one is worse than the other, but able to take a few steps on it before needing a rest. Does not see painful or distressed. Rec continue lasix 1/4 tab SID for now until appetite is consistent, then may consider incresasing. Continue pimo and taurine. Will put refill through for max cal. Emailed Client: I put through a prescription for 7 cans of food for (b) (6) . She needs just under 1 can per day (although if she eats a whole can per day that is fine). There are also refills on the prescription if you need more. I will call to check in on her in a few days. Please call me with any concerns.
5/29/2016	B		7.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) by (b) (6)
5/28/2016	C		PHARMACY NOTE Returned O call, left voice message that medication is ready for pick up
5/28/2016	P	(b) (6)	21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472) Rx #: 2569385 1 Of 12 Refills Filled by: (b) (6) Give 1 tablet by mouth twice daily with food.
5/28/2016	B		21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472) by (b) (6)
5/26/2016	TC		COMMUNICATIONS WITH CLIENT - TENTATIVE 5/26/2016 10:12 SWO- (b) (6) back to licking gravy, not eating a lot of solid food. Hind legs are weak. Owner not able to get sRR yet but seems comfortable. A/o to continue with current meds. If stops eating, then stop lasix. Otherwise will touch base in a few days. Gave owner my cell phone number if they need anything.

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Patient: (b) (6)
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Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/25/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/25/2016 18:38 SWO- BW wnl, kidney values have decresed to normal. A/o to give lasix 12.5mg tabs- 1/4 tab SID. Will adjust based on appetite and breathing. Continue with other meds (pimo, taurine and app stimulant). Owner thankful.
5/25/2016	D		Pleural Effusion Final
5/25/2016	C		CARDIAC EVALUTION - CLOSED 05/28/2016 - Cardiac Evaluation

Date of evaluation: Wednesday, May 25, 2016

Date of previous evaluation: Sunday, May 15, 2016

CHIEF COMPLAINT: heavy breathing

HISTORY: Owners noted heavy breathing yesterday. Decreased appetite yesterday and today. Prior to that her appetite was improving. Owners transitioned her to royal canin and she started eating small amounts of solid food, previously only licking gravy.
Previous hx: Diagnosed with DCM (b) (6)16. Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.
Taurine level confirmed taurine deficiency

PHYSICAL EXAM: The patient was bright, alert and responsive. No murmur on auscultation. The patient was tachypnic with mild increased effort, RR 48. Normal BV sounds, no crackles on auscultation. Faint referred upper airway noise. Femoral pulses were fair and synchronous. Heart rate was 180 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals)(b) (6)16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Brief Echo 5/25/16: moderate volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle unchanged from previous.
Brief Echo 5/15/16: small volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle.
ECHOCARDIOGRAM (b) (6)/2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia, LV thrombus

THORACOCENTESIS 4/10/16: a total of 180ml of yellow fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed scant effusion remained in the patient.
THORACOCENTESIS (b) (6) 6: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.
ER thoracocentesis 5/8/16: 25ml yellow tinged fluid from the right side.

SUMMARY: (b) (6) had 180ml fluid removed from her chest today. A renal panel showed normal renal values (BUN 31, creatinine 1.3)- previous azotemia. Will start with very low dose of lasix since decreased appetite right now (decreased appetite seemed to correlate with onset of heavy breathing). If appetite improves, can consider increasing lasix dose. Continue other medications as below. Recheck in 2 weeks, sooner if concerns.

MEDICATIONS:
START:
Lasix 12.5mg tablets- give ¼ tablet by mouth once daily

CONTINUE:
Taurine 250mg by mouth twice daily
Mirtazepine 15mg tablets: Give ¼ tablet by mouth every 3 days as needed.
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

5/25/2016	I	(b) (6)	Cardiology Discharge Instructions (b) (6) (b) (6) (b) (6) 5/25/2016 (b) (6) had 160ml of fluid removed from her chest tonight. The clot in her heart appears similar to previous. I will call you with the bloodwork results and we can determine what to do with her lasix dose.

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Recheck in 2 weeks, sooner if concerns.

MEDICATIONS:
CONTINUE:
Taurine 250mg by mouth twice daily
Mirtazepine 15mg tablets: Give ¼ tablet by mouth every 3 days as needed.
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

WAIT TO BE INSTRUCTED FURTHER ON LASIX DOSE

Watch (b) (6) for the following clinical signs and call a veterinarian if you see any of these:
Initiation of or increase in cough
Excessive panting or wheezing
Restlessness, unable to get comfortable
Decreased appetite
Lethargy/weakness
Collapse or fainting

It has been a pleasure caring for (b) (6) . Thank you for entrusting us with his care. If you have any further questions or problems, please don't hesitate to call.

5/25/2016 L (b) (6)

(b) (6)

Cardiac Panel #10 results from (b) (4) Requisition ID: 0 Posted Final

Test	Result	Reference Range
HCT =	34 %	
NA+ =	147.3 mmol/L	146.2 – 156.2
K+ =	6.65 mmol/L H	3.41 – 4.71
CL– =	115.1 mmol/L L	117.0 – 125.3
BUN =	31 mg/dL	22 – 33
CREA =	1.3 mg/dL	0.07 – 1.9

Manually entered.
PCV = 32% TS = 6.0g/dL

5/25/2016 P (b) (6)

30.00 ml of DNULsix 10mg/ml/ML (M0568)
Rx #: 2576809 0 Of 12 Refills
Give 0.5ml by mouth once daily or as directed by your veterinarian.

5/25/2016 V (b)

May 25, 2016 04:26 PM Staff: (b) (6)

Weight : 5.30 kilograms
Rm. 14

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Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/25/2016	CK	(b) (6)	breathing heavy Reason for Visit: Recheck Date Patient Checked Out: 05/25/16 Practice (b) (6)
5/25/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/25/2016 14:40 SWO- (b) (6) breathing heavy today. No interest in food yesterday or today. Owner to bring in this afternoon.
5/25/2016	B	(b) (6)	1.00 Specialty/Referral Exam Level 2 (REF02) by (b) (6)
5/25/2016	B		Echo Guided Thoracocentesis Group (EGT) by (b) (6)
5/25/2016	B		1.00 EGT Procedure (USSC50) by (b) (6)
5/25/2016	B		1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
5/25/2016	B		1.00 Thoracocentesis Therapeutic (R33) by (b) (6)
5/25/2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
5/25/2016	B		1.00 In-house lab (XNBALIX) by (b) (6)
5/25/2016	B		1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/25/2016	B		1.00 Lab Sample Label (TL) by (b) (6)
5/25/2016	B		1.00 Cardiac (b) (6) Panel #10 ((b) (6)) by (b) (6)
5/25/2016	B		1.00 Cared for by (b) (6) ((b)) by (b) (6)
5/25/2016	B		30.00 ml of DNULsix 10mg/ml/ML (M0568) by (b) (6)
5/25/2016	B		-30.00 ml of DNULsix 10mg/ml/ML (M0568) b
5/22/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/22/2016 16:23 SWO- Appetite is still the same, but now (b) (6) will go to the food on her own instead of owners bringing it to her. Still only eating gravy, no solid food yet. Owner has not tried Max Cal, recommended trying that. Cats who go prolonged period without eating at risk for hepatic lipidosis. Personatlity wise, she is much improved, almost back to normal self. Ambulating around the house as before. Very social. Owner bought Royal Canin as new diet. Gets taurine and pimo BID now. Told owner to continue appetite stimulant for now (had stopped this). Urinating and defecating outside of the litter box, not a SE of meds, likley behavioral. Soft stools. A/o let me know if soft stools continue.
5/19/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT 5/19/2016 16:18

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			SWO - updating that I did talk with Merrick customer service today to take my complaint and are filing it with their quality assurance. I am not sure when they will get back with me, but I will let them know as soon as I hear anything. Owner thankful for call.
5/19/2016	C	(b) (6)	MEDICAL COMMENTS ***ADDENDUM 5/19/2016 5/19/2016 11:49 Called Merrick at 1(800)664-7387 to report taurine deficiency possibly related to consumption of their product, Merrick Purrfect Bistro Grain Free Real Chicken Recipe feline dry (best by 7/26/2017, lot #16025 DL1 38310 14131 - lost # difficult to read), USB# 22808 38310). Owner has been feeding this food for approximately 3 years, 5 cats total in household, product has been purchased from the (b) (6) in (b) (6) Requesting that the company investigate this possible deficiency, also discussed that I would like for the other cats in the household to be tested. SW (b) (6) @ Merrick - said I could expect call back in 2 weeks, let her know I would like to know when to expect a call. She will submit complaint and let me know. ADDENDUM on 5/19/2016 at 15:28:19 from (b) (6) Merrick called back - additional questions of how long the cat has been sick - presented to ER (b) (6) and sick day before; also wanted to know if bag was new - yes bag was purchased about 2 weeks prior per owner. My concern however is that it takes several months for this to develop and I do not believe this is a single bag/lot issue.
5/19/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT 5/19/2016 10:10 SWO - introduce3d myself, asked owner about diet history, has been feeding Merrick Purrfect Bistro Grain Free Real Chicken Recipe for approximately 3 years and purchasing from (b) (6) in (b) (6) . Prior to this feeding Dick Van Pattons Indoor Formula Dry, chicken and salmon flavor. Discussed with owner that I will contact the company and also report to the FDA. Will let owner know of communication. In my experience sometimes the company will also want to reach out to the client. Owner thankful for call.
5/18/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/18/2016 18:16 SWO- (b) (6) still not eating, had a little gravy this morning. Drank a lot of water today. Breathing is normal. Owner dropping food off tonight.

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/18/2016	TC	(b) (6)	COMMUNICATIONS WITH DOCTOR - TENTATIVE 5/18/2016 18:15 Imom for (b) (6) regarding appt on Satruday with other cats in house. Would like whole blood taurine levels sent to the UC Davis amino acid lab. Call me or speak with nutrition regarding any questions.
5/17/2016	P		3.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) Rx #: 2573240 0 Of 3 Refills Feed as directed
5/17/2016	TC		COMMUNICATIONS WITH CLIENT - TENTATIVE 5/17/2016 15:58 SWO- did not eat much this morning. O gave appetite stimulant this morning and then left her alone with some food. Has not checked on her yet. Normal BM last night. sRR6brs/15sec last night. Vet coming for house call Saturday morning to take taurine sample for other cats. A/o to transition after that- recommended Hills Science Diet, Purina, Royal Canin. Will also rx Iams max cal for her to pick up here and offer (b) (6) .
5/17/2016	B	(b) (6)	3.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) by (b) (6)
5/16/2016	TC		COMMUNICATIONS WITH CLIENT - TENTATIVE 5/16/2016 18:04 SWO- (b) (6) seemed to be doing better last night. A little brighter when they got home. Started taurine supplementation last night. Not eating solid foods yet, but licking gravy- had the gravy from almost 3 cans last night. Owner put solid food in blender, but (b) (6) not interested (may have been too thick still). Drinking water. No BM, not a concern becuase she is not eating. Asked owner to bring in the food in original package as soon as possible, owner was planning on dropping off tomorrow. Also discussed to get other 4 cats tested for taurine levels tis week, as since we are changing their diet we would like to know levels on current diet. Owner will call to either have mobile vet come to house or schedule here with GP this week. Told owner I will talk to nutrition about recommended diets.
5/15/2016	R	(b) (6)	Referral Letter - Cardio Resident Eval and labs - FINAL 05/15/2016 - REF

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6)

TO: (b) (6)
FAX #: (b) (6)
FROM: (b) (6)
DATE: Sunday, May 15, 2016
RE:
Client: (b) (6)
Patient: (b) (6)
Breed: Shorthair, Domestic
Age: 12 Yrs. 4 Mos. **Sex:** Spayed Female
Current Weight: 5.2 kilograms as of 5/15/2016

Thank you for referring (b) (6). The following is a case summary.

