Hindawi Publishing Corporation
International Journal of Genomics
Volume 2015, Article ID 204823, 13 pages
http://dx.doi.org/10.1155/2015/204823

*Review Article*

# Genetics of Human and Canine Dilated Cardiomyopathy

**Siobhan Simpson,[1] Jennifer Edwards,[1] Thomas F. N. Ferguson-Mignan,[1] Malcolm Cobb,[1] Nigel P. Mongan,[1,2] and Catrin S. Rutland[1]**

[1] *Faculty of Medicine and Health Sciences, School of Veterinary Medicine and Science, The University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK*
[2] *Department of Pharmacology, Weill Cornell Medical College, 1300 York Avenue, New York, NY 10065, USA*

Correspondence should be addressed to Nigel P. Mongan; nigel.mongan@nottingham.ac.uk and Catrin S. Rutland; catrin.rutland@nottingham.ac.uk

Received 28 May 2015; Accepted 23 June 2015

Academic Editor: Giulia Piaggio



Cardiovascular disease is a leading cause of death in both humans and dogs. Dilated cardiomyopathy (DCM) accounts for a large number of these cases, reported to be the third most common form of cardiac disease in humans and the second most common in dogs. In human studies of DCM there are more than 50 genetic loci associated with the disease. Despite canine DCM having similar disease progression to human DCM studies into the genetic basis of canine DCM lag far behind those of human DCM. In this review the aetiology, epidemiology, and clinical characteristics of canine DCM are examined, along with highlighting possible different subtypes of canine DCM and their potential relevance to human DCM. Finally the current position of genetic research into canine and human DCM, including the genetic loci, is identified and the reasons many studies may have failed to find a genetic association with canine DCM are reviewed.

## 1. Dilated Cardiomyopathy Aetiology and Epidemiology

Cardiovascular disease is the fourth most common cause of death in dogs [1] and one of the most common causes of death in humans [2]. Dilated cardiomyopathy (DCM) is the second most prevalent form of heart disease in dogs, accounting for 10% of cardiac diagnoses [3], and is estimated to be the third most common inherited type of heart disease in humans, reported to affect 35.6 in 100,000 people, although this is thought to be an underestimation [4, 5].

Due to the similar nature of DCM in humans and dogs in terms of disease phenotype and progression, it has been suggested that canine DCM can act as a model for human DCM [6]. Conversely, knowledge obtained from the clinical management of people with DCM may guide improvements in the clinical care and outcomes of companion animals with DCM.

Animal models of DCM are useful in providing insights into the molecular and cellular progression of the disease and thus lead to potential new treatments [7]. While there are many animal models where DCM is induced, for example [8–11], naturally occurring cases of canine DCM are also valuable, in particular with regard to natural disease progression, especially where the underlying cause can be shown to be similar in dogs and people (e.g., similar genetic function) [12]. In addition to providing a potential natural model for human DCM, canine cardiovascular health is an important issue in its own right. Understanding the disorder will impact veterinary care, treatment, and prognosis and may also influence pedigree breeding, health, and welfare. Here we review the clinically distinct types of canine DCM and relate these to clinical heterogeneity seen in human DCM. Furthermore we provide a review of the known genetic contributions to DCM and discuss how these factors may inform future clinical management and breeding strategies in the dog.

## 2. Clinical Characteristics of DCM

DCM is characterised by cardiac ventricular chamber enlargement and systolic dysfunction which often leads

to congestive heart failure and death [13]. The aetiology of DCM is complex in that genetic factors, myocardial ischemia, hypertension, toxins, infections, and metabolic defects have been implicated in human disease [14]. Both human and canine DCM have a number of phases of progression starting with a long asymptomatic period before clinical signs appear [6, 15]. During this asymptomatic period, no functional changes in cardiac tissue have yet been reported, but it is possible that the underlying causes (e.g., genetic factors, toxins, and infections) are already initiating the disease [6]. During the next stage, there are again no reported outward clinical signs and the individual usually appears to be healthy, but cardiovascular electrical and morphological changes can be observed [6, 15–17]. Cardiovascular electrical changes may be detected using Holter monitoring for 24 hours, with individuals that go on to develop canine DCM often displaying ventricular arrhythmias [18]. Echocardiography can identify individuals that have an enlarged left ventricle which ultimately leads to symptomatic canine DCM [18]. Due to the apparently asymptomatic nature of this stage it is often termed the occult or preclinical stage and can last for several years in dogs [6, 17, 18]. In the final stage of DCM patients present with clinical signs of heart failure, commonly including cough, depression, dyspnoea, weight loss, and syncope, the individual requires treatment for heart failure, but prognosis is often poor [6, 19]. In humans, mortality 10 years following diagnosis is roughly 40%, although there is a wide variation with some individuals remaining asymptomatic, conversely many individuals suffer from sudden death [20]. Dogs also have significantly shortened lifespan following diagnosis, mean survival time following diagnosis, usually at the point of developing overt clinical symptoms, being 34 weeks, although, similar to humans, large variations are observed, with some surviving for several months while others only live for a few weeks [21–23].

Treatment of DCM in humans is aimed at minimising the effect of heart failure on the patient and delaying disease progression [24]. Standard medical treatment for human DCM consists of ACE inhibitors and $\beta$-blockade, often with a diuretic agent and, in the latter stages of disease progression, inotropic agents are frequently prescribed [24, 25]. Heart transplants are often the last resort in treating human heart disease; however the proportion of heart transplants in humans due to nonischemic cardiomyopathy, of which DCM is the second most common form, has increased to become the leading cause of heart transplant in recent years: 51% of transplant cases had nonischemic cardiomyopathy [26]. Canine DCM is treated in a similar manner to human DCM, in that treatment is aimed at minimising the effect of heart failure [27]. This treatment usually consists of diuretics, ACE inhibitors, positive inotropes, and other vasodilators [27, 28]. There is evidence that treatment when preclinical symptoms appear can increase lifespan, but this requires screening of individuals for preclinical DCM [29]. Heart transplants and other cardiac assist devices are not generally available to canine DCM patients.

*2.1. Evidence for Different Types of Canine DCM.* Although dogs within all breeds have the potential to develop DCM, there are some breeds that are particularly afflicted by DCM [30]. These include Newfoundlands, St. Bernards, Doberman Pinschers, Great Danes, Irish Wolfhounds, Boxers, and English Cocker Spaniels [3]. While these breeds, as well as other less frequently affected breeds, can be diagnosed as having DCM, there is evidence that different breeds may present with distinct types of DCM. This evidence consists of differential survival times from diagnosis, histopathology, inheritance patterns, and age of onset [21, 31–35].

Within canine DCM, two distinct types of histopathological variations have been described: "attenuated wavy fibre type" and "fatty infiltration type" [32]. While this evidence may be subjective, it adds to the evidence suggesting that there are different types of canine DCM. The fatty infiltration type is less subjective and has only been reported in Doberman Pinschers, Estrela Mountain Dogs, Great Danes, and Boxers [32, 36–38]; while the wavy fibre type is more ubiquitous, it does not seem to be restricted to specific breeds and can also occur in breeds which display the fatty infiltration type [32, 37]. As the wavy fibre type is found across breeds and in many individuals, it could be the tissue's response to the other processes of DCM. In particular atrophy, or attenuation, of muscle fibres is a frequent result of processes that prevent normal contractile ability: contractile ability is consistently compromised in DCM [19]. The prevalence and clinical significance of these histopathological variants remain to be established, although the phenotype can only be established post mortem and thus is unlikely to be useful in a clinical setting.

Human DCM is generally inherited in an autosomal dominant fashion [39], but autosomal recessive, X-linked recessive, and mitochondrial modes of inheritance have all been reported [40]. In common with human inheritance patterns there are several types of inheritance suggested in canine DCM. These include autosomal recessive [34], X-linked [33], and the common autosomal dominant inheritance [35, 38]; although often with reduced penetrance, not all dogs with the DCM genotype will develop the disease [41, 42]. X-linked and autosomal inheritance patterns show that the genetic basis of the disease is different. Recessive and dominant inheritance patterns also suggest the presence of different mutations leading to DCM and reduced penetrance indicates that there are likely to be additional factors involved in the formation of the disease phenotype. These additional factors may involve additional genes, epigenetic effects, and environmental effects including, but not limited to, diet, exercise, stress and toxins, or a combination of any number of these.

There is a wide variation in the long term prognosis of canine DCM. Some dogs, with appropriate disease management, can have a good quality of life for many years following a DCM diagnosis, whereas others die within weeks despite medical intervention [21–23]. Within this variation there are prognosis trends within breeds. Doberman Pinschers are a breed with particularly poor prognosis, and mean time to death (from diagnosis) is in the range of 7.4 to 9.7 weeks [21, 31], while the mean time for other

breeds is reported to be about four times that at 34 weeks [21]. Great Danes also suffer from a poor prognosis with Martin et al. [23] finding that they have the lowest median survival time of breeds included in their analysis, while Doberman Pinschers had the lowest upper quartile range.