Date of evaluation: Sunday, May 15, 2016

Date of previous cardiac evaluation: Monday, (b) (6) 2016

CHIEF COMPLAINT: Recheck, not eating

HISTORY: (b) (6) has not eaten since discharge on (b) (6) Owner gave mirtazapine yesterday. Today licking some of the liquid off the food and very polydyspic, but no interest in eating any solid food. Will take a few steps and then lay down, very weak. Owner not able to get sRR, awake RR 6breaths/15sec. No heavy breathing noted.
Previous hx: Diagnosed with DCM (b) (6) 16. Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.

PHYSICAL EXAM: The patient was quiet, alert and responsive. No murmur on auscultation. The patient was eupnic, RR 32. Normal BV sounds, no crackles on auscultation. Femoral pulses were fair and synchronous. Heart rate was 180 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) (b) (6) 16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Brief Echo 5/15/16: small volume pleural effusion. No pericardial effusion. Large mass noted

in left ventricle.
ECHOCARDIOGRAM (b) (6)/2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm
Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia, LV thrombus

THORACOCENTESIS (b) (6) **6**: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the pati
ER thoracocentesis (b) (6) 6: 25ml yellow tinged fluid from the right side.

SUMMARY: The small amount of pleural effusion was not enough to warrant thoracocentesis, especially since patient eupnic. (b) (6) taurine level came back low (24nmol/ml). Recommended supplementing taurine at below dose. Discussed with owner it can take up to 2-3 weeks to see an effect of this and even longer (3 months) to see changes on echocardiogram. There is a chance (b) (6) will succumb to congestive heart failure before we see the positive effects of the taurine. A renal panel revealed azotemia. Discussed with owner the challenge going forward of managing the heart failure with azotemia. If (b) (6) develops trouble breathing, may not have options to treat. Euthanasia may be the most humane option for (b) (6) at that time.
The echocardiogram showed a large mass in the left ventricle, consistent with a thrombus. There is a risk that this clot, or a piece of it, leaves the heart and causes a thromboembolic event. The most common place for thromboembolic disease in cats is the aortic bifurcation, but can occur anywhere including the lungs which can be fatal. Advised owners, if FATE suspected at home, bring (b) (6) to closest emergency facility. At that time, euthanasia is recommended. We discussed holding off on an asprin or Plavix for now, as it will not affect the current clot, and (b) (6) is not yet eating.
Owners willing to start taurine supplementation and take it day by day. Owners understand the possible outcomes we discussed today. I will call to check in tomorrow.

MEDICATIONS:
START:
Taurine 250mg by mouth twice daily

CONTINUE:
Mirtazepine 15mg tablets: Give ¼ tablet by mouth every 3 days as needed.

HOLD FOR NOW:
Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet once daily.
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

Thank you for the courtesy of this interesting referral. Please feel free to contact me with any questions or comments.

Sincerely,

(b) (6)

(b) (6)
Sent electronically - no signature required

(b) (6)

Client ID: (b) (6) Patient ID: (b) (6) Patient Name: (b) (6)

DATE/TIME	TEST	RESULT	REFERENCE RANGE
5/15/2016	BUN	= 61 mg/dL (H)	22 - 33
5/15/2016	CL-	= 104.5 mmol/L (L)	117.0 - 125.3
5/15/2016	CREA	= 3.1 mg/dL (H)	0.07 - 1.9
5/15/2016	HCT	= 43 %	
5/15/2016	K+	= 3.33 mmol/L (L)	3.41 - 4.71
5/15/2016	NA+	= 145.3 mmol/L (L)	146.2 - 156.2

Patient History Report

Client: (b) (6)
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Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Lab Comments: Manually entered.

Additional Comments: BUN/Cre=19.7mg/mg PCV=39% TS=7.8g/dL Normal

Pain Condition Scores
Have you noticed the "PCS" aka Pain Condition Score in our medical records? As part of our AAHA specialty accreditation, we evaluate the level of pain or discomfort for each and every patient we see. You will see this reflected in the physical exam recordings as a "PCS". We follow the guidelines created and published by Colorado State University. If you have questions about interpreting pain or treatment options, or you need to schedule a pain management consult, please speak with our Anesthesiology & Pain Management Department by calling (b) (6) .

Clinical Studies
We are recruiting for a variety of clinical studies across multiple specialties and are looking for eligible patients. For a complete list, please visit our website at and click on Veterinary Professionals and then Current Clinical Studies.

Avian & Exotics
(b) (6) is happy to re-introduce our Avian & Exotics Department and welcome (b) (6) to our team! We offer advanced diagnostics and treatment methods to complement the care provided by referring veterinarians. In addition to referral services, we provide primary care and medical boarding. Our facility maintains a dedicated exotics ward and a 24-hour monitored critical care unit. The Avian & Exotics Department accepts appointments 5 days a week (Tuesday-Saturday) and our emergency service receives patients, including exotic species, 24 hours a day. You can contact (b) (6) at (b) (6)

5/15/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/15/2016 16:34 SWO- discussed azotemia. Since (b) (6) is eupnic, would hold off on lasix for now. Hope would be that she may be able to breathe comfortably without lasix for enough time that taurine may start to help. Otherwise may give low dose of lasix, but going to be a big challange with azotemia. Owners are to start taurine tonight. Discussed case with nutrition. Will file a complaint about the food. Will have more

Patient History Report

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Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			information on this tomorrow. Will call to check in tomorrow.
5/15/2016	D	(b) (6)	Taurine Deficiency Final
5/15/2016	D		Azotemia Tentative
5/15/2016	D		Pleural Effusion Final
5/15/2016	C	(b) (6)	CARDIAC EVALUTION - CLOSED 05/18/2016 - Cardiac Evaluation

Date of evaluation: Sunday, May 15, 2016

Date of previous cardiac evaluation: Monday, (b) (6) 2016

CHIEF COMPLAINT: Recheck, not eating

HISTORY: (b) (6) has not eaten since discharge on (b) (6) Owner gave mirtazapine yesterday. Today licking some of the liquid off the food and very polydyspic, but no interest in eating any solid food. Will take a few steps and then lay down, very weak. Owner not able to get sRR, awake RR 6breaths/15sec. No heavy breathing noted.
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PHYSICAL EXAM: The patient was quiet, alert and responsive. No murmur on auscultation. The patient was eupnic, RR 32. Normal BV sounds, no crackles on auscultation. Femoral pulses were fair and synchronous. Heart rate was 180 bpm, regular rhythm. PCS 0/4. BCS 8/9.

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The echocardiogram showed a large mass in the left ventricle, consistent with a thrombus. There is a risk that this clot, or a piece of it, leaves the heart and causes a thromboembolic event. The most common place for thromboembolic disease in cats is the aortic bifurcation, but can occur anywhere including the lungs which can be fatal. Advised owners, if FATE suspected at home, bring (b) (6) to closest emergency facility. At that time, euthanasia is recommended. We discussed holding off on an asprin or Plavix for now, as it will not affect the current clot, and (b) (6) is not yet eating.
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Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

5/15/2016	I	CDS	Cardiology Discharge Instructions (b) (6) 5/15/2016

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6) has a small amount of fluid in her chest today. It was not enough to warrant draining today.

Her taurine levels came back low. Please start supplementing taurine as below. It can take up to 2-3 weeks to see an effect of this.

The echocardiogram showed a large mass in one of the chambers of her heart (the left ventricle). There is a risk that this clot, or a piece of it, leaves the heart. If that happens, it can travel to any part of the body (lungs, hind legs, etc) and this can be fatal. We discussed holding off on an asprin or Plavix medication for now, as it will not do anything for the current clot, and (b) (6) is not yet eating. I will call you with her bloodwork results this afternoon.

MEDICATIONS:
START:
Taurine 250mg by mouth twice daily

Watch (b) (6) for the following clinical signs and call a veterinarian if you see any of these:
Initiation of or increase in cough
Excessive panting or wheezing
Restlessness, unable to get comfortable
Decreased appetite
Lethargy/weakness
Collapse or fainting

It has been a pleasure caring for (b) (6). Thank you for entrusting us with his care. If you have any further questions or problems, please don't hesitate to call.

5/15/2016 L (b) (6)

(b) (6)
Cardiac Panel #10 results from (b) (4) Requisition ID: 0 Posted Final

Test	Result	Reference Range
HCT =	43 %	
NA+ =	145.3 mmol/L L	146.2 - 156.2
K+ =	3.33 mmol/L L	3.41 - 4.71
CL- =	104.5 mmol/L L	117.0 - 125.3
BUN =	61 mg/dL H	22 - 33
CREA =	3.1 mg/dL H	0.07 - 1.9

Manually entered.
BUN/Cre=19.7mg/mg PCV=39% TS=7.8g/dL Normal

5/15/2016 V (b) (6) May 15, 2016 03:24 PM Staff: (b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			Weight : 5.20 kilograms cardio baby scale
5/15/2016	CK	(b) (6)	Reason for Visit: Recheck Date Patient Checked Out: 05/15/16 Practice (b)
5/15/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/15/2016 13:29 SWO (mrs)- owner gave mirtazapine, no improvement in appetite. Drinking excesivly. Having a hard time walking, very weak. Owner not able to get sRR, awake breathing 6breaths/15sec. Offered to see (b) (6) today. Made appt for 3pm
5/15/2016	B	(b) (6)	1.00 Specialty/Referral Exam Level 2 (REF02) by (b) (6)
5/15/2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
5/15/2016	B		1.00 In-house lab (XNBALIX) by (b) (6)
5/15/2016	B		1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/15/2016	B		1.00 Lab Sample Label (TL) by (b) (6)
5/15/2016	B		1.00 Cardiac (b) (6) Panel #10 (b) (6) by (b) (6)
5/15/2016	B		Echo Guided Thoracocentesis Group (EGT) by (b) (6)
5/15/2016	B		1.00 EGT Procedure (USSC50) by (b) (6)
5/15/2016	B		1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
5/15/2016	B		1.00 Cared for by (b) (6) ((b)) by (b) (6)
5/14/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/14/2016 17:42 Mrs called and lmovm that (b) (6) hasn't been eating well. I called back and sw Mr. He said she is eating only very tiny amounts and not improving, wanted to know if I had suggestions. Owners feel breathing is still ok, 6 breaths/15 seconds but coughed a little today. I told Mr she could have poor app due to fluid reforming or azotemia or her heart disease in general. She may need to be rechecked sooner than later to evaluate this and r/o fluid and worsening azo. Owners plan to discuss w/ (b) (6) but wanted to know if there is something they could give her before morning. I offered to prescribe appetite stimulant, explained that this may not work b/c it doesn't override what is causing the inappetance in the 1st place but it's fine to try. Mr was thankful, said he may or may not pick it up tonight but is glad to have the option.
5/14/2016	P	(b) (6)	2.00 tablet of Mirtazapine 15mg Tablet (M1052)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
			A/G Ratio 1.4 Ratio 0.35 - 1.5 B/C Ratio 28 Ratio 4 - 33 Na/K Ratio 33
5/21/2016	L		Endocrinology results from (b) (6) ID: 209396 Posted Final Test Result Reference Range T4 20.2 ug/dL H 0.8 - 4.0 Ascn: (b) (6) Profile: Total T4 Result verified.
5/21/2016	L		Miscellaneous results from (b) (6) ID: 209396 Posted Final Ascn: (b) (6) Profile: Superchem RE: 1045 PrecisionP 28 U/L 8 - 26 PresisionPSL elevations correlate closely with abnormal PLI concentrations. In cats with appropriate clinical signs, this PrecisionPSL is supportive of, but not definitive, for a diagnosis of pancreatitis. In cats without clinical signs of pancreatitis, a mild elevation is an insignificant finding. RE: 11067 Comment Hemolysis 1+ No significant interference.
5/21/2016	B	(b) (6)	1.00 Superchem Cbc T4 (b) (6) Sa120 (L85) by (b) (6)
5/21/2016	B		1.00 House Call Travel Level 2 (HC06) by (b) (6)
5/21/2016	B		1.00 At Home Appointment (HC04) by (b) (6)
5/21/2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016	B		1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016	B		1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016	B		1.00 Lab Sample Label (b) (6) by (b) (6)
5/21/2016	B		1.00 Cared for by (b) (6) (b) (6) by (b) (6)
5/20/2016	C		COMMUNICATIONS WITH CLIENT - Closed May 21/2016 5/20/2016 15:00 Called to confirm tomorrow's appointment fro (b) (6) , (b) (6) , (b) (6) and (b) (6) at 9 am. I also mentioned in my message that we should use the address (b) (6) in the GPS. If any questions please call (b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: [redacted]
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			critical level <200 nmol/mL. **TEST PERFORMED AT THE UNIVERSITY OF WISCONSIN** **FELINE PLASMA NORMALS: 60–120 nmol/ml WHOLE BLOOD NORMALS: 300–600 nmol/ml** **PLASMA CRITICAL: LESS THAN 40 nmol/ml WHOLE BLOOD CRITICAL: LESS THAN 200** **nmol/ml** **K9 PLASMA NORMALS: 60–120 nmol/ml WHOLE BLOOD NORMALS: 200–350 nmol/ml PLASMA** **CRITICAL: LESS THAN 40 nmol/ml WHOLE BLOOD CRITICAL: LESS THAN 150 nmol/ml**
(b) (6) 2016	R	(b) (6)	Referral Letter - Cardio Resident Eval and labs - FINAL 05/09/2016 - REF