Age of onset can also affect prognosis. There is a juvenile form of DCM in Portuguese water dogs, where age of onset is measured in weeks from birth [43, 44], while in most other cases age of onset is measured in years [45]. It would seem from this that DCM in Portuguese water dogs is a distinct condition. Even within adult canine DCM there is variation between breeds as to when individuals present with outward clinical signs. For example, Great Dane mean age of onset is 4.8 (SD ± 2.3) years [33], which is comparable to Irish Wolfhound mean age of onset of 4.40 (SD ± 2.03) years [42]; however, Doberman Pinscher's mean age of onset is in 7.3 years in males and 8.6 years in females [31]. This variation in mean age of onset could further suggest that there are different types of canine DCM.

There also appears to be different types of human DCM, with different inheritance patterns and age of onset reported [46]. If canine DCM can be appropriately matched to human DCM in terms of age of onset, inheritance pattern, survival time, and histopathology, they could provide appropriate models for each other. In particular some cases of childhood DCM have been shown to have an autosomal recessive pattern of inheritance [47], and in this instance the juvenile DCM observed in Portuguese water dogs [44] could be an appropriate model. There are currently several types of DCM identified in humans [39], but additional studies of canine DCM phenotypes are required to allow appropriate matching of canine and human DCM categories. Once identified, knowledge about canine DCM types could benefit current and future potential treatments and support for both human and canine DCM patients, in addition to elucidating other clinically important factors in canine DCM, such as longevity and prognosis.

*2.2. Genomic Research of DCM in Humans.* While there are many implicated causes or risk factors related to developing DCM and disease progression, genetics is a common one, with the disease often affecting several individuals within a family. To date mutations in over 50 genes have been associated with DCM in humans; however mutations in the most prevalent DCM related genes only account for approximately 50% of patients with DCM [39]. Genetic testing of individuals related to DCM patients can allow those that are at high risk of developing DCM to be more closely monitored [48]. This genetic testing is carried out on a panel of about 50 loci and more than one locus can be implicated in the disease [14] suggesting a dose effect, whereby the more DCM alleles an individual carries, the more severe the phenotype [39]. Gene penetrance has also been reported to affect disease expression and severity, and likewise the type of mutation and the specific gene which is affected often lead to differing features, age of onset or severity, and prognosis [49, 50].

Human DCM-associated genes identified to date are involved in a range of functions but can usually be placed into one of six functional groups: sarcomeric protein genes, cytoskeletal protein genes, nuclear envelope protein, desmosomal protein genes, calcium/sodium-handling genes, and transcription factor genes [39]. Cardiac muscle consists of striated muscle, and the sarcomere is the smallest unit of contractile muscle within this and thus alterations to this could lead to heart disease [51]. The cytoskeleton forms the majority of the cytoplasm, enabling cells to maintain their shape and facilitating communication within the cell [52, 53]. The nuclear envelope provides a barrier between nucleic acid synthesis and the rest of the cell but must remain permeable to allow the cell to function [54], a large number of proteins within the nuclear envelope have been implicated in chromatin organization and gene regulation [55]. The desmosome provides mechanical strength to tissues and potentially has cell signalling capacity, both of which are essential for cardiac function [56]. $Na^+/Ca^{2+}$ are important in the contraction of muscle [57] and as such calcium/sodium-handling genes are important in maintaining the correct concentration of $Na^+/Ca^{2+}$ for contraction of the heart. Transcription factors regulate the rate at which transcription of DNA to mRNA occurs; this rate is important in controlling the expression of genes and therefore the amount of a protein produced [58]. The breakdown of any of these functions has the capacity to lead to disease, including DCM. Table 1 shows the genes with mutations associated with DCM in humans, including the group into which the gene falls (where appropriate).

*2.3. Genetics of Canine DCM.* Canine DCM has often been used as a model for human DCM, but it is also a major clinical challenge in companion animals [3, 18, 22, 59]. It is has been established that, in common with human DCM, canine DCM frequently has a familial basis [33–35, 42]. Despite this, current understanding of the genetics of canine DCM is limited, in particular compared to the depth of genetic information available for human DCM. Indeed it is only recently that any loci have been associated with canine DCM [6, 60–62]. Genes associated with canine DCM are *DMD* in German short-haired pointers [63], *PDK4* in Doberman Pinschers [60], and *STRN* in Boxers [62], in addition to a locus on chromosome 5 in Doberman Pinschers [6]. Additional polymorphisms on chromosomes 1, 10, 15, 17, 21, and 37 have also been implicated in Irish Wolfhounds [61]. There are two methods that have been employed in attempts to identify genes associated with canine DCM, candidate gene studies, and genome wide association studies (GWAS).

## 3. Canine Candidate Gene Studies

Candidate gene studies for canine DCM primarily involve examining genes with variants associated with human DCM or associated conditions, for example [64–69]. The majority of canine DCM genetic studies have been of this type; however, only one mutation associated with canine DCM has been identified in this manner, which is that of a deletion in the *Striatin* gene in Boxers, a gene previously associated

Table 1: Genes with mutations associated with DCM in humans.

| Gene | Location/role | Reference |
|---|---|---|
| ABCC9 | Calcium/sodium-handling | [81] |
| ACTC1 | Sarcomere & cytoskeleton | [82] |
| ACTN2 | Sarcomere & cytoskeleton | [83] |
| ANKRD1 | Sarcomere & transcription factor | [84] |
| BAG3 | Sarcomere | [85–87] |
| CAV3 | Other | [88] |
| CHRM2 | Other | [89] |
| CRYAB | Cytoskeleton | [90] |
| CSRP3 | Sarcomere & cytoskeleton | [83] |
| CTF1 | Other | [91] |
| DES | Cytoskeleton | [92, 93] |
| DMD | Cytoskeleton | [94, 95] |
| DNAJC19 | Other | [96] |
| DOLK | Other | [97] |
| DSC2 | Desmosome | [98] |
| DSG2 | Desmosome | [99] |
| DSP | Desmosome | [100] |
| EYA4 | Other | [101] |
| FHL2 | Sarcomere & cytoskeleton | [102] |
| FKTN | Cytoskeleton | [103] |
| FKRP | Cytoskeleton | [104] |
| FOXD4 | Transcription factor | [105] |
| GATAD1 | Other | [106] |
| HCG22 | Other | [107] |
| HLA-DQB1 | Other | [108] |
| HSPB7 | Other | [109] |
| ILK | Cytoskeleton | [110] |
| LAMA2 | Other | [111] |
| LAMA4 | Cytoskeleton | [110] |
| LAMP2 | Other | [112] |
| LDB3 | Sarcomere & cytoskeleton | [113] |
| LMNA | Nuclear envelope | [114] |
| MURC | Other | [115] |
| MYBPC3 | Sarcomere | [116, 117] |
| MYH6 | Sarcomere | [117, 118] |
| MYH7 | Sarcomere | [116] |
| MYPN | Cytoskeleton | [119] |
| NEBL | Sarcomere | [120] |
| NEXN | Sarcomere | [121] |
| NOS3 | Other | [122] |
| PKP2 | Desmosome | [98] |
| PLN | Calcium/sodium-handling | [123] |
| PRDM16 | Transcription factor | [124] |
| PSEN1 | Other | [125] |
| PSEN2 | Other | [125] |
| RBM20 | Other | [126] |
| RYR2 | Calcium/sodium-handling | [127] |
| SCN5A | Calcium/sodium-handling | [128] |
| SDHA | Other | [129] |
| SGCD | Cytoskeleton | [130] |
| SYNE1 | Nuclear envelope | [131] |
| TAZ | Other | [132] |
| TBX20 | Transcription factor | [133] |
| TCAP | Sarcomere & cytoskeleton | [134] |
| TMPO | Nuclear envelope | [135] |
| TNNC1 | Sarcomere | [117] |
| TNNI3 | Sarcomere | [136] |
| TNNT2 | Sarcomere | [137] |
| TPM1 | Sarcomere | [117] |
| TXNRD2 | Other | [138] |
| TTN | Sarcomere & cytoskeleton | [139] |
| VCL | Sarcomere & cytoskeleton | [140] |
| ZBTB17 | Other | [141] |

with Boxer arrhythmogenic right ventricular cardiomyopathy using GWAS [62]. All other candidate gene studies have failed to find an association with canine DCM in the cohort examined (see Table 2), and unfortunately the small sample sizes frequently utilised could have limited the power to detect an association. In addition to small sample sizes in a number of studies, control (non-DCM cases) dogs have been limited or have not been appropriate (see Table 2 for exact numbers). Suitable controls should be breed matched and over a certain age to ensure that they are unlikely to develop DCM. Table 2 shows the genes examined for mutations associated with canine DCM in a variety of breeds, sample sizes, and control dogs, in the published literature to date.