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6)

TO: (b) (6)
FAX #: (b) (6)
FROM: (b) (6)
DATE: Monday, May 09, 2016
RE:
Client: (b) (6)
Patient: (b) (6)
Breed: Shorthair, Domestic
Age: 12 Yrs. 4 Mos. **Sex:** Spayed Female
Current Weight: 15.6 pounds as of 12/28/2009

Thank you for referring (b) (6). The following is a case summary.

Date of evaluation: Monday, May 09, 2016

CHIEF COMPLAINT: pleural effusion

HISTORY: Presented to ER last night for lethargy and ADR. Cursory ultrasound revealed pleural effusion. Thoracocentesis yielded 25ml yellow tinged fluid from the right side. Patient received 12mg lasix IV and was placed in oxygen overnight. Her RR was wnl, with slight effort noted overnight. She ate a small amount. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3).

PHYSICAL EXAM: The patient was bright, alert and responsive. No murmur on auscultation, but heart sound slightly muffled. The patient was eupnic, RR 30 with slight effort. Normal BV sounds, no crackles on auscultation. Pulses were fair and synchronous. Heart rate was 170 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) (b) (6) **16:** Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM). Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial

effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia

THORACOCENTESIS (b) (6) **/16**: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.

SUMMARY: (b) (6) has dilated cardiomyopathy (DCM) and has developed pleural effusion due to congestive heart failure. The pleural effusion was removed today and we are starting the medications as described below. Although taurine deficiency is a rare cause for dilated cardiomyopathy in cats, we submitted a taurine level to the lab today to look for this. The test results can take up to 2 weeks; we will fax results when they are available. Unfortunately the prognosis for DCM (with no taurine deficiency) in cats is poor, with survival time 3-6 weeks or in rare cases 4-6 months. A renal panel was recheck today and showed elevated BUN (74) and normal creatinine (1.4). Discussed with owner the azotemia may make treating the congestive heart failure challenging.
We discussed that with heart enlargement there is a risk for developing a blood clot, or stroke. Although aspirin +/- plavix can be given in hopes of reducing clot formation, they have not been definitively proven to prevent blood clot formation in cats. If the owners elect to start this medication I would recommend waiting until we know she is eating and feeling well on the below medications as aspirin/plavix can cause GI side effects (vomiting, inappetance) in some cats. A recheck with cardiology is recommended in 2 weeks, or sooner if clinical signs.
Advised owner congestive heart failure likely contributing to lethargy, but concern that weakness may be secondary to the DCM and low cardiac output. If that is the case, may not improve once we get her out of failure. Owner comfortable taking (b) (6) home to see how she does. (b) (6) was discharged on the below medications. If she does not improve at home over the next few days, owner to consider euthanasia.

MEDICATIONS:
START: Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet once daily.

START in 3 DAYS IF EATING
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.
If eating and easy to medicate, start: Taurine 250 mg by mouth twice a day with food.

Thank you for the courtesy of this interesting referral. Please feel free to contact me

with any questions or comments.

Sincerely,

(b) (6)

(b) (6)
Sent electronically - no signature required

(b) (6)

Client ID: (b) (6) Patient ID: (b) (6) Patient Name: (b) (6)

DATE/TIME	TEST	RESULT	REFERENCE RANGE
(b) (6)	**CREA**	**= 1.4 mg/dL**	**0.8 - 2.4**

Lab Comments: CREA: Test results for the latest analyzer run have been multiplied by the dilution factor for a dilution of 1 in 4 total.

DATE/TIME	TEST	RESULT	REFERENCE RANGE
(b) (6)	**ALB**	**= 2.8 g/dL**	**2.3 - 3.9**
	ALB/GLOB	**= 0.8**	
	ALKP	**= 11 U/L (L)**	**14 - 111**
	ALT	**= 140 U/L (H)**	**12 - 130**
	BUN/UREA	**= 74 mg/dL (H)**	**16 - 36**
	Chloride	**= 100 mmol/L (L)**	**112 - 129**
	CREA	**- --.-- mg/dL**	**0.8 - 2.4**
	GLOB	**= 3.3 g/dL**	**2.8 - 5.1**
	GLU	**= 105 mg/dL**	**71 - 159**
	Na/K	**= 29**	
	OSM calc	**= 298 mmol/kg**	
	PHOS	**= 7.0 mg/dL**	**3.1 - 7.5**
	Potassium	**= 4.7 mmol/L**	**3.5 - 5.8**
	Sodium	**= 138 mmol/L (L)**	**150 - 165**

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6) **= 6.1 g/dL** **5.7 - 8.9**

Pain Condition Scores
Have you noticed the "PCS" aka Pain Condition Score in our medical records? As part of our AAHA specialty accreditation, we evaluate the level of pain or discomfort for each and every patient we see. You will see this reflected in the physical exam recordings as a "PCS". We follow the guidelines created and published by Colorado State University. If you have questions about interpreting pain or treatment options, or you need to schedule a pain management consult, please speak with our Anesthesiology & Pain Management Department by calling (b) (6).

Clinical Studies
We are recruiting for a variety of clinical studies across multiple specialties and are looking for eligible patients. For a complete list, please visit our website at and click on Veterinary Professionals and then Current Clinical Studies.

Avian & Exotics
(b) (6) is happy to re-introduce our Avian & Exotics Department and welcome (b) (6) to our team! We offer advanced diagnostics and treatment methods to complement the care provided by referring veterinarians. In addition to referral services, we provide primary care and medical boarding. Our facility maintains a dedicated exotics ward and a 24-hour monitored critical care unit. The Avian & Exotics Department accepts appointments 5 days a week (Tuesday-Saturday) and our emergency service receives patients, including exotic species, 24 hours a day. You can contact (b) (6) at b) (6).

(b) (6) 2016 I (b) (6) Cardiology Discharge Instructions
(b) (6)
(b) (6) 2016

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

A cardiologist has evaluated (b) (6) and has diagnosed her with Dilated Cardiomyopathy (DCM). DCM means she has poor muscle contraction of the heart and she has developed significant heart enlargement over time. Her clinical signs were due to congestive heart failure (fluid buildup around the lungs called pleural effusion), which developed secondary to the enlarged heart. We removed all the pleural effusion today. The fluid will reform but how fast this occurs is unpredictable. Please start the medications as below to help clear fluid and slow the fluid formation.

Although taurine deficiency is a rare cause for cardiomyopathy in cats, we submitted a taurine level to the lab today to look for this. The test results can take up to 2 weeks; we will call you when the results are available.

As we discussed, (b) (6) has elevations in her kidney values. This can make treating her heart disease challenging because she may not tolerate the lasix. If her kidney values become elevated to a certain degree, it will make her feel sick and she will likely have a decreased appetite or stop eating. We will monitor her kidney values with bloodwork over time.

Cats with heart enlargement are at risk for developing a blood clot, or stroke. Although aspirin and/or plavix can be given in hopes of reducing clot formation, they have not been proven to prevent blood clot formation in cats. If you elect to start this medication I would recommend waiting until she is eating and feeling well at home as these medications can cause GI side effects (vomiting, inappetance) in some cats.

Please periodically take a sleeping respiratory rate (sRR) at home. WHILE (b) (6) IS SLEEPING, count the number of times she breathes in over 15 seconds. She should breathe 8 or fewer breaths in 15 seconds.

A recheck with cardiology is recommended in 2 weeks, or sooner if you see any of the below signs.

MEDICATIONS:
START TODAY: Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet by mouth once a day
Furosemide: Also called Salix or Lasix. This is a diuretic and will help clear the fluid from your pet's lungs. Side effects include electrolyte abnormalities (if they stop eating), dehydration and kidney enzyme elevations. Blood work can be done to monitor these. This medication will be probably given for the life of your pet.

START IN 3 DAYS IF EATING:
Pimobendan 1.5 mg tiny tabs: Give 1 tablet by mouth two times a day WITH

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

FOOD.
Pimobendan is a phosphodiesterase inhibitor that gives increased contractility and arterial vasodilation. This will help the heart function better, allow your cat to feel better and live longer. Any medication can upset the stomach. This drug does not typically cause this, but if you see any changes, please stop the drug till you talk to a doctor here at (b) (6). Please give this with (b) (6) meals. Giving on empty stomach is more likely to make her nauseous.

We have called this medication into (b) (6). Please call them to order it and they will mail it to you.

If eating, start: Taurine 250 mg by mouth twice a day with food.
I have submitted blood for a taurine level. The result may not return for 2 weeks. In the meantime, please start Taurine at home, 250 mg two times a day with food. This can be purchased at any health food store. If she is not eating well or if it is difficult to give her this medication, you can skip this until we get the taurine result from the blood work.

Watch (b) (6) for the following clinical signs and call a veterinarian if you see any of these:
Initiation of or increase in cough
Excessive panting or wheezing
Restlessness, unable to get comfortable
Decreased appetite
Lethargy/weakness
Collapse or fainting

It has been a pleasure caring for (b) (6). Thank you for entrusting us with his care. If you have any further questions or problems, please don't hesitate to call.

(b) (6) L

(b) (6)

Chemistry results from (b) (6) In-clinic
Laboratory Requisition ID: 197 Posted Final

Test	Result	Reference Range
CREA =	1.4 mg/dL	0.8 - 2.4

CREA: Test results for the latest analyzer run have been multiplied by the dilution factor for a dilution of 1 in 4 total.