## 4. Genome Wide Association Studies (GWAS)

Genome wide association studies are a method of screening the genomes of many individuals for variants or regions that are associated with a trait [70]. Some variants will fall within genes and some outside of genes. When variants associated with a trait are found outside of genes it can be more difficult to establish their mode of action.

There have been three GWAS looking for an association with canine DCM. One of these led to the identification of a deletion in a splice site of *PDK4* associated with DCM in Doberman Pinschers [60]. A separate GWAS in Doberman Pinschers revealed a single SNP associated with DCM in a different location to the *PDK4* gene [6]. The only other GWAS undertaken with regard to canine DCM is that by Philipp et al. [61] which found one significantly associated SNP and five suggestively associated SNPs in Irish Wolfhounds. Of all the loci identified as associated with canine DCM only two are on the same chromosome, one of the Irish Wolfhound SNPs and the *Striatin* genes are both on chromosome 17, but even these are far apart. This indicates that there may be many loci involved in the development of canine DCM.

Table 2: All genes investigated in relation to canine DCM.

| Gene | Associated with DCM in humans | Associated with canine DCM | DCM dogs | | Control dogs | Human reference | Canine study reference |
|---|---|---|---|---|---|---|---|
| | | | Number | Breed | | | |
| ACTC1 | Y | N | 16 | Doberman Pinscher | 12 mixed breeds | [39, 142] | [77] |
| | | | 64 | Irish Wolfhound | 25 Irish Wolfhounds | | [143] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |
| | | | 5 | Doberman Pinscher | 5 unspecified dogs without overt heart disease | | [144] |
| ACTN2 | Y | N | 5 | Doberman Pinscher | 5 unspecified dogs without overt heart disease | [39, 142] | [144] |
| CAV1 | N | N | 38 | Newfoundland | 36 Newfoundlands | na | [69] |
| CSRP3 | Y | N | 64 | Irish Wolfhound | 25 Irish Wolfhounds | [39, 142] | [143] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |
| | | | 5 | Doberman Pinscher | 2 Labradors | | [67] |
| | | | 5 | Doberman Pinscher | 5 unspecified dogs without overt heart disease | | [144] |
| DES | Y | N | 25 | Doberman Pinscher | 10 Doberman Pinschers | [39, 142] | [35] |
| | | | 64 | Irish Wolfhound | 25 Irish Wolfhounds | | [143] |
| | | | 18 | Doberman Pinscher | 10 Doberman Pinschers | | [68] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |
| DMD | Y | Y | 2 | German short-haired pointers | 2 German short-haired pointers with reduced dystrophin | [39, 142] | [63] |
| LDB3 | Y | N | 38 | Newfoundland | 36 Newfoundlands | [39, 142] | [69] |
| LMNA | Y | N | 38 | Newfoundland | 36 Newfoundlands | [39, 142] | [69] |
| | | | 5 | Doberman Pinscher | 2 Labradors | | [67] |
| MYBPC3 | Y | N | 5 | Doberman Pinscher | 5 unspecified dogs without overt heart disease | [39, 142] | [144] |
| MYH7 | Y | N | 38 | Newfoundland | 36 Newfoundlands | [39, 142] | [69] |
| | | | 5 | Doberman Pinscher | 2 Labradors | | [67] |
| | | | 5 | Doberman Pinscher | 5 unspecified dogs without overt heart disease | | [144] |
| PDK4 | N | Y | 66 | Doberman Pinscher | 66 Doberman Pinschers + 100 others from 11 breeds | na | [60] |
| PLN | Y | N | 25 | Doberman Pinscher | 10 Doberman Pinschers | [39, 142] | [35] |
| | | | 2 Doberman Pinscher, 2 Newfoundland, 2 great dane | | computer database only | | [66] |
| | | | 64 | Irish Wolfhound | 25 Irish Wolfhounds | | [143] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |
| SGCD | Y | N | 25 | Doberman Pinscher | 10 Doberman Pinschers | [39, 142] | [35] |
| | | | 64 | Irish Wolfhound | 25 Irish Wolfhounds | | [143] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |
| | | | 25 | Doberman Pinscher | 13 Doberman Pinschers | | [145] |
| STRN | N | Y | 33 | Boxer | 16 Boxers | na | [62] |
| TAZ | Y | N | 64 | Irish Wolfhound | 25 Irish Wolfhounds | [39] | [143] |
| TCAP | Y | N | 8 | Irish Wolfhound | 5 Irish Wolfhounds | [39, 142] | [146] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |
| | | | 5 | Doberman Pinscher | 5 unspecified dogs without overt heart disease | | [144] |

Table 2: Continued.

| Gene | Associated with DCM in humans | Associated with canine DCM | DCM dogs | | Control dogs | Human reference | Canine study reference |
|---|---|---|---|---|---|---|---|
| | | | Number | Breed | | | |
| TMOD | N | N | 64 | Irish Wolfhound | 25 Irish Wolfhounds | na | [143] |
| TNNC1 | Y | N | 5 | Doberman Pinscher | 2 Labradors | [39, 142] | [67] |
| TNNI3 | Y | N | 38 | Newfoundland | 36 Newfoundlands | [39, 142] | [69] |
| TNNT2 | Y | N | 5 | Doberman Pinscher | 2 Labradors | [39, 142] | [67] |
| | | | 5 | Doberman Pinscher | 5 dogs without overt heart disease | | [144] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |
| TPM1 | Y | N | 38 | Newfoundland | 36 Newfoundlands | [39] | [69] |
| | | | 5 | Doberman Pinscher | 5 dogs without overt heart disease | | [144] |
| TTN | Y | N | 38 | Newfoundland | 36 Newfoundlands | [39, 142] | [69] |
| | | | 2 | Doberman Pinscher | 5 mixed breeds | | [147] |
| VCL | Y | N | 5 | Doberman Pinscher | 5 dogs without overt heart disease | [39, 142] | [144] |
| | | | 38 | Newfoundland | 36 Newfoundlands | | [69] |

Y = yes, N = no.

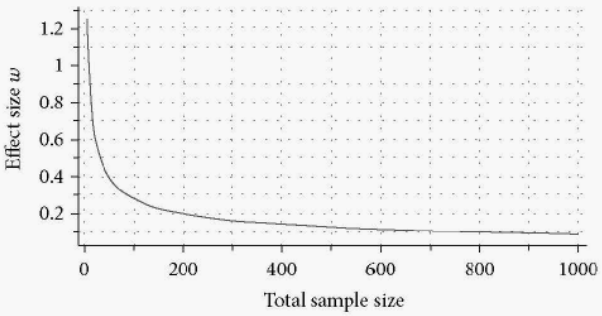



FIGURE 1: $\chi^2$goodness of fit tests: contingency tables Df = 1, $\alpha$ err prob = 0.05, and power (1 − $\beta$ err prob) = 0.8.

## 5. The Effects of Multiple Loci on DCM

Thus far in both canine and human genetic DCM studies loci have only been considered for an association with disease individually. There have been indications that multiple loci may influence the development of DCM [6]. In human DCM where a pannel of more than 50 loci are tested concurrently, often several loci are implicated. Simpson et al. [71] have shown theoretically that multiple loci affect the development of DCM in Doberman Pinschers. While this still requires valiadation, it is possible that similar effects occur in other breeds and species.

## 6. Power to Detect an Association with Canine DCM

The majority of studies undertaken with the aim of identifying causal genetic variants of canine DCM have only utilised small samples (5–40 individuals) which is unlikely to be large enough to detect an effect. To establish appropriate study sizes and indicate the effect size that can be detected in published studies G*Power 3.1.7 Chi-squared goodness of fit tests were used (using the methods from [72]). This takes known input parameters, including sample size, and calculates estimated effect sizes based on assumed power and can be used to indicate minimum sample size for prescribed power, alpha error rate, and effect size. This was done to indicate minimum sample sizes needed to detect various effect sizes (Figure 1).