(b) (6) C (b) (6)

PHARMACY NOTE
Called (b) (6) and spoke to (b) (6). Ordered Pimobendan

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

1.5mg tiny tabs. Give 1 tablet by mouth twice daily with food. #100, 12 refills

---

(b) (6) L

Chemistry results from (b) (6) In-clinic
Laboratory Requisition ID: 197 Posted Final

Test	Result	Reference Range
ALB =	2.8 g/dL	2.3 - 3.9
ALKP =	11 U/L L	14 - 111
ALT =	140 U/L H	12 - 130
BUN/UREA =	74 mg/dL H	16 - 36
Chloride =	100 mmol/L L	112 - 129
CREA -	--.-- mg/dL	0.8 - 2.4
GLU =	105 mg/dL	71 - 159
PHOS =	7.0 mg/dL	3.1 - 7.5
Potassium =	4.7 mmol/L	3.5 - 5.8
Sodium =	138 mmol/L L	150 - 165
TP =	6.1 g/dL	5.7 - 8.9
GLOB =	3.3 g/dL	2.8 - 5.1
ALB/GLOB =	0.8	
Na/K =	29	
OSM calc =	298 mmol/kg	

(b) (6) TC (b) (6)

COMMUNICATIONS WITH CLIENT - TENTATIVE
(b) (6) 2016 12:35
10am- SWO (mrs)- Discussed echo confirmed heart disease, DCM. Reviewed causes of DCM (unlikley taurine def, but will submit for levels) and prognosis with owner. Risk of future episodes of CHF, when is unpredictable. Oenwer consented to thoracocentesis. If continues to breath comfortably out of oxygen can go home this afternoon.

12:30pm- SWO- (b) (6) breathing is stable out of oxygen. Very weak and lethargic. Ate a small amount of food this morning. Discussed since breathing is comfortable, can try at home. If energy level does not improve at home over the next few days, may consider euthanasia. Discussed elevated kidney values and how that is giong to make treating CHF with lasix challenging. Owner is comfortable with trying (b) (6) at home to see how she does. Will have husband call back to set up a time.

(b) (6) P (b) (6)
P

21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472)
Rx #: 2569385 0 Of 12 Refills
Give 1 tablet by mouth twice daily with food.
60.00 tablet of Lasix (Salix / Furosemide) 12.5mg Tablet (M568)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	D D D	(b) (6)	Rx #: 2569382 0 Of 0 Refills Give 1/2 tablet by mouth twice daily. Pleural Effusion Final Left Atrial Enlargement Final Dilated Cardiomyopathy Final

(b) (6) C (b) (6) RADIOLOGY REVIEW - FINAL 05/09/2016

The DV view of the thorax obtained on May 8, 2016 has been reviewed and there is a moderate to large amount of pleural effusion that obscures visualization of the cardiac silhouette. There is also an area of increased opacity in the left hemithorax in the region of the caudal segment of left cranial lung lobe. The remaining lung parenchyma appears to be within normal limits and the pulmonary vessels appear normal. This combination of findings may be the result of heart failure or neoplasia and a cardiac consult is recommended for further evaluation.

This review was written by: (b) (6)

(b) (6) C (b) (6) CARDIAC EVALUTION - CLOSED 05/12/2016 - Cardiac Evaluation

Date of evaluation: Monday, May 09, 2016

CHIEF COMPLAINT: pleural effusion

HISTORY: Presented to ER last night for lethargy and ADR. Cursory ultrasound revealed pleural effusion. Thoracocentesis yielded 25ml yellow tinged fluid from the right side. Patient received 12mg lasix IV and was placed in oxygen overnight. Her RR was wnl, with slight effort noted overnight. She ate a small amount. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3).

PHYSICAL EXAM: The patient was bright, alert and responsive. No murmur on auscultation, but heart sound slightly muffled. The patient was eupnic, RR 30 with slight effort. Normal BV sounds, no crackles on auscultation. Pulses were fair and synchronous. Heart rate was 170 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals (b) (6) 16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

ECHOCARDIOGRAM (b) (6) 2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm

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Date	Type	Staff	History

IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm
Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia

THORACOCENTESIS(b) (6)**16**: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.

SUMMARY: (b) (6) has dilated cardiomyopathy (DCM) and has developed pleural effusion due to congestive heart failure. The pleural effusion was removed today and we are starting the medications as described below. Although taurine deficiency is a rare cause for dilated cardiomyopathy in cats, we submitted a taurine level to the lab today to look for this. The test results can take up to 2 weeks; we will fax results when they are available. Unfortunately the prognosis for DCM (with no taurine deficiency) in cats is poor, with survival time 3-6 weeks or in rare cases 4-6 months. A renal panel was recheck today and showed elevated BUN (74) and normal creatinine (1.4). Discussed with owner the azotemia may make treating the congestive heart failure challenging.
We discussed that with heart enlargement there is a risk for developing a blood clot, or stroke. Although aspirin +/- plavix can be given in hopes of reducing clot formation, they have not been definitively proven to prevent blood clot formation in cats. If the owners elect to start this medication I would recommend waiting until we know she is eating and feeling well on the below medications as aspirin/plavix can cause GI side effects (vomiting, inappetance) in some cats. A recheck with cardiology is recommended in 2 weeks, or sooner if clinical signs.
Advised owner congestive heart failure likely contributing to lethargy, but concern that weakness may be secondary to the DCM and low cardiac output. If that is the case, may not improve once we get her out of failure. Owner comfortable taking (b) (6) home to see how she does. (b) (6) was discharged on the below medications. If she does not improve at home over the next few days, owner to consider euthanasia.

MEDICATIONS:
START: Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet once daily.

START in 3 DAYS IF EATING
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

If eating and easy to medicate, start: Taurine 250 mg by mouth twice a day with food.

(b) (6) 2016 L (b) (6)

Basic Metabolic Profile, (b) (6) results from (b) (6)
In-Clinic Requisition ID: 0 Posted Final

Test	Result	Reference Range
HCT =	40 %	12 - 70
HB =	13.1 g/dL	9.9 - 14.9
NA+ =	146.3 mmol/L	146.2 - 156.2
K+ =	4.99 mmol/L H	3.41 - 4.71
CL+ =	107.8 mmol/L L	117.0 - 125.3
CA++ =	1.17 mmol/L	1.16 - 1.35
MG++ =	1.08 mmol/L H	0.33 - 0.49
GLU =	156 mg/dL H	72 - 132
LAC =	9.7 mg/dL H	0.7 - 1.9
BUN =	67 mg/dL H	22 - 33
CREAT =	5.3 mmo/L H	1.1 - 3.5
O2CAP =	18.2 mL/dL	
TCO2 =	19.9 mmol/L	
GAP =	20.3 mmol/L	
CA/MG =	1.1 mol/mol	
OSM =	313.5 mOsm/kg	
BUN/CREA =	12.7 mg/mg	

Manually entered.
PCV: 43% T.S: 6.6mg/dl

(b) (6) TC (b) (6)

LAB RESULTS - NOTES - TENTATIVE
(b) (6) 00:00
Lab Results: PCV: 42% TS g/dl: 6.8 Serum: Normal
Original Lab Date:

	B	Laboratory Request / Sample Handling (LABS) by (b) (6)
	B	1.00 Sample Handling & Disposal (LFEE) by (b) (6)
	B	1.00 Basic Metabolic (b) (6) Panel # 2 ((b) (6)) by (b) (6)
	B	1.00 Specialty/Referral Exam Level 3 (REF03) by (b) (6)
	B	Echocardiogram Level 3 Group (USSC19) by (b) (6)
	B	1.00 VRC04 Procedure (VRC04) by (b) (6)
	B	1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
	B	1.00 Thoracocentesis Therapeutic (R33) by (b) (6)

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Patient History Report

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Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6) 2016	B	(b) (6)	60.00 tablet of Lasix (Salix / Furosemide) 12.5mg Tablet (M568) by (b) (6)
2016	B		21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472) by (b) (6)
2016	B		Oxygen Therapy (Caged)/Hour (Group) (O2CAGE) by (b) (6)
2016	B		7.00 O2 Therapy Per Hour (T044) by (b) (6)
2016	B		7.00 Oxygen-related Patient Care / Hour (O2CARE) by (b) (6)
2016	B		1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
2016	B		Hospitalization Hours Smart Group (HOSPIT) by (b) (6)
2016	B		17.00 Hospitalization Hours- Feline (H01) by (b) (6)
2016	B		1.00 In-House Nutrition Assessment Level 1 (NTR012) by (b) (6)
2016	B		17.00 Critical Care Level 2- Hours (CCU2) by (b) (6)
2016	B		.20 ml of DNULsix 50mg/ml/ML (T106) by (b) (6)
2016	B		1.00 Cared for by (b) (6) ((b) (6)) by (b) (6)
2016	B		1.00 Cared by (b) (6) - Cardiology (CDS) by (b) (6)
2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
2016	B		1.00 Taurine U of Wisc (via (b) (6) S16755) (L0245) by (b) (6)
2016	B		1.00 Taurine U of Wisc (via (b) (6) S16755) (L0245) by (b) (6)
2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
2016	B		1.00 Chemistry IV Renal Panel RBVH (CH25) by (b) (6)
2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
2016	B		1.00 Dilution Verification Catalyst CREA (CH11DV) by (b) (6)

(b) (6) C (b) (6)

EMERGENCY PHYSICAL EXAM - Closed May 10/2016
(b) (6)

Chief Complaint: Lethargic

History: Starting yesterday patient was noted to be lethargic and not herself. 4 other cats so difficult to say if she was eating but they think she was. Not sure about U/BM. Indoor only. Did not notice she was having issues breathing.

Other Medical Problems: None

Medications/Supplements: None

Environment: Indoor only

Vaccination Status:

Current Diet (Type):
- Frequency:
- Amount:

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Patient History Report

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Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Physical Examination:

S(ubjective): QAR, hydration WNL, BCS 7/9, pain score: 0/4

O(bjective):
Weight: 15.6 pounds
TPR: [temp - 93]F, [HR - 150] bpm, [RR - 60] bpm
EENT: clear AU/OU, no nasal discharge, normal cervical palpation, mm pink, moist/CRT<2 sec
INTEG: Hair coat ok
PLN: WNL
CV: Heart sounds muffled
RESP: Increased RE, dull lung sounds
GI: soft, nonpainful, no masses
UG: SF, WNL
M/S: amb x 4
Neuro: alert/appropriate, cranial nerves intact

Problems/Differential Diagnoses:
Dyspnea
Lethargy

Diagnostics:
Cursory ultrasound - mild to moderate amount of pleural effusion R>L
DV thoracic radiograph - cardiac silhouette difficult to visualize, pleural effusion
(b) (6) 2

Assessment:
12 yr SF DSH
1. Pleural effusion, dyspnea - r/o cardiac (HCM) vs neoplasia (lymphoma vs other)

Treatment:
12 mg Lasix IM at 10 PM
Place in O2 cage
Thoracocentesis - 25 mL clear to yellow fluid removed from the right side
Place IVC, 12 mg Lasix IV at 2 AM

Plan/Recommendations:
Discussed differentials for pleural effusion - cardiac vs neoplasia. Due to pleural effusion cannot tell on radiographs if this is cardiac over neoplastic. Rec thoracocentesis to make (b) (6) breath more comfortably - o consents. Rec echocardiogram in the morning to see if this is heart disease. If this is (b) (6) spoke about disease process and prognosis. If this is neoplasia owner's may decide to

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			stop. Discussed possibility of taking repeat radiographs tomorrow if needed.

(b) (6) R (b) (6) Tx Template (blank)- Old WTS - TENTATIVE

WARD TREATMENT SHEET

DATE: (b) (6)	WARD: **ccu**
LAST NAME: (b) (6)	CLINICIAN: (b) (6) (b) (6)
PATIENT NAME: (b) (6)	TRANSFER DOCTOR:
BREED: **Shorthair, Domestic** COLOR:**Black**	**LEGEND:**
AGE: **12 Yrs. 4 Mos.** SEX: **Spayed Female**	**O** = scheduled **X** = performed **D/C** = discontinued
WEIGHT: 15.6 pounds as of: 12/28/2009	
PROBLEM/WORKING DIAGNOSIS:	**inc** = increase **dec** = decrease ⊖ = not given
Pleural effusion	SURGERY:
ALERT: **increased RR**	IV CATHETER: CODE: **RED**

			Technician STAFF ID ==>																							
DR Staff ID	TREATMENTS	Call Parameters	8	9	10	11	N	1	2	3	4	5	6	7	8	9	10	11	M	1	2	3	4	5	6	7
	Temperature	<99, >102.5																								
	HR/MM/CRT	<140, >240																								
	RR/RE	>40, increased RE	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
	Pulse Quality	wk																								
	Assess Pain & Note Score	2																								
	WEIGH																									
	RER: 262 kcal/day Diet: 115 g EN BID - any -				O										O											
	Quantify Food Intake						O										O									
	Lasix 12 mg IV - ask		O																		O					
	O2 cage																									

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Primary Infusion	Additives	ml/hr	
		Daily Cumulative	
OBSERVATION COMMENTS ON REVERSE		**ADDITIONAL BAGS**	

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	AM SHIFT
	MID SHIFT
	OVER

TIME	Init. TECH	Temp	HR	Pulse qual.	RR/ effort	MM color	CRT	PCV/ ts	BG	Turn	Blood Pressure	Pain Score	BM	Urine	Vomit	Grams Consumed	Init. AA
8 a																	
9 a																	
10 a																	
11a																	
N																	
1 p																	
2 p																	
3 p																	
4 p																	
5 p																	
6 p																	
7 p																	
8 p																	
9 p																	
10 p																	
11 p																	
M																	
1 a																	
2 a																	
3 a																	
4 a																	
5 a																	
6 a																	
7 a																	

TIME	ADDITIONAL COMMENTS

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(b) (6)

Image: Thorax
Received via DICOM C-STORE on Sun May 08 22:41:15 EDT 2016.
Client First Name: (b) (6)
Client Last Name: (b) (6)
Patient Id: '(b) (6) '
Patient Name: (b) (6)
Patient DOB: 'Thu Jan 01 00:00:00 EST 2004'
Patient Sex: 'Female'

lack of energy
Reason for Visit: Emergency
Date Patient Checked Out: 05/09/16 Practice (b) (6)
Hospitalization Hours Smart Group (HOSPIT) by (b) (6)
0.00 Admission time was 10:10 PM (ADMTIME) by (b) (6)
3.00 Hospitalization Hours- Feline (H01) by (b) (6)
3.00 Critical Care Level 2- Hours (CCU2) by (b) (6)
1.00 Emergency Exam Level 4 (EE04) by (b) (6)
.24 ml of DNULsix 50mg/ml/ML (T106) by (b) (6)
1.00 Thoracocentesis Therapeutic (R33) by (b) (6)
Oxygen Therapy (Caged)/Hour (Group) (O2CAGE) by (b) (6)

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Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)			3.00 O2 Therapy Per Hour (T044) by (b) (6) 3.00 Oxygen-related Patient Care / Hour (O2CARE) by (b) (6) 1.00 Equipment Service & Preparation (USEQPT) by (b) (6) IV Catheter with Injection Cap (IVCATCP) by (b) (6) 1.00 IV Catheter Placement (CATH) by (b) (6) 1.00 each of Tx Catheter IV 20g x 2" Surflo (PINK) (H0112) by (b) (6) 1.00 each of Tx IV Ext T Set Hospira 1265028 (H027) by (b) (6) 1.00 each of Tx Injection Cap/Plug Termo 007110 (H118) by (b) (6) 1.00 Cared for by (b) (6) ((b) (6)) by (b) (6) Thorax Radiographic Study Group (RADTH) by (b) (6) 1.00 Radiograph Preparation (XFEE) by (b) (6) 1.00 One view rdgh stdy (RAD1V) by (b) (6) 1.00 Radiologist Review Fee (RADGN) by (b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
6/7/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 6/7/2016 12:33 On the phone with client discussing (b) (6), also discussed (b) (6) T4 and liver values need to be rechecked 6 weeks after his meds began to test for any needed dose adjustments. We can come to the home or he can schedule with a GP in the hospital. We discussed that he's enjoyed working with (b) (6) before.
6/6/2016	C	(b) (6)	MEDICAL COMMENTS 6/6/2016 11:47 FDA complaint submitted: Pet Food Safety Report, ID 54405, was successfully submitted on 6/6/2016 11:44:41 AM EST to the FDA, and it was issued an Individual Case Safety Report Number (ICSR) of 1053339
(b) (6)	TC	(b) (6)	MEDICAL COMMENTS - TENTATIVE (b) (6) 10:46 SW (b) (6) at Merrick - updated company that we have documented taurine deficiency in 2 other cats in house hold. The quality assurance team indicated that the level of taurine in the lot # I gave them was sufficient - discussed that this likely takes 3-6 month to develop and likely to be related to earlier lot and they need to investigate further. Asked if the level they gave me was from them retesting the food after I called or from an earlier test - not sure. Asked for information from the taurine tests - told I can send summary of lab results. Also indicated that I am reporting this to the FDA. She give new info to her manager and quality assurance. Told them I expect them to follow up with me. Below email sent to Merrick: Taurine Levels (b) (6) To: (b) (6) @merrickpetcare.com Hi (b) (6), Thank you for your help with these cases. Here is the summary of the lab results: 12yr female spayed domestic short hair diagnosed and clinical for dilated cardiomyopathy (b) (6)016 Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016 5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on

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Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016
-9yr male neutered domestic long hair: 196 nmol/ml
-8y female spayed domestic short hair: 368 nmol/ml
-9yr male neutered domestic long hair: 124 nmol/ml
-9yr male neutered domestic long hair: 536 nmol/ml

Please let me know if you have any other questions.

Sincerely,

(b) (6)

(b) (6)

5/31/2016 C (b) (6)

COMMUNICATIONS WITH CLIENT - Closed (b) (6)
5/31/2016 16:14
Spoke with husband, he confirmed email was received and (b) (6) and (b) (6) have begun taurine supplementation. Discussed results are a different normal range for the whole blood testing I did vs the plasma testing the cardiologist did on (b) (6). He notes (b) (6) had been getting some Fancy Feast so that likely explains why his values are top of the normal range. Discussed (b) (6) elevated globulins, need protein electrophoresis to better define the issue, can be chronic inflammatory response or a few types of cancer. If cancer, because they are RB clients they can consult with oncology at no charge to hear what the treatment options would be. They can bring (b) (6) into the office with a GP doctor for the bloodwork or I can do the bloodwork at the house. If I am doing it, I would not charge any recheck exam, just the travel and diagnostic test costs. If a GP in the hospital, they shouldn't have to charge for an exam either because she was just checked in late May. Owner likes (b) (6); advised he could schedule that with her. Discussed rechecking (b) (6) T4 and liver values 6 weeks after starting meds to check if dose needs adjusting; can be done at the house or in the office as well. Advised Nutrition is contacting Merrick about the taurine results and she feels the owner shouldn't have to pay for the taurine testing; she wants Merrick to have to pay for it directly. So I

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			told the owner that first and foremost, they are responsible for payment of the testing to (b) (6) and once we advise them of a charge, they would be required to pay it. If the Nutritionist is able to circumvent that by having Merrick pay us directly, that would be a nice advantage for the client. He understands.
5/31/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed Jun 02/2016 5/31/2016 15:39 LMOM on husband cell making sure they received my treatment advice in the email from over the weekend. Please call back or reply to email so I can be certain the treatment guidelines were received.
5/28/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 30/2016 5/28/2016 15:46 Email to client: Hi Mr and Mrs (b) (6) – I left a (long-winded) message on Mr (b) (6) voicemail earlier today. The nutritionist has since been in contact with me and advised that both (b) (6) and (b) (6) should be started on taurine supplementation. She recommends 250mg taurine twice daily for 2-3 weeks. Because you've already switched them to another diet, after 2-3 weeks, supplementation can be discontinued. (b) (6) and (b) (6) tested safely within the normal range for taurine, so they do not require any supplementation. I presume since you have already been treating (b) (6), you likely have a supply of taurine supplement. If not, feel free to contact me ((b) (6)) or the nutritionist or cardiologist to get a larger supply in order to treat the brothers. The nutritionist also advised she'll be contacting Merrick again now that the data has been received. Once she has heard more from them, she'll be in contact with you, as well. I also mentioned in the voicemail that (b) (6) blood test was repeated and verified that she does have elevated globulins. The most harmless reason would be chronic inflammation, but since she's been otherwise healthy, it is valuable to pursue further diagnostic inquiry. Unfortunately, elevated globulins can also indicate cancer, so we want to determine precisely what is happening with her. We can collect another blood sample from her at any time in order to perform a test called protein electrophoresis which further defines which specific immunoglobulins are elevated. You may choose to bring her into the office or have us out to the home again. (b) (6) will need repeat bloodwork after he's been on his thyroid supplement for 6 weeks, we could collect her second sample at that time as well, if

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

you choose.

Feel free to contact me with any questions. I will next be in the office on Tuesday May 31st.
Happy Memorial Day weekend –
(b) (6)

5/28/2016 C (b) (6)

COMMUNICATIONS WITH DOCTOR - Closed May 30/2016
5/28/2016 15:45
Email response from (b) (6) to (b) (6)
I'm also going to talk with (b) (6) this week about the cost of the taurine test. In my opinion this should be paid for by the company. I don't want the owner to pay the cost yet until I talk with (b) and the company again.

(b) (6)

From: (b) (6)
Sent: Saturday, May 28, 2016 2:16 PM
To: (b) (6)
Subject: RE: price for taurine test
There is a risk of deficiency with anything <200, so that's why I would go ahead and supplement both cats...and it's harmless:-)

From: (b) (6)
Sent: Saturday, May 28, 2016 2:13 PM
To: (b) (6)
Subject: RE: price for taurine test
It is really interesting...probably the same reason some puppies raised on an unbalanced home cooked diet never have issues and other do.

Great the diet has been changed. We should get the cats that tested low on some supplementation for 2-3 weeks just to cover our bases. 250mg taurine PO BID...if she needs to use a powder form and mix with the cats food that's fine

Let the owner know I will touch base with the company after Memorial day...I have not heard back from them yet. This will also give me much more to go on when reporting to the FDA...who know this might turn into a pet food recall (it should turn into a recall)!

Thank you so much for the update!