Published studies that have identified genetic variants associated with DCM have used sample sizes of 180 [6], 132 [60], and 49 [62]. Assuming these studies had enough power to identify a positive effect (0.8), the effect sizes of these variants in these studies are 0.2088, 0.2438, and 0.4002, respectively, calculated using the sensitivity power analysis in G*Power 3.1.7 [72]. These effect sizes, while not large, are larger than the standard effect size for small effect of 0.1. None of these variants explain all incidences of DCM, suggesting that other factors, which may be additional genetic variants of smaller effect, are involved. The sample size required to obtain a positive result from variants with small effect size (0.1) is 785, a number possibly not obtainable for all breeds but could be aimed for in future studies. It is likely that earlier studies concentrated on simple Mendelian recessive, dominant traits and even a multiplicative risk models where Karlsson & Lindblad-Toh [73] had suggested that affected and control groups of 20, 50, and 100, respectively, may suffice. Despite these suggestions, the authors indicated that higher group sizes (around 500 samples) would likely provide sufficient power to map an allele conferring a two-fold risk.

*6.1. Discussion of Selected Breeds.* While there are many breeds affected by canine DCM only a few have had genetic loci identified as associated with the disease. Here we discuss breeds with adolescent and adult onset DCM associated loci. The juvenile DCM that Portuguese water dogs develop is not discussed because it is already considered to be a distinct condition [34].

*6.2. Boxers: Striatin (STRN).* The Boxer breed of dog was developed in the late 1800's primarily from the now extinct hunting dog the Bullenbeisser [74]. As with the development of most modern breeds there is documented evidence of inbreeding to produce the desired characteristics. In the case of the boxer this included a mating of a son to his mother, and following the creation of a breed standard in 1902 it is likely that usually Boxers will have exclusively been mated to other Boxers [74]. This limited genetic diversity is likely to have led to Boxers being prone to developing a number of diseases including heart disease, of which they frequently develop both arrhythmogenic right ventricular cardiomyopathy (ARVC) and DCM [62]. Since boxer cardiomyopathy was described by Harpster [75] there have been several subtypes described, of the two displaying overt clinical symptoms these most closely align to human ARVC and DCM [62]. Recently Meurs et al. [62] tested a deletion in the striatin (*STRN*) gene for an association with DCM in boxers. This deletion has previously been associated with ARVC and it was hypothesised that ARVC and DCM are variants of the same disease in Boxers and the homozygous genotype leads to DCM rather than ARVC [62]. They found a significant association with the deletion in its homozygous form and DCM, but there were three cases of DCM where there was no deletion in the gene, thus indicating that there is at least one more cause of DCM in the breed to be established [62].

*6.3. Doberman Pinschers: PDK4 and Chromosome 5 SNP.* The Doberman Pinscher breed was developed at the end of the 1800's in Germany [76] when a number of individuals from established breeds were used to improve various characteristics. According to Gruenig [76] these include the Manchester terrier, Greyhound, Rottweiler, Gordon Setter, Old English Sheepdog, Beauceron, Pinscher (probably German Pinscher), Weimaraner, and other less specific breeds such as Mastiff (possibly Great Dane), Hound, and Sporting dogs. The development of the breed happened rapidly, over a period of about 30 years, and since then Doberman Pinschers have only been bred to Doberman Pinschers [76], leading to a closed gene pool. Although a number of breeds contributed to the Doberman Pinscher it is likely that relatively few individuals of each breed were used likely leading to low genetic diversity. In addition to relatively few founders there is evidence of

some individuals contributing a greater number of offspring to the breeding population than others [76].

Doberman Pinschers can develop a particularly severe type of DCM with rapid disease progression following the diagnosis of DCM with mean survival time of less than 10 weeks [21, 31]. Poor survival time following diagnosis combined with the high prevalence of the disease with estimates ranging from 45% to 63% means DCM in this breed is a particular problem for clinicians [59]. Doberman Pinschers display the fatty infiltration type of histopathology [32]. Despite these poor statistics, age of onset of clinical signs is often later than in other commonly affected breeds (7.3 years in males and 8.6 years in females, compared to 4.8 (SD ± 2.3) years in Great Danes), giving individuals a good quality of life up until overt DCM clinical signs [31, 77]. Across age groups there is no difference in clinical signs associated with DCM between the sexes including echocardiographic changes, presence and number of ventricular premature contractions, and overt DCM [59]. Unfortunately, however, males are more likely to have overt DCM than females with 73.7% of all observed males becoming clinically overt while only 26.3% of females observed became clinically overt [59].

DCM in Doberman Pinschers appears to be inherited in an autosomal dominant fashion with equal numbers of males and females affected, male-male transmission, and the mating of two affected individuals producing unaffected offspring [35]. There have been two loci identified as associated with DCM in the breed, a deletion of a splice site in pyruvate dehydrogenase kinase, isozyme 4 (*PDK4*), and a SNP on chromosome 5 [6, 60]. Unfortunately neither of these loci explains all incidences of DCM, and the PDK4 deletion is not significantly associated with DCM in a separate Doberman Pinscher population [78]. There are still additional causes of DCM to be identified in Doberman Pinschers and the function of the SNP on chromosome 5 needs to be established.

*6.4. German Short-Haired Pointers: Dystrophin (DMD).* The only gene associated with canine DCM in German short-haired pointers is Dystrophin (*DMD*) [63]. German short-haired pointers are not considered a breed particularly afflicted by heart disease and the deletion was only identified in two male litter mates [3, 63]. This could be an isolated case which is unlikely to have implications in other breeds, particularly as the affected individuals also had skeletal myopathies, whereas in most cases of canine DCM there are not any other myopathies present [63].

*6.5. Irish Wolfhounds.* Although Irish Wolfhounds have a long history, this includes a period when they were close to extinction. As part of conserving the breed, Great Danes, Scottish deerhounds, Borzoi, and Mastiffs were crossed with the few remaining Irish Wolfhounds [61, 79]. While this will have introduced some degree of genetic diversity to the breed, by necessity a large amount of inbreeding will have been required to retain the Irish Wolfhound phenotype and so, like most modern breeds, genetic diversity is low [80].

Irish Wolfhounds do not usually develop a particularly severe form of DCM and with appropriate management can live with the disease for many months or years [22]. Unfortunately, however, the prevalence of heart disease, including DCM, within the breed is very high, with 41% of individuals presenting with cardiac abnormalities, of which 58% have DCM [22]. This high prevalence combined with early onset of clinical signs at around 4 years old [42] means that DCM in Irish Wolfhounds is of concern and so identifying genetic causes of the disease could have a large impact on the health of breed.

The mode of inheritance of DCM in Irish Wolfhounds has been shown to be autosomal dominant major gene effect, but with reduced penetrance indicating that multiple factors influence disease progression [42]. Of the six SNPs associated with DCM in Irish Wolfhounds to date, only three lie within known genes [61]. Further work is therefore required to establish the functional significance of the alleles and to confirm the associations with DCM.

## 7. Conclusions: *Impact of Genetics on Canine DCM*

In the short term, the identification of the genetic contributors to DCM will enable targeted heart monitoring prior to the onset of clinical signs and clinical management of those dogs with increased risk of developing DCM. In the longer term, knowledge of the genetic factors which predispose to DCM will allow for selective breeding strategies to be considered and may identify novel therapeutic and diagnostic approaches. Individuals likely to develop DCM, identified through robust genetics, could be removed from breeding programmes with the ultimate goal of reducing the number of affected animals within the population and promoting the long term welfare of the breed. Understanding the genetic causes may also aid the stratification of distinct clinical subtypes of DCM. This knowledge may also permit the development of novel DCM management programmes, help to guide prognosis, and assist with future drug and intervention research. Furthermore, investigations into causative genes in canine DCM may prove beneficial for other species, including humans. Novel mutations in canine breeds may serve as candidate genes in affected humans. For these reasons a more detailed understanding of the genetic basis of DCM in diverse dog breeds is now required.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgments

The authors gratefully acknowledge generous funding from the BBSRC University of Nottingham Doctoral Training Programme BB/J014508/1 and the School of Veterinary Medicine and Science and, in part, by an unrestricted educational research grant provided by Boehringer Ingelheim Vetmedica, all to Catrin S. Rutland, Nigel P. Mongan, and Malcolm Cobb.

## References


[1] J. M. Fleming, K. E. Creevy, and D. E. L. Promislow, "Mortality in north american dogs from 1984 to 2004: an investigation into age-, size-, and breed-related causes of death," *Journal of Veterinary Internal Medicine*, vol. 25, no. 2, pp. 187–198, 2011.

[2] C. D. Mathers and D. Loncar, "Projections of global mortality and burden of disease from 2002 to 2030," *PLoS Medicine*, vol. 3, no. 11, article e442, 2006.

[3] A. Egenvall, B. N. Bonnett, and J. Häggström, "Heart disease as a cause of death in insured Swedish dogs younger than 10 years of age," *Journal of Veterinary Internal Medicine*, vol. 20, no. 4, pp. 894–903, 2006.

[4] H. Raju, C. Alberg, G. S. Sagoo, H. Burton, and E. R. Behr, "Inherited cardiomyopathies," *British Medical Journal*, vol. 343, Article ID d6966, 2011.