(b) (6)

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
			From: (b) (6) Sent: Saturday, May 28, 2016 1:55 PM To: (b) (6) Subject: RE: price for taurine test Hi (b) (6) – Thanks for providing this justification for the lab decision; I really appreciate it! I left you a voicemail earlier today – the results are in. 2 cats tested within the normal range [(b) (6) 368, (b) (6) 536 (300-600)]. (b) (6) was 196 and (b) (6) was way down at 124. All 4 cats were switched to Royal Canin food about 7 days ago. I left a voicemail for the client advising of the results, but told him I wanted your input before devising a treatment strategy. I would think of these 4, only (b) (6) would benefit substantially from taurine supplementation. I presume (b) (6) levels are sufficient now that he's been put on a properly formulated diet. Do you agree? This case is so interesting… how the cats fall all along the clinical spectrum, including some that have sufficient taurine, despite all eating the same presumably flawed diet. Thanks for your input, (b) (6)
5/28/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 30/2016 5/28/2016 13:44 LMOM - advised client taurine results have been received, I have notified nutrition dept who will weigh in on treatment decision-making. Normal range is 300-600 and (b) (6) and (b) (6) tested within that range. Clinical signs are unlikely above 200, (b) (6) was 196, so it is likely he wouldn't show any issues. (b) (6) tested at 124 so he might be the one to benefit from additional supplementation, aside from just the diet change to the Royal Canin food. We will wait to initiate any therapy until the nutritionist has a chance to comment; we are working as a team on this. Since we have results, we likely have an invoice from the lab as well, so we should be able to advise of the cost of this testing in the short-term. I had spoken with his wife about (b) (6) having elevated globulins and on the re-test that status persists, was verified. Recommend additional blood testing for further work-up, could be collected when we visit (b) (6) for bloodwork 6 weeks after starting his thyroid meds. Please call back to discuss these results.
5/27/2016	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE 5/27/2016 15:32 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
5/24/2016	P	(b) (6)	100.00 tablet of Tapazole (Methimazole) 5mg Tablet (M066) Rx #: 2576298 0 Of 0 Refills Give 1 tablet by mouth twice daily. Check bloodwork for dose adjustment 6 weeks after starting medication.
5/24/2016	C		COMMUNICATIONS WITH CLIENT - Closed May 26/2016 5/24/2016 16:41 Spoke with Mrs; advised hyperthyroid with some liver elevations. Reviewed life-long treatment, bloodwork 6 weeks after med started and then twice yearly if stable. If dose is changed after first bloodwork, we repeat bloodwork again 6 weeks later until properly regulated. Med can be tablet, liquid or transdermal. Owner wants to crush tablet into canned food; advised this is fine as long as we're certain he's the only one who might consume the medicated food within their group-housing situation. Owner feels she can guarantee that. Meds will be at 197 pharmacy. Taurine pending, will call. Advised final pricing on taurine at (b) (6) lab not yet determined, will be in touch with that info as soon as finalized. Owner asked why use a diff lab; advised nutritionists recommended this lab, specialized testing at university, two labs finding low levels strengthens case against food company.
5/24/2016	B	(b) (6)	100.00 tablet of Tapazole (Methimazole) 5mg Tablet (M066) by (b) (6)
5/24/2016	B		1.00 Cared for by (b) (6) (RHS) by (b) (6)
5/21/2016	C		GP PHYSICAL EXAM - Closed May 26/2016 Date Presented: 5/21/2016 Chief Complaint: Weight loss/Taurine check History: Owner notes chronic weight loss across the recent months. Was losing hair for over a year, but was told it was related to anxiety. Eats with voracious appetite. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; he is one of 4 cats that live together in a room above the garage. Indoor only. (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 - slightly feisty O: MM / ORPH: Pink, moist, crt <2 sec, mild tartar E/E: mild black debris in outer cartilages of left ear, deep canal WNL, right ear WNL. ophtho WNL.

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
			INT: alopecia caudal dorsum, ventrum, lateral thighs. no ectoparasites observed. PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses MS/NS: Normal amb x4 BCS: 1.5-2/5 4.4kg A: 9yr9mo MN DLH 1) weight loss - r/o hyperthyroid, diabetes mellitus, organ dz (kidney, liver), other endocrinopathy, neoplasia, nutritional problem 2) alopecia - r/o FAD, other derm issue, psychogenic (stress, pain-related) 3) dental disease 4) otic debris - r/o infection vs inadequate grooming P: PE Taurine level CBC/Superchem/T4 Advised client of marked weight loss from last documented weight. Systemic bloodwork may illuminate the reason; will call with results next week. Taurine level will take 7-10 days. Advised client we are sending taurine test to a different lab than the one that tested (b) (6) sample, at the advice of the nutrition service. We do not have a price in our computer system for this test through this lab, so the client will be invoiced for the taurine level (for all 4 cats) once that is established. Client paid today's services during the visit and is aware of the pending charge; advised the (b) (6) charge was $214 and the charge at the other lab will likely be within $50 under/over that fee. He commits to paying taurine test fees once advised of final fee. Stated we want to submit samples for testing ASAP and he understands fee structure will not be set until after tests are underway.
5/21/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 23/2016 5/21/2016 16:25 mailed welcome card, magnet, Rabies certificates (b) (6) , (b) (6)) and feedback postcard
5/21/2016	V	(b) (6)	May 21, 2016 11:21 AM Staff: (b) (6) Weight : 4.40 kilograms HC-RS scale
5/21/2016	L		**Hematology results from** (b) (6) **ID: 209396 Posted Final**

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

Test	Result	Reference Range
HCT	42 %	29 – 48
HGB	15.0 g/dL	9.3 – 15.9
MCHC	35.7 g/dL	30 – 38
WBC	14.2 10^3/uL	3.5 – 16.0
Bands	0 %	0 – 3
RBC	9.5 10^6/uL	5.92 – 9.93
MCV	44 fL	37 – 61
MCH	15.8 pg	11 – 21
ABS BASO	0 /uL	0 – 150
ABS NEUTB	0 /uL	0 – 150
Platelet C	254 10^3/uL	200 – 500
Platelet E	ADEQUATE	ADEQUATE –
Neutrophil	53 %	35 – 75
Lymphocyte	41 %	20 – 45
Monocytes	2 %	1 – 4
Eosinophil	4 %	2 – 12
Basophils	0 %	0 – 1
Absolute N	7526 /uL	2500 – 8500
Absolute L	5822 /uL	1200 – 8000
Absolute M	284 /uL	0 – 600
Absolute E	568 /uL	0 – 1000

Ascn: (b) (6) Profile: Complete Blood Count

5/21/2016 L

Chemistry results from (b) (6)
ID: 209396 Posted Final

Test	Result	Reference Range
ALB	3.4 g/dL	2.5 – 3.9
ALKP	174 U/L H	6 – 102
ALT	243 U/L H	10 – 100
AMYL	882 U/L	100 – 1200
AST	46 U/L	10 – 100
BUN/UREA	17 mg/dL	14 – 36
Ca	9.2 mg/dL	8.2 – 10.8
Chloride	111 mEq/L	104 – 128
CHOL	181 mg/dL	75 – 220
CK	124 U/L	56 – 529
CREA	0.6 mg/dL	0.6 – 2.4
GGT	3 U/L	1 – 10
GLU	80 mg/dL	64 – 170
Mg	1.7 mEq/L	1.5 – 2.5
PHOS	5.9 mg/dL	2.4 – 8.2
Potassium	4.5 mEq/L	3.4 – 5.6
Sodium	150 mEq/L	145 – 158
TBIL	0.1 mg/dL	0.1 – 0.4
TP	5.9 g/dL	5.2 – 8.8
TRIG	58 mg/dL	25 – 160
GLOB	2.5 g/dL	2.3 – 5.3

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

```
A/G Ratio      1.4 Ratio            0.35 - 1.5
B/C Ratio      28 Ratio             4 - 33
Na/K Ratio     33
```

5/21/2016 L

```
Endocrinology results from (b) (6)
                          ID:  209396      Posted      Final
Test           Result                  Reference Range
T4             20.2 ug/dL H            0.8 - 4.0
 Ascn:          (b) (6)  Profile: Total T4

 Result verified.
```

5/21/2016 L

```
Miscellaneous results from (b) (6)
                          ID:  209396      Posted      Final
Ascn:          (b) (6)  Profile: Superchem
RE: 1045 PrecisionP 28 U/L 8 - 26
PresisionPSL elevations correlate closely with abnormal PLI
concentrations. In
cats with appropriate clinical signs, this PrecisionPSL is
supportive of, but
not definitive, for a diagnosis of pancreatitis. In cats
without clinical signs
of pancreatitis, a mild elevation is an insignificant
finding.
RE: 11067 Comment
Hemolysis 1+ No significant interference.
```

5/21/2016	B	(b) (6)
5/21/2016	B	
5/21/2016	B	
5/21/2016	B	
5/21/2016	B	
5/21/2016	B	
5/21/2016	B	
5/21/2016	B	

1.00 Superchem Cbc T4 (b) (6) Sa120 (L85) by (b) (6)
1.00 House Call Travel Level 2 (HC06) by (b) (6)
1.00 At Home Appointment (HC04) by (b) (6)
Laboratory Request / Sample Handling (LABS) by (b) (6)
1.00 Outside Lab (XTBALUO) by (b) (6)
1.00 Sample Handling & Disposal (LFEE) by (b) (6)
1.00 Lab Sample Label (b) (6) by (b) (6)
1.00 Cared for by (b) (6) (b) (6) by (b) (6)

5/20/2016 C

COMMUNICATIONS WITH CLIENT - Closed May 21/2016
5/20/2016 15:00
Called to confirm tomorrow's appointment fro (b) (6) , (b) (6) , (b) (6) and (b) (6) at 9 am. I also mentioned in my message that we should use the address (b) (6) in the GPS. If any questions please call (b) (6)

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
5/17/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 19/2016 5/17/2016 12:29 Responding to owner's message, booked (b) (6), (b) (6), (b) (6), (b) (6) for House Call on Saturday 5/21. (b) (6) was seen on emergency and diagnosed with low taurine, so all cats need to be screened. Had been eating Merrick dry food. Cats are kept in a finished room above the garage; he thinks they won't need to be confined/isolated more than that in order to work on them. Discussed senior bloodwork as well. He notes this emergency with (b) (6) was a wake-up call and he'd like to thoroughly have everyone checked out. (b) (6) is losing weight. (b) (6) and (b) (6) have been to another vet and have a current Rabies; (b) (6) and (b) (6) haven't been to the vet in a long time. Discussed PureVax 1yr vs 3yr vs standard RabVac, vaccine-associated sarcoma issue - owner wants the purified vaccine, prefers the one year since they should be examined annually anyway. Advised if (b) (6) status progresses and we need to be checking her as well, please call to inform us in case we need special items/supplies for her care. Owner notes we should use (b) (6) with the GPS; his home address was renamed/renumbered a few years ago, but GPS cannot often find (b)