[5] R. E. Hershberger, A. Morales, and J. D. Siegfried, "Clinical and genetic issues in dilated cardiomyopathy: a review for genetics professionals," *Genetics in Medicine*, vol. 12, no. 11, pp. 655–667, 2010.

[6] T.-B. Mausberg, G. Wess, J. Simak et al., "A locus on chromosome 5 is associated with dilated cardiomyopathy in doberman pinschers," *PLoS ONE*, vol. 6, no. 5, Article ID e20042, 2011.

[7] J. A. Dixon and F. G. Spinale, "Large animal models of heart failure: a critical link in the translation of basic science to clinical practice," *Circulation: Heart Failure*, vol. 2, no. 3, pp. 262–271, 2009.

[8] F. G. Akar, R. C. Wu, G. J. Juang et al., "Molecular mechanisms underlying $K^+$ current downregulation in canine tachycardia-induced heart failure," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 288, no. 6, pp. H2887–H2896, 2005.

[9] M. Argenziano and M. L. Dickstein, "Cardiovascular effects of inhaled nitric oxide in a canine model of cardiomyopathy," *The Annals of Thoracic Surgery*, vol. 69, no. 5, pp. 1644–1645, 2000.

[10] Y. Toyoda, M. Okada, and M. A. Kashem, "A canine model of dilated cardiomyopathy induced by repetitive intracoronary doxorubicin administration," *Journal of Thoracic and Cardiovascular Surgery*, vol. 115, no. 6, pp. 1367–1373, 1998.

[11] K. Huntington, P. Picard, G. Moe, D. J. Stewart, A. Albernaz, and J. C. Monge, "Increased cardiac and pulmonary endothelin-1 mRNA expression in canine pacing-induced heart failure," *Journal of Cardiovascular Pharmacology*, vol. 31, no. 1, pp. S424–S426, 1998.

[12] M. Hongo, T. Ryoke, and J. Ross Jr., "Animal models of heart failure: recent developments and perspectives," *Trends in Cardiovascular Medicine*, vol. 7, no. 5, pp. 161–167, 1997.

[13] O. P. Guttmann, S. A. Mohiddin, and P. M. Elliott, "Almanac 2014: cardiomyopathies," *Heart*, vol. 100, no. 10, pp. 756–764, 2014.

[14] E. M. McNally, J. R. Golbus, and M. J. Puckelwartz, "Genetic mutations and mechanisms in dilated cardiomyopathy," *The Journal of Clinical Investigation*, vol. 123, no. 1, pp. 19–26, 2013.

[15] N. G. Mahon, R. T. Murphy, C. A. MacRae, A. L. Caforio, P. M. Elliott, and W. J. McKenna, "Echocardiographic evaluation in asymptomatic relatives of patients with dilated cardiomyopathy reveals preclinical disease," *Annals of Internal Medicine*, vol. 143, no. 2, pp. 108–115, 2005.

[16] M. K. Baig, J. H. Goldman, A. L. P. Caforio, A. S. Coonar, P. J. Keeling, and W. J. McKenna, "Familial dilated cardiomyopathy: cardiac abnormalities are common in asymptomatic relative and may represent early disease," *Journal of the American College of Cardiology*, vol. 31, no. 1, pp. 195–201, 1998.

[17] S. E. Brownlie and M. A. Cobb, "Observations on the development of congestive heart failure in Irish wolfhounds with dilated cardiomyopathy," *Journal of Small Animal Practice*, vol. 40, no. 8, pp. 371–377, 1999.

[18] J. Dukes-McEwan, M. Borgarelli, A. Tidholm, A. C. Vollmar, and J. Häggström, "Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy," *Journal of Veterinary Cardiology*, vol. 5, no. 2, pp. 7–19, 2003.

[19] A. Tidholm, J. Häggström, M. Borgarelli, and A. Tarducci, "Canine idiopathic dilated cardiomyopathy. Part I: Aetiology, clinical characteristics, epidemiology and pathology,," *Veterinary Journal*, vol. 162, no. 2, pp. 92–107, 2001.

[20] S. Lehrke, D. Lossnitzer, M. Schöb et al., "Use of cardiovascular magnetic resonance for risk stratification in chronic heart failure: prognostic value of late gadolinium enhancement in patients with non-ischaemic dilated cardiomyopathy," *Heart*, vol. 97, no. 9, pp. 727–732, 2011.

[21] A. D. Petric, P. Stabej, and A. Zemva, "Dilated cardiomyopathy in doberman pinschers: survival, causes of death and a pedigree review in a related line," *Journal of Veterinary Cardiology*, vol. 4, no. 1, pp. 17–24, 2002.

[22] A. C. Vollmar, "The prevalence of cardiomyopathy in the Irish wolfhound: a clinical study of 500 dogs," *Journal of the American Animal Hospital Association*, vol. 36, no. 2, pp. 125–132, 2000.

[23] M. W. S. Martin, M. J. S. Johnson, G. Strehlau, and J. N. King, "Canine dilated cardiomyopathy: a retrospective study of prognostic findings in 367 clinical cases," *Journal of Small Animal Practice*, vol. 51, no. 8, pp. 428–436, 2010.

[24] J. L. Jefferies and J. A. Towbin, "Dilated cardiomyopathy," *The Lancet*, vol. 375, no. 9716, pp. 752–762, 2010.

[25] M. Merlo, A. Pivetta, B. Pinamonti et al., "Long-term prognostic impact of therapeutic strategies in patients with idiopathic dilated cardiomyopathy: changing mortality over the last 30 years," *European Journal of Heart Failure*, vol. 16, no. 3, pp. 317–324, 2014.

[26] D. O. Taylor, J. Stehlik, L. B. Edwards et al., "Registry of the international society for heart and lung transplantation: twenty-sixth official adult heart transplant report-2009," *Journal of Heart and Lung Transplantation*, vol. 28, no. 10, pp. 1007–1022, 2009.

[27] J. D. McEwan, "Canine dilated cardiomyopathy 2. Pathophysiology and treatment," *In Practice*, vol. 22, no. 10, pp. 620–628, 2000.

[28] M. Borgarelli, A. Tarducci, A. Tidholm, and J. Häggström, "Canine idiopathic dilated cardiomyopathy. Part II: pathophysiology and therapy," *Veterinary Journal*, vol. 162, no. 3, pp. 182–195, 2001.

[29] N. J. Summerfield, A. Boswood, M. R. O'Grady et al., "Efficacy of pimobendan in the prevention of congestive heart failure or sudden death in Doberman Pinschers with preclinical dilated cardiomyopathy (the PROTECT Study)," *Journal of Veterinary Internal Medicine*, vol. 26, no. 6, pp. 1337–1349, 2012.

[30] A. Tidholm and L. Jönsson, "A retrospective study of canine dilated cardiomyopathy (189 cases)," *Journal of the American Animal Hospital Association*, vol. 33, no. 6, pp. 544–550, 1997.

[31] C. A. Calvert, C. W. Pickus, G. J. Jacobs, and J. Brown, "Signalment, survival, and prognostic factors in Doberman pinschers with end-stage cardiomyopathy," *Journal of Veterinary Internal Medicine*, vol. 11, no. 6, pp. 323–326, 1997.

[32] A. Tidholm and L. Jönsson, "Histologic characterization of canine dilated cardiomyopathy," *Veterinary Pathology*, vol. 42, no. 1, pp. 1–8, 2005.

[33] K. M. Meurs, M. W. Miller, and N. A. Wright, "Clinical features of dilated cardiomyopathy in Great Danes and results of a pedigree analysis: 17 cases (1990–2000)," *Journal of the American Veterinary Medical Association*, vol. 218, no. 5, pp. 729–732, 2001.

[34] M. M. Sleeper, P. S. Henthorn, C. Vijayasarathy et al., "Dilated cardiomyopathy in juvenile Portuguese water dogs," *Journal of Veterinary Internal Medicine*, vol. 16, no. 1, pp. 52–62, 2002.

[35] K. M. Meurs, P. R. Fox, M. Norgard et al., "A prospective genetic evaluation of familial dilated cardiomyopathy in the Doberman pinscher," *Journal of Veterinary Internal Medicine*, vol. 21, no. 5, pp. 1016–1020, 2007.

[36] L. Lobo, J. Carvalheira, N. Canada, C. Bussadori, J. L. Gomes, and A. M. R. Faustino, "Histologic characterization of dilated cardiomyopathy in Estrela mountain dogs," *Veterinary Pathology*, vol. 47, no. 4, pp. 637–642, 2010.

[37] H. Aupperle, I. Marz, K. Baldauf, N. Roggon, and J. G. Kresken, "Pathology of DCM in Great Danes," *Journal of Veterinary Internal Medicine*, vol. 28, no. 2, p. 730, 2014.