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
6/7/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 6/7/2016 12:25 Spoke with owner, advised of specialist's comments about protein electrophoresis. Can conduct further testing through our Internal Medicine service of ultrasounds of chest and abdomen, essentially searching for origin of chronic inflammation. It is possible that testing will come back normal, despite the bloodwork indicating the elevated globulins. Another option is to track her body weight at home once monthly and report any weight loss promptly; if none noted, recheck bloodwork in 3 months to see if globulins are resolved. Specific cancers cause specific spikes and her results do not show those elevations, so that is good news. But we don't have a definite reason for her chronic inflammation. Owner understands and will relate to wife.
6/7/2016	TC	(b) (6)	COMMUNICATIONS WITH DOCTOR - TENTATIVE 6/7/2016 12:11 Spoke with (b) (6) at (b) (6) ; she notes monoclonal globulin spikes are most concerning because they define lymphoma, myeloma. Polyclonal spikes can be FIP, but also any cause of chronic inflammation. There is a chance chronic inflammation can be associated with neoplastic process though. She notes the degree of elevation is mild. If the owners want to work this up aggressively, full body imaging, best with ultrasound, to search for cancer. If they would like to monitor, recheck chemistry panel in 3 months and assess globulin count at that time. Only repeat electrophoresis if significantly higher elevation. If pet is losing weight, neoplasia moves up the list of differentials. Advised chronic otitis externa in this otherwise healthy patient; doctor concurs that wouldn't be sufficient to cause systemic response.
(b) (6)	L		**Chemistry results from** (b) (6) **ID: 209396 Posted Final** **Test Result Reference Range** **ALB 2.9 g/dL 2.5 – 3.9** **TP 8.2 g/dL 5.2 – 8.8** **GLOB 5.3 g/dL 2.3 – 5.3** **ALPHA 1 0.3 g/dL 0.2 – 1.1** **ALPHA 2 0.7 g/dL 0.4 – 0.9** **BETA 0.6 g/dL 0.3 – 0.9** **GAMMA 3.6 g/dL H 0.3 – 2.5**
(b) (6)	L		**Miscellaneous results from** (b) (6)

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			(b) (6) **ID: 209396 Posted Final** **Ascn:** (b) (6) **Profile: Protein Electrophoresis,Serum** **RE: 1140 Interpreta** **The gamma globulin fraction is elevated, characterized by a broad polyclonal band, resulting from a mixture of increased immunoglobulins associated with an immune response. Potential causes include suppurative disease, chronic infectious disease (bacterial; protozoal; viral; rickettsial; fungal), connective tissue disease, chronic granulomatous disease, etc. Correlate with clinical findings.** **PATHOLOGIST:** (b) (6) **, BVSc (Hons 1), DACVP** (b) (6) **Due to difference in method of analysis, there may be slight differences in the quantitative albumin and calculated globulin results between serum electrophoresis results compared to a generalchemistry panel.**
(b) (6)	C	(b) (6)	MEDICAL COMMENTS - Closed Jun 04/2016 (b) (6) 18:35 Drew sample for protein electrophoresis while at the home for EOL care for b) (6) .
(b) (6)	B	(b) (6)	1.00 House Call Travel Level 2 (HC06) by (b) (6)
	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
	B		1.00 Outside Lab (XTBALUO) by (b) (6)
	B		1.00 Protein Electrophor. Serum (b) (6) T240 (L018) by (b) (6)
	B		1.00 Sample Handling & Disposal (LFEE) by (b) (6)
	B		1.00 Lab Sample Label (TL) by (b) (6)
	B		1.00 Cared for by (b) (6) (RHS) by (b) (6)
(b) (6)	C	RHS	COMMUNICATIONS WITH CLIENT - Closed Jun 02/2016 (b) (6) 16:21 (See full phone call under (b) (6) record)

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Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			Discussed (b) (6) elevated globulins, need protein electrophoresis to better define the issue, can be chronic inflammatory response or a few types of cancer. If cancer, because they are (b) (6) clients they can consult with oncology at no charge to hear what the treatment options would be. They can bring (b) (6) into the office with a GP doctor for the bloodwork or I can do the bloodwork at the house. If I am doing it, I would not charge any recheck exam, just the travel and diagnostic test costs. If a GP in the hospital, they shouldn't have to charge for an exam either because she was just checked in late May. Owner likes (b) (6); advised he could schedule that with her.
5/27/2016	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE 5/27/2016 15:36 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)
5/24/2016	C	(b) (6)	COMMUNICATIONS WITH DOCTOR - Closed May 26/2016 5/24/2016 16:48 Spoke with doctor at (b) (6) consult line - she opted to rerun the full chemistry profile to validate the results since (b) (6) remaining profile is so normal. If globulins are truly elevated, protein electrophoresis is the next step. Ddx: myeloma, lymphoma, FIP, other neoplasia, chronic inflammatory condition. Asked specifically about taurine based on (b) (6) and current investigation into whole household's taurine status; not aware of any relationship between globulins and taurine.
5/24/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 26/2016 5/24/2016 16:46 Spoke with Mrs; (b) (6) has elevated globulins which can indicate cancer or a chronic inflammatory condition. Spoke with specialist and no correlation with taurine deficiency. Lab is going to re-run her full profile to validate the results. Expect an update in 1-2 days. If verified, we may need to collect additional blood for the next level of testing which tells us which specific pattern of globulins is elevated. Taurine pending, will call.
5/21/2016	C	(b) (6)	GP PHYSICAL EXAM - Closed May 26/2016 Date Presented: 5/21/2016 Chief Complaint: Wellness/Taurine check History: Doing well. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; she is one of 4 cats that live together in a room above the

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			garage. Indoor only. (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 O: MM / ORPH: Pink, moist, crt <2 sec, small suspect FORL right upper PM3, mild tartar overall. E/E: copious black debris AU, mildly pruritic while cleaning. ophtho WNL. INT: WNL; no evidence of ectoparasites observed PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses MS/NS: Normal amb x4 BCS: 3/5 4.15kg A: 8yr FS DSH 1) otitis externa - r/o bacterial/fungal vs ear mites 2) dental disease P: PE Taurine level CBC/Vetscreen Disp Tresaderm 7.5ml - apply 2-3 drops in each ear twice daily for 7-10 days, keep in fridge ear cleaning PureVax Rabies 1yr SQ right hind (lot#17390B, exp 12/11/2016) Discussed ear infection and treatment. Will call with lab results; systemic early next week, taurine in 7-10 days.
5/21/2016	I	(b) (6)	An animal is not considered immunized for at least 28 days after the initial or primary vaccination is administered. For this reason, pets receiving their first rabies vaccine should not be left outdoors unattended.
5/21/2016	P		1.00 bottle of Tresaderm 7.5ml (Merial] (M225) Rx #: 2574865 0 Of 0 Refills Apply 2-3 drops in each ear twice daily for 7-10 days.
5/21/2016	V		May 21, 2016 11:15 AM Staff: (b) (6) Weight : 4.15 kilograms HC-RS scale
5/21/2016	L		**Hematology results from** (b) (6) **ID: 209396 Posted Final** **Test Result Reference Range**

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

HCT	31 %	29 - 48
HGB	11.0 g/dL	9.3 - 15.9
MCHC	35.5 g/dL	30 - 38
WBC	13.4 10^3/uL	3.5 - 16.0
Bands	0 %	0 - 3
RBC	6.7 10^6/uL	5.92 - 9.93
MCV	46 fL	37 - 61
MCH	16.4 pg	11 - 21
ABS BASO	0 /uL	0 - 150
ABS NEUTB	0 /uL	0 - 150
Platelet C	375 10^3/uL	200 - 500
Platelet E	ADEQUATE	ADEQUATE -
Neutrophil	55 %	35 - 75
Lymphocyte	37 %	20 - 45
Monocytes	2 %	1 - 4
Eosinophil	6 %	2 - 12
Basophils	0 %	0 - 1
Absolute N	7370 /uL	2500 - 8500
Absolute L	4958 /uL	1200 - 8000
Absolute M	268 /uL	0 - 600
Absolute E	804 /uL	0 - 1000

Ascn: (b) (6) Profile: Complete Blood Count

5/21/2016 L

Chemistry results from (b) (6)
ID: 209396 Posted Final

Test	Result	Reference Range
ALB	2.6 g/dL	2.5 - 3.9
ALKP	18 U/L	6 - 102
ALT	14 U/L	10 - 100
AST	11 U/L	10 - 100
BUN/UREA	29 mg/dL	14 - 36
Ca	8.8 mg/dL	8.2 - 10.8
Chloride	113 mEq/L	104 - 128
CHOL	94 mg/dL	75 - 220
CK	62 U/L	56 - 529
CREA	1.2 mg/dL	0.6 - 2.4
GLU	79 mg/dL	64 - 170
PHOS	5.7 mg/dL	2.4 - 8.2
Potassium	4.8 mEq/L	3.4 - 5.6
Sodium	148 mEq/L	145 - 158
TBIL	0.1 mg/dL	0.1 - 0.4
TP	9.1 g/dL H	5.2 - 8.8
GLOB	6.5 g/dL H	2.3 - 5.3
A/G Ratio	0.4 Ratio	0.35 - 1.5
B/C Ratio	24 Ratio	4 - 33
Na/K Ratio	31	

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/21/2016	L		**Miscellaneous results from** (b) (6) **ID: 209396 Posted Final** **Ascn:** (b) (6) **Profile: Vet Screen** **RE: 11067 Comment** **Hemolysis 1+ No significant interference.**
5/21/2016	B	(b) (6)	1.00 At Home Additional Pet Appointment (HC03) by (b) (6)
5/21/2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016	B		1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016	B		1.00 Vetscreen Cbc Antec SA030 (L00030) by (b) (6)
5/21/2016	B		1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016	B		1.00 Lab Sample Label (TL) by (b) (6)
5/21/2016	B		1.00 Fel Vax Rabies 1 Year Purevax (Merial) (V21) by (b) (6)
5/21/2016	B		1.00 bottle of Tresaderm 7.5ml (Merial] (M225) by (b) (6)
5/21/2016	B		1.00 Cared for by (b) (6) ((b) (6)) by (b) (6)
5/20/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 21/2016 5/20/2016 15:04 Called to confirm tomorrow's appointment fro (b) (6), (b) (6), (b) (6) and (b) (6) at 9 am. I also mentioned in my message that we should use the address (b) (6) in the GPS. If any questions please call (b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History
(b) (6)	TC	(b) (6)	MEDICAL COMMENTS - TENTATIVE (b) (6) 10:46 SW (b) (6) at Merrick - updated company that we have documented taurine deficiency in 2 other cats in house hold. The quality assurance team indicated that the level of taurine in the lot # I gave them was sufficient - discussed that this likely takes 3-6 month to develop and likely to be related to earlier lot and they need to investigate further. Asked if the level they gave me was from them retesting the food after I called or from an earlier test - not sure. Asked for information from the taurine tests - told I can send summary of lab results. Also indicated that I am reporting this to the FDA. She give new info to her manager and quality assurance. Told them I expect them to follow up with me. Below email sent to Merrick: Taurine Levels (b) (6) To: (b) (6) @merrickpetcare.com Hi (b) (6) , Thank you for your help with these cases. Here is the summary of the lab results: 12yr female spayed domestic short hair diagnosed and clinical for dilated cardiomyopathy (b) (6) 2016 Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016 5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016 -9yr male neutered domestic long hair: 196 nmol/ml -8y female spayed domestic short hair: 368 nmol/ml -9yr male neutered domestic long hair: 124 nmol/ml -9yr male neutered domestic long hair: 536 nmol/ml Please let me know if you have any other questions. Sincerely, (b) (6) (b) (6) Clinical Nutrition Department

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient:
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico
Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History
			(b) (6)
5/27/2016	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE 5/27/2016 15:34 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)
5/24/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 26/2016 5/24/2016 16:50 Spoke with Mrs; systemic blood results WNL for (b) (6). Taurine pending.
5/21/2016	C	(b) (6)	GP PHYSICAL EXAM - Closed May 26/2016 Date Presented: 5/21/2016 Chief Complaint: Wellness/Taurine check History: Doing well. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; he is one of 4 cats that live together in a room above the garage. Indoor only. (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 O: MM / ORPH: Pink, moist, crt <2 sec, moderate tartar overall E/E: ophtho/otoscopic exams WNL INT: no evidence of ectoparasites observed. matted hair present. PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses MS/NS: Normal amb x4 BCS: 4/5 9.5kg A: 9yr9mo MN DLH 1) overweight 2) dental disease

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient:
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico
Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History

3) matted hair
P: PE
Taurine level
CBC/Vetscreen

Recommend weight loss, frequent brushing +/- clippers to remove mats or using a groomer. Dental condition warrants treatment. Will call with systemic blood results early next week, taurine level in 7-10 days.