[38] H. M. Stephenson, S. Fonfara, J. López-Alvarez, P. Cripps, and J. Dukes-McEwan, "Screening for dilated cardiomyopathy in Great Danes in the United Kingdom," *Journal of Veterinary Internal Medicine*, vol. 26, no. 5, pp. 1140–1147, 2012.

[39] A. Posafalvi, J. C. Herkert, R. J. Sinke et al., "Clinical utility gene card for: dilated cardiomyopathy (CMD)," *European Journal of Human Genetics*, vol. 21, no. 10, p. 1185, 2013.

[40] G. W. Dec and V. Fuster, "Idiopathic dilated cardiomyopathy," *The New England Journal of Medicine*, vol. 331, no. 23, pp. 1564–1575, 1994.

[41] J. Dukes-McEwan and I. J. Jackson, "The promises and problems of linkage analysis by using the current canine genome map," *Mammalian Genome*, vol. 13, no. 11, pp. 667–672, 2002.

[42] O. Distl, A. C. Vollmar, C. Broschk, H. Hamann, and P. R. Fox, "Complex segregation analysis of dilated cardiomyopathy (DCM) in Irish wolfhounds," *Heredity*, vol. 99, no. 4, pp. 460–465, 2007.

[43] P. Werner, M. G. Raducha, U. Prociuk, M. M. Sleeper, T. J. van Winkle, and P. S. Henthorn, "A novel locus for dilated cardiomyopathy maps to canine chromosome 8," *Genomics*, vol. 91, no. 6, pp. 517–521, 2008.

[44] D. M. Dambach, A. Lannon, M. M. Sleeper, and J. Buchanan, "Familial dilated cardiomyopathy of young Portuguese water dogs," *Journal of Veterinary Internal Medicine*, vol. 13, no. 1, pp. 65–71, 1999.

[45] M. W. S. Martin, M. J. Stafford Johnson, and B. Celona, "Canine dilated cardiomyopathy: a retrospective study of signalment, presentation and clinical findings in 369 cases," *Journal of Small Animal Practice*, vol. 50, no. 1, pp. 23–29, 2009.

[46] L. Mestroni, C. Rocco, D. Gregori et al., "Familial dilated cardiomyopathy: evidence for genetic and phenotypic heterogeneity," *Journal of the American College of Cardiology*, vol. 34, no. 1, pp. 181–190, 1999.

[47] J. A. Towbin, A. M. Lowe, S. D. Colan et al., "Incidence, causes, and outcomes of dilated cardiomyopathy in children," *Journal of the American Medical Association*, vol. 296, no. 15, pp. 1867–1876, 2006.

[48] D. Jacoby and W. J. McKenna, "Genetics of inherited cardiomyopathy," *European Heart Journal*, vol. 33, no. 3, pp. 296–304, 2012.

[49] L. Mestroni and M. R. G. Taylor, "Genetics and genetic testing of dilated cardiomyopathy: a new perspective," *Discovery Medicine*, vol. 15, no. 80, pp. 43–49, 2013.

[50] A. Morales and R. E. Hershberger, "Genetic evaluation of dilated cardiomyopathy," *Current Cardiology Reports*, vol. 15, no. 7, article 375, 2013.

[51] N. Hamdani, V. Kooij, S. van Dijk et al., "Sarcomeric dysfunction in heart failure," *Cardiovascular Research*, vol. 77, no. 4, pp. 649–658, 2008.

[52] E. Frixione, "Recurring views on the structure and function of the cytoskeleton: a 300-year epic," *Cell Motility and the Cytoskeleton*, vol. 46, no. 2, pp. 73–94, 2000.

[53] S. Hein, S. Kostin, A. Heling, Y. Maeno, and J. Schaper, "The role of the cytoskeleton in heart failure," *Cardiovascular Research*, vol. 45, no. 2, pp. 273–278, 2000.

[54] I. G. Macara, "Transport into and out of the nucleus," *Microbiology and Molecular Biology Reviews*, vol. 65, no. 4, pp. 570–594, 2001.

[55] M. W. Hetzer, "The nuclear envelope," *Cold Spring Harbor Perspectives in Biology*, vol. 2, no. 3, Article ID a000539, 2010.

[56] K. J. Green and C. A. Gaudry, "Are desmosomes more than tethers for intermediate filaments?" *Nature Reviews Molecular Cell Biology*, vol. 1, no. 3, pp. 208–216, 2000.

[57] R. DiPolo and L. Beaugé, "Sodium/calcium exchanger: influence of metabolic regulation on ion carrier interactions," *Physiological Reviews*, vol. 86, no. 1, pp. 155–203, 2006.

[58] D. S. Latchman, "Transcription factors: an overview," *The International Journal of Biochemistry & Cell Biology*, vol. 29, no. 12, pp. 1305–1312, 1997.

[59] G. Wess, A. Schulze, V. Butz et al., "Prevalence of dilated cardiomyopathy in Doberman Pinschers in various age groups," *Journal of Veterinary Internal Medicine*, vol. 24, no. 3, pp. 533–538, 2010.

[60] K. M. Meurs, S. Lahmers, B. W. Keene et al., "A splice site mutation in a gene encoding for PDK4, a mitochondrial protein, is associated with the development of dilated cardiomyopathy in the Doberman pinscher," *Human Genetics*, vol. 131, no. 8, pp. 1319–1325, 2012.

[61] U. Philipp, A. Vollmar, J. Häggström, A. Thomas, and O. Distl, "Multiple loci are associated with dilated cardiomyopathy in irish wolfhounds," *PLoS ONE*, vol. 7, no. 6, Article ID e36691, 2012.

[62] K. M. Meurs, J. A. Stern, D. D. Sisson et al., "Association of dilated cardiomyopathy with the striatin mutation genotype in boxer dogs," *Journal of Veterinary Internal Medicine*, vol. 27, no. 6, pp. 1437–1440, 2013.

[63] S. J. Schatzberg, N. J. Olby, M. Breen et al., "Molecular analysis of a spontaneous dystrophin 'knockout' dog," *Neuromuscular Disorders*, vol. 9, no. 5, pp. 289–295, 1999.

[64] U. Philipp, C. Broschk, A. Vollmar, and O. Distl, "Evaluation of tafazzin as candidate for dilated cardiomyopathy in Irish wolfhounds," *Journal of Heredity*, vol. 98, no. 5, pp. 506–509, 2007.

[65] A. C. Wiersma, P. A. Leegwater, B. A. van Oost, W. E. Ollier, and J. Dukes-McEwan, "Canine candidate genes for dilated cardiomyopathy: annotation of and polymorphic markers for 14 genes," *BMC Veterinary Research*, vol. 3, article 28, 2007.

[66] P. Stabej, P. A. Leegwater, A. A. Stokhof, A. Domanjko-Petrič, and B. A. van Oost, "Evaluation of the phospholamban gene in purebred large-breed dogs with dilated cardiomyopathy," *American Journal of Veterinary Research*, vol. 66, no. 3, pp. 432–436, 2005.

[67] K. M. Meurs, K. P. Hendrix, and M. M. Norgard, "Molecular evaluation of five cardiac genes in Doberman Pinschers with dilated cardiomyopathy," *American Journal of Veterinary Research*, vol. 69, no. 8, pp. 1050–1053, 2008.

[68] P. Stabej, S. Imholz, S. A. Versteeg et al., "Characterization of the canine desmin (DES) gene and evaluation as a candidate gene for dilated cardiomyopathy in the Dobermann," *Gene*, vol. 340, no. 2, pp. 241–249, 2004.

[69] A. C. Wiersma, P. Stabej, P. A. J. Leegwater, B. A. van Oost, W. E. Ollier, and J. Dukes-McEwan, "Evaluation of 15 candidate genes for dilated cardiomyopathy in the Newfoundland dog," *Journal of Heredity*, vol. 99, no. 1, pp. 73–80, 2008.

[70] T. A. Manolio, "Genomewide association studies and assessment of the risk of disease," *The New England Journal of Medicine*, vol. 363, no. 2, pp. 166–176, 2010.

[71] S. Simpson, J. Edwards, R. D. Emes, M. A. Cobb, N. P. Mongan, and C. S. Rutland, "A predictive model for canine dilated cardiomyopathy—a meta-analysis of Doberman Pinscher data," *PeerJ*, vol. 3, article e842, 2015.

[72] F. Faul, E. Erdfelder, A. Buchner, and A.-G. Lang, "Statistical power analyses using G* power 3.1: tests for correlation and regression analyses," *Behavior Research Methods*, vol. 41, no. 4, pp. 1149–1160, 2009.