5/21/2016 V (b) (6)

May 21, 2016 11:21 AM Staff: (b) (6)

Weight : 9.50 kilograms
HC-RS scale

5/21/2016 L (b) (6)

Hematology results from (b) (6)
ID: 209396 Posted Final

Test	Result	Reference Range
HCT	40 %	29 - 48
HGB	12.3 g/dL	9.3 - 15.9
MCHC	30.8 g/dL	30 - 38
WBC	11.6 10^3/uL	3.5 - 16.0
Bands	0 %	0 - 3
RBC	7.9 10^6/uL	5.92 - 9.93
MCV	51 fL	37 - 61
MCH	15.6 pg	11 - 21
ABS BASO	0 /uL	0 - 150
ABS NEUTB	0 /uL	0 - 150
Platelet C	188 10^3/uL L	200 - 500
Platelet E	ADEQUATE	ADEQUATE -
Neutrophil	72 %	35 - 75
Lymphocyte	21 %	20 - 45
Monocytes	3 %	1 - 4
Eosinophil	4 %	2 - 12
Basophils	0 %	0 - 1
Absolute N	8352 /uL	2500 - 8500
Absolute L	2436 /uL	1200 - 8000
Absolute M	348 /uL	0 - 600
Absolute E	464 /uL	0 - 1000

Ascn: (b) (6) **Profile: Complete Blood Count Ascn:** (b) (6) **Profile: Complete Blood Count**

Platelet count reflects the minimum number due to platelet clumping.

5/21/2016 L (b) (6)

Chemistry results from (b) (6)

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient:
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico
Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History

ID: 209396 Posted Final

Test	Result	Reference Range
ALB	3.1 g/dL	2.5 – 3.9
ALKP	27 U/L	6 – 102
ALT	64 U/L	10 – 100
AST	44 U/L	10 – 100
BUN/UREA	26 mg/dL	14 – 36
Ca	9.3 mg/dL	8.2 – 10.8
Chloride	112 mEq/L	104 – 128
CHOL	98 mg/dL	75 – 220
CK	157 U/L	56 – 529
CREA	1.2 mg/dL	0.6 – 2.4
GLU	90 mg/dL	64 – 170
PHOS	5.9 mg/dL	2.4 – 8.2
Potassium	5.1 mEq/L	3.4 – 5.6
Sodium	150 mEq/L	145 – 158
TBIL	0.1 mg/dL	0.1 – 0.4
TP	8.4 g/dL	5.2 – 8.8
GLOB	5.3 g/dL	2.3 – 5.3
A/G Ratio	0.6 Ratio	0.35 – 1.5
B/C Ratio	22 Ratio	4 – 33
Na/K Ratio	29	

5/21/2016 L (b) (6)

Miscellaneous results from (b) (6)
ID: 209396 Posted Final
Ascn: (b) (6) **Profile: Vet Screen**
RE: 11067 Comment
Hemolysis 1+ No significant interference.
Ascn: (b) (6) **Profile: Vet Screen**
RE: 11067 Comment
Hemolysis 1+ No significant interference.

(b) (6)

5/21/2016 B 1.00 At Home Additional Pet Appointment (HC03) by (b) (6)
5/21/2016 B Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016 B 1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016 B 1.00 Vetscreen Cbc Antec SA030 (L00030) by (b) (6)
5/21/2016 B 1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016 B 1.00 Lab Sample Label ((b) (6) by (b) (6)
5/21/2016 B 1.00 Cared for by (b) (6) ((b) (6) by (b) (6)

5/20/2016 C (b) (6) COMMUNICATIONS WITH CLIENT - Closed May 21/2016
5/20/2016 15:04
Called to confirm tomorrow's appointment fro (b) (6) , (b) (6) , (b) (6) and (b) (6) at 9

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient:
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History
			am. I also mentioned in my message that we should use the address (b) (6) in the GPS. If any questions please call (b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
6/7/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 6/7/2016 12:32 While speaking with owner about (b) (6), discussed (b) (6) dental. Spends the day at 197, but most often home that same night after procedure. Bloodwork is good for 2 months. Can schedule with GP or dentistry according to owner's preference.
5/27/2016	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE 5/27/2016 15:38 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)
5/24/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 26/2016 5/24/2016 16:51 Spoke with Mrs; bloodwork WNL, excellent news for planning anesthesia and dental work. Important that taurine status is addressed prior to anesthesia, but dental work should be planned for the next 4-8 weeks. Taurine pending, will call.
5/21/2016	C	(b) (6)	GP PHYSICAL EXAM - Closed May 26/2016 Date Presented: 5/21/2016 Chief Complaint: Wellness/Taurine check History: Doing well. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; he is the one cat who lives in the house ((b) (6) is aggressive toward (b) (6), so he lives away from other cats). Indoor only. (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 O: MM / ORPH: Pink, moist, crt <2 sec; right upper canine tooth loose, significant gingivitis locally. heavy tartar on PM3s bilaterally. missing incisors. E/E: brown debris in outer ear cartilages bilaterally, but canals clean/free of debris. ophtho exam WNL. INT: matted hair. no evidence of ectoparasites observed. PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

MS/NS: Normal amb x4
BCS: 3-3.5/5 6.7kg
A: 9yr7mo MN DLH
1) dental disease
2) matted hair
P: PE
Taurine level
CBC/Superchem
PureVax Rabies 1yr SQ right hind (lot# 17390B, exp 12/11/2016)

Advised dental status is poor and likely painful; recommend prompt dental cleaning under general anesthesia with extraction of canine +/- other teeth. Can use dental specialists or general practitioner depending on owner's preference. Recommend waiting for taurine level and any management pertaining to that issue before scheduling anesthetic procedure. Can be shaved down during anesthesia; frequent brushing +/- intermittent clipping or taking to a groomer is needed. Will call with systemic blood results early next week; taurine level will take 7-10 days.

5/21/2016 I (b) (6)

An animal is not considered immunized for at least 28 days after the initial or primary vaccination is administered. For this reason, pets receiving their first rabies vaccine should not be left outdoors unattended.

5/21/2016 V (b) (6)

May 21, 2016 11:24 AM Staff: (b) (6)

Weight : 6.70 kilograms
HC-RS scale

5/21/2016 L

Hematology results from (b) (6)
ID: 209396 Posted Final

Test	Result	Reference Range
HCT	36 %	29 - 48
HGB	11.8 g/dL	9.3 - 15.9
MCHC	32.8 g/dL	30 - 38
WBC	9.8 10^3/uL	3.5 - 16.0
Bands	0 %	0 - 3
RBC	7.9 10^6/uL	5.92 - 9.93
MCV	46 fL	37 - 61
MCH	14.9 pg	11 - 21
ABS BASO	0 /uL	0 - 150
ABS NEUTB	0 /uL	0 - 150
Platelet C	490 10^3/uL	200 - 500
Platelet E	ADEQUATE	ADEQUATE -
Neutrophil	59 %	35 - 75
Lymphocyte	33 %	20 - 45
Monocytes	2 %	1 - 4
Eosinophil	6 %	2 - 12

Patient History Report

Client: (b) (6)
Phone:
Address:

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

Basophils	0 %	0 – 1
Absolute N	5782 /uL	2500 – 8500
Absolute L	3234 /uL	1200 – 8000
Absolute M	196 /uL	0 – 600
Absolute E	588 /uL	0 – 1000

Ascn: (b) (6) Profile: Complete Blood Count

5/21/2016 L

Chemistry results from (b) (6)
ID: 209396 Posted Final

Test	Result	Reference Range
ALB	3.8 g/dL	2.5 – 3.9
ALKP	25 U/L	6 – 102
ALT	32 U/L	10 – 100
AMYL	1067 U/L	100 – 1200
AST	14 U/L	10 – 100
BUN/UREA	32 mg/dL	14 – 36
Ca	9.9 mg/dL	8.2 – 10.8
Chloride	112 mEq/L	104 – 128
CHOL	125 mg/dL	75 – 220
CK	76 U/L	56 – 529
CREA	1.3 mg/dL	0.6 – 2.4
GGT	1 U/L	1 – 10
GLU	99 mg/dL	64 – 170
Mg	2.2 mEq/L	1.5 – 2.5
PHOS	5.5 mg/dL	2.4 – 8.2
Potassium	5.1 mEq/L	3.4 – 5.6
Sodium	150 mEq/L	145 – 158
TBIL	0.1 mg/dL	0.1 – 0.4
TP	7.8 g/dL	5.2 – 8.8
TRIG	97 mg/dL	25 – 160
GLOB	4.0 g/dL	2.3 – 5.3
A/G Ratio	1.0 Ratio	0.35 – 1.5
B/C Ratio	25 Ratio	4 – 33
Na/K Ratio	29	

5/21/2016 L

Miscellaneous results from
ID: 209396 Posted Final
(b) (6) Profile: Superchem
RE: 1045 PrecisionP 17 U/L 8 – 26
Acute pancreatitis is unlikely. Chronic pancreatitis is not excluded by a
normal PrecisionPSL.
RE: 11067 Comment
Hemolysis 1+ No significant interference.

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
5/21/2016	B	(b) (6)	1.00 At Home Additional Pet Appointment (HC03) by (b) (6)
5/21/2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016	B		1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016	B		1.00 Superchem Cbc (b) (6) Sa020 (L07) by (b) (6)
5/21/2016	B		1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016	B		1.00 Lab Sample Label (b) (6) by (b) (6)
5/21/2016	B		1.00 Fel Vax Rabies 1 Year Purevax (Merial) (V21) by (b) (6)
5/21/2016	B		1.00 Cared for by (b) (6) ((b) (6) by (b) (6)
5/20/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 21/2016 5/20/2016 15:05 Called to confirm tomorrow's appointment fro (b) (6) , (b) (6) , (b) (6) and (b) (6) at 9 am. I also mentioned in my message that we should use the address (b) (6) in the GPS. If any questions please call (b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

14770

Sample Submission Form

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/labs/aal/index.cfm

Vet/Tech Cont (b) (6)
Company Nam
Address:
Email: (b) (6)
Tel: **Fax:** (b) (6)

Billing C (b) (6) **TAX ID:**
Email: **Tel:** (b) (6)

Patient Name: (b) (6)
Species: Feline
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☒ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☐ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 368 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

14769

Sample Submission Form

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
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Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/labs/aal/index.cfm

Vet/Tech Conta (b) (6)
Company Name
Address:

Email: (b) (6)
Tel: **Fax:** (b) (6)

Billing (b) (6) **TAX ID:**
Email: **Tel:** (b) (6)

Patient Name: (b) (6)
Species: Feline
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☑ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☐ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 124 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

14771

Sample Submission Form

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/labs/aal/Index.cfm

Vet/Tech Contact: (b) (6)
Company Name:
Address:

Email: (b) (6)
Tel: **Fax:** (b) (6)

Billing Co (b) (6) **TAX ID:**
Email: **Tel:** (b) (6)

Patient Name: (b) (6)
Species: Feline
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☒ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☐ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood 536 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150