[73] E. K. Karlsson and K. Lindblad-Toh, "Leader of the pack: gene mapping in dogs and other model organisms," *Nature Reviews Genetics*, vol. 9, no. 9, pp. 713–725, 2008.

[74] J. P. Wagner, *The Boxer*, 1939.

[75] N. Harpster, "Boxer cardiomyopathy," in *Current Veterinary Therapy VIII*, W.B. Saunders Company, Philadelphia, Pa, USA, 1983.

[76] P. Gruenig, *The Doberman Pinscher: History and Development of the Breed*, Orange Judd Publishing Company Inc, 1939.

[77] K. M. Meurs, A. L. Magnon, A. W. Spier, M. W. Miller, L. B. Lehmkuhl, and J. A. Towbin, "Evaluation of the cardiac actin gene in Doberman Pinschers with dilated cardiomyopathy," *American Journal of Veterinary Research*, vol. 62, no. 1, pp. 33–36, 2001.

[78] M. Owczarek-Lipska, T.-B. Mausberg, H. Stephenson, J. Dukes-McEwan, G. Wess, and T. Leeb, "A 16-bp deletion in the canine PDK4 gene is not associated with dilated cardiomyopathy in a European cohort of Doberman Pinschers," *Animal Genetics*, vol. 44, no. 2, p. 239, 2013.

[79] J. Samaha, *The New Complete Irish Wolfhound*, Howell Book House, 1991.

[80] M. Jansson and L. Laikre, "Recent breeding history of dog breeds in Sweden: modest rates of inbreeding, extensive loss of genetic diversity and lack of correlation between inbreeding and health," *Journal of Animal Breeding and Genetics*, vol. 131, no. 2, pp. 153–162, 2014.

[81] M. Bienengraeber, T. M. Olson, V. A. Selivanov et al., "ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating," *Nature Genetics*, vol. 36, no. 4, pp. 382–387, 2004.

[82] T. M. Olson, V. V. Michels, S. N. Thibodeau, Y.-S. Tai, and M. T. Keating, "Actin mutations in dilated cardiomyopathy, a heritable form of heart failure," *Science*, vol. 280, no. 5364, pp. 750–752, 1998.

[83] B. Mohapatra, S. Jimenez, J. H. Lin et al., "Mutations in the muscle LIM protein and α-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis," *Molecular Genetics and Metabolism*, vol. 80, no. 1-2, pp. 207–215, 2003.

[84] M. Moulik, M. Vatta, S. H. Witt et al., "ANKRD1, the gene encoding cardiac ankyrin repeat protein, is a novel dilated cardiomyopathy gene," *Journal of the American College of Cardiology*, vol. 54, no. 4, pp. 325–333, 2009.

[85] N. Norton, D. Li, M. J. Rieder et al., "Genome-wide studies of copy number variation and exome sequencing identify rare variants in *BAG3* as a cause of dilated cardiomyopathy," *The American Journal of Human Genetics*, vol. 88, no. 3, pp. 273–282, 2011.

[86] E. Villard, C. Perret, F. Gary et al., "A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy," *European Heart Journal*, vol. 32, no. 9, pp. 1065–1076, 2011.

[87] T. Arimura, T. Ishikawa, S. Nunoda, S. Kawai, and A. Kimura, "Dilated cardiomyopathy-associated BAG3 mutations impair Z-disc assembly and enhance sensitivity to apoptosis in cardiomyocytes," *Human Mutation*, vol. 32, no. 12, pp. 1481–1491, 2011.

[88] M. Catteruccia, T. Sanna, F. M. Santorelli et al., "Rippling muscle disease and cardiomyopathy associated with a mutation in the CAV3 gene," *Neuromuscular Disorders*, vol. 19, no. 11, pp. 779–783, 2009.

[89] L. Zhang, A. Hu, H. Yuan et al., "A missense mutation in the CHRM2 gene is associated with familial dilated cardiomyopathy," *Circulation Research*, vol. 102, no. 11, pp. 1426–1432, 2008.

[90] N. Inagaki, T. Hayashi, T. Arimura et al., "αB-crystallin mutation in dilated cardiomyopathy," *Biochemical and Biophysical Research Communications*, vol. 342, no. 2, pp. 379–386, 2006.

[91] J. Erdmann, S. Hassfeld, H. Kallisch, E. Fleck, and V. Regitz-Zagrose, "Genetic variants in the promoter (g983G>T) and coding region (A92T) of the human cardiotrophin-1 gene (CTF1) in patients with dilated cardiomyopathy," *Human Mutation*, vol. 16, no. 5, p. 448, 2000.

[92] J. Schaper, R. Froede, S. Hein et al., "Impairment of the myocardial ultrastructure and changes of the cytoskeleton in dilated cardiomyopathy," *Circulation*, vol. 83, no. 2, pp. 504–514, 1991.

[93] D. Li, T. Tapscoft, O. Gonzalez et al., "Desmin mutation responsible for idiopathic dilated cardiomyopathy," *Circulation*, vol. 100, no. 5, pp. 461–464, 1999.

[94] F. Muntoni, M. Cau, A. Ganau et al., "Brief report: deletion of the dystrophin muscle-promoter region associated with X-linked dilated cardiomyopathy," *The New England Journal of Medicine*, vol. 329, no. 13, pp. 921–925, 1993.

[95] R. Ortiz-Lopez, H. Li, J. Su, V. Goytia, and J. A. Towbin, "Evidence for a dystrophin missense mutation as a cause of X-linked dilated cardiomyopathy," *Circulation*, vol. 95, no. 10, pp. 2434–2440, 1997.

[96] K. M. Davey, J. S. Parboosingh, D. R. McLeod et al., "Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition," *Journal of Medical Genetics*, vol. 43, no. 5, pp. 385–393, 2006.

[97] D. J. Lefeber, A. P. M. de Brouwer, E. Morava et al., "Autosomal recessive dilated cardiomyopathy due to DOLK mutations results from abnormal dystroglycan O-mannosylation," *PLoS Genetics*, vol. 7, no. 12, Article ID e1002427, 2011.

[98] P. Elliott, C. O'Mahony, P. Syrris et al., "Prevalence of desmosomal protein gene mutations in patients with dilated cardiomyopathy," *Circulation: Cardiovascular Genetics*, vol. 3, no. 4, pp. 314–322, 2010.

[99] M. G. Posch, M. J. Posch, C. Geier et al., "A missense variant in desmoglein-2 predisposes to dilated cardiomyopathy," *Molecular Genetics and Metabolism*, vol. 95, no. 1-2, pp. 74–80, 2008.

[100] E. E. Norgett, S. J. Hatsell, L. Carvajal-Huerta et al., "Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma," *Human Molecular Genetics*, vol. 9, no. 18, pp. 2761–2766, 2000.

[101] J. Schönberger, L. Wang, J. T. Shin et al., "Mutation in the transcriptional coactivator EYA4 causes dilated cardiomyopathy and sensorineural hearing loss," *Nature Genetics*, vol. 37, no. 4, pp. 418–422, 2005.

[102] T. Arimura, T. Hayashi, Y. Matsumoto et al., "Structural analysis of four and half LIM protein-2 in dilated cardiomyopathy," *Biochemical and Biophysical Research Communications*, vol. 357, no. 1, pp. 162–167, 2007.

[103] T. Murakami, Y. K. Hayashi, S. Noguchi et al., "Fukutin gene mutations cause dilated cardiomyopathy with minimal muscle weakness," *Annals of Neurology*, vol. 60, no. 5, pp. 597–602, 2006.

[104] T. Müller, M. Krasnianski, R. Witthaut, M. Deschauer, and S. Zierz, "Dilated cardiomyopathy may be an early sign of the C826A Fukutin-related protein mutation," *Neuromuscular Disorders*, vol. 15, no. 5, pp. 372–376, 2005.

[105] P. Minoretti, M. Arra, E. Emanuele et al., "A W148R mutation in the human FOXD4 gene segregating with dilated cardiomyopathy, obsessive-compulsive disorder, and suicidality," *International Journal of Molecular Medicine*, vol. 19, no. 3, pp. 369–372, 2007.

[106] J. L. Theis, K. M. Sharpe, M. E. Matsumoto et al., "Homozygosity mapping and exome sequencing reveal GATAD1 mutation in autosomal recessive dilated cardiomyopathy," *Circulation: Cardiovascular Genetics*, vol. 4, no. 6, pp. 585–594, 2011.

[107] B. Meder, F. Rühle, T. Weis et al., "A genome-wide association study identifies 6p21 as novel risk locus for dilated cardiomyopathy," *European Heart Journal*, vol. 35, no. 16, pp. 1069–1077, 2014.

[108] S. Pankuweit, V. Ruppert, T. Jónsdóttir, H.-H. Müller, and T. Meyer, "The HLA class II allele DQB1*0309 is associated with dilated cardiomyopathy," *Gene*, vol. 531, no. 2, pp. 180–183, 2013.

[109] K. Stark, U. B. Esslinger, W. Reinhard et al., "Genetic association study identifies *HSPB7* as a risk gene for idiopathic dilated cardiomyopathy," *PLoS genetics*, vol. 6, no. 10, 2010.

[110] R. Knöll, R. Postel, J. Wang et al., "Laminin-alpha4 and integrin-linked kinase mutations cause human cardiomyopathy via simultaneous defects in cardiomyocytes and endothelial cells," *Circulation*, vol. 116, no. 5, pp. 515–525, 2007.

[111] N. Carboni, G. Marrosu, M. Porcu et al., "Dilated cardiomyopathy with conduction defects in a patient with partial merosin deficiency due to mutations in the laminin-$\alpha$2-chain gene: a chance association or a novel phenotype?" *Muscle & Nerve*, vol. 44, no. 5, pp. 826–828, 2011.

[112] B. J. Maron, W. C. Roberts, M. Arad et al., "Clinical outcome and phenotypic expression in LAMP2 cardiomyopathy," *The Journal of the American Medical Association*, vol. 301, no. 12, pp. 1253–1259, 2009.

[113] T. Arimura, T. Hayashi, H. Terada et al., "A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C," *The Journal of Biological Chemistry*, vol. 279, no. 8, pp. 6746–6752, 2004.

[114] D. Fatkin, C. Macrae, T. Sasaki et al., "Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease," *The New England Journal of Medicine*, vol. 341, no. 23, pp. 1715–1724, 1999.

[115] G. Rodriguez, T. Ueyama, T. Ogata et al., "Molecular genetic and functional characterization implicate muscle-restricted coiled-coil gene (MURC) as a causal gene for familial dilated cardiomyopathy," *Circulation: Cardiovascular Genetics*, vol. 4, no. 4, pp. 349–358, 2011.

[116] S. Daehmlow, J. Erdmann, T. Knueppel et al., "Novel mutations in sarcomeric protein genes in dilated cardiomyopathy," *Biochemical and Biophysical Research Communications*, vol. 298, no. 1, pp. 116–120, 2002.

[117] R. E. Hershberger, N. Norton, A. Morales, D. Li, J. D. Siegfried, and J. Gonzalez-Quintana, "Coding sequence rare variants identified in MYBPC3, MYH6, TPM1, TNNC1, and TNNI3 from 312 patients with familial or idiopathic dilated cardiomyopathy," *Circulation: Cardiovascular Genetics*, vol. 3, no. 2, pp. 155–161, 2010.

[118] E. Carniel, M. R. G. Taylor, G. Sinagra et al., "$\alpha$-myosin heavy chain: a sarcomeric gene associated with dilated and hypertrophic phenotypes of cardiomyopathy," *Circulation*, vol. 112, no. 1, pp. 54–59, 2005.

[119] L. Duboscq-Bidot, P. Xu, P. Charron et al., "Mutations in the Z-band protein myopalladin gene and idiopathic dilated cardiomyopathy," *Cardiovascular Research*, vol. 77, no. 1, pp. 118–125, 2008.

[120] E. Purevjav, J. Varela, M. Morgado et al., "Nebulette mutations are associated with dilated cardiomyopathy and endocardial fibroelastosis," *Journal of the American College of Cardiology*, vol. 56, no. 18, pp. 1493–1502, 2010.

[121] D. Hassel, T. Dahme, J. Erdmann et al., "Nexilin mutations destabilize cardiac Z-disks and lead to dilated cardiomyopathy," *Nature Medicine*, vol. 15, no. 11, pp. 1281–1288, 2009.

[122] L. S. Matsa, A. Rangaraju, V. Vengaldas et al., "Haplotypes of NOS3 gene polymorphisms in dilated cardiomyopathy," *PLoS ONE*, vol. 8, no. 7, Article ID e70523, 2013.

[123] K. Haghighi, F. Kolokathis, L. Pater et al., "Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human," *Journal of Clinical Investigation*, vol. 111, no. 6, pp. 869–876, 2003.

[124] A.-K. Arndt, S. Schafer, J.-D. Drenckhahn et al., "Fine mapping of the 1p36 deletion syndrome identifies mutation of PRDM16 as a cause of cardiomyopathy," *American Journal of Human Genetics*, vol. 93, no. 1, pp. 67–77, 2013.

[125] D. Li, S. B. Parks, J. D. Kushner et al., "Mutations of presenilin genes in dilated cardiomyopathy and heart failure," *The American Journal of Human Genetics*, vol. 79, no. 6, pp. 1030–1039, 2006.

[126] K. M. Brauch, M. L. Karst, K. J. Herron et al., "Mutations in ribonucleic acid binding protein gene cause familial dilated cardiomyopathy," *Journal of the American College of Cardiology*, vol. 54, no. 10, pp. 930–941, 2009.

[127] Z. A. Bhuiyan, M. P. van den Berg, J. P. van Tintelen et al., "Expanding spectrum of human RYR2-related disease: new electrocardiographic, structural, and genetic features," *Circulation*, vol. 116, no. 14, pp. 1569–1576, 2007.

[128] W. P. McNair, L. Ku, M. R. G. Taylor et al., "SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia," *Circulation*, vol. 110, no. 15, pp. 2163–2167, 2004.

[129] A. Levitas, E. Muhammad, G. Harel et al., "Familial neonatal isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase," *European Journal of Human Genetics*, vol. 18, no. 10, pp. 1160–1165, 2010.

[130] S. Tsubata, K. R. Bowles, M. Vatta et al., "Mutations in the human δ-sarcoglycan gene in familial and sporadic dilated cardiomyopathy," *Journal of Clinical Investigation*, vol. 106, no. 5, pp. 655–662, 2000.

[131] Q. Zhang, C. Bethmann, N. F. Worth et al., "Nesprin-1 and -2 are involved in the pathogenesis of Emery–Dreifuss muscular dystrophy and are critical for nuclear envelope integrity," *Human Molecular Genetics*, vol. 16, no. 23, pp. 2816–2833, 2007.

[132] S. Bione, P. D'Adamo, E. Maestrini, A. K. Gedeon, P. A. Bolhuis, and D. Toniolo, "A novel X-linked gene, G4.5. is responsible for Barth syndrome," *Nature Genetics*, vol. 12, no. 4, pp. 385–389, 1996.

[133] E. P. Kirk, M. Sunde, M. W. Costa et al., "Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy," *American Journal of Human Genetics*, vol. 81, no. 2, pp. 280–291, 2007.

[134] T. Hayashi, T. Arimura, M. Itoh-Satoh et al., "Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy," *Journal of the American College of Cardiology*, vol. 44, no. 11, pp. 2192–2201, 2004.

[135] M. R. G. Taylor, D. Slavov, A. Gajewski et al., "Thymopoietin (lamina-associated polypeptide 2) gene mutation associated with dilated cardiomyopathy," *Human Mutation*, vol. 26, no. 6, pp. 566–574, 2005.

[136] R. T. Murphy, J. Mogensen, A. Shaw, T. Kubo, S. Hughes, and W. J. McKenna, "Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy," *The Lancet*, vol. 363, no. 9406, pp. 371–372, 2004.

[137] M. Kamisago, S. D. Sharma, S. R. DePalma et al., "Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy," *The New England Journal of Medicine*, vol. 343, no. 23, pp. 1688–1696, 2000.

[138] D. Sibbing, A. Pfeufer, T. Perisic et al., "Mutations in the mitochondrial thioredoxin reductase gene TXNRD2 cause dilated cardiomyopathy," *European Heart Journal*, vol. 32, no. 9, pp. 1121–1133, 2011.

[139] B. Gerull, M. Gramlich, J. Atherton et al., "Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy," *Nature Genetics*, vol. 30, no. 2, pp. 201–204, 2002.

[140] T. M. Olson, S. Illenberger, N. Y. Kishimoto, S. Huttelmaier, M. T. Keating, and B. M. Jockusch, "Metavinculin mutations alter actin interaction in dilated cardiomyopathy," *Circulation*, vol. 105, no. 4, pp. 431–437, 2002.

[141] X. Li, R. Luo, X. Mo et al., "Polymorphism of ZBTB17 gene is associated with idiopathic dilated cardiomyopathy: a case control study in a Han Chinese population," *European Journal of Medical Research*, vol. 18, no. 1, article 10, 2013.

[142] C. Andreasen, J. B. Nielsen, L. Refsgaard et al., "New population-based exome data are questioning the pathogenicity of previously cardiomyopathy-associated genetic variants," *European Journal of Human Genetics*, vol. 21, no. 9, pp. 918–928, 2013.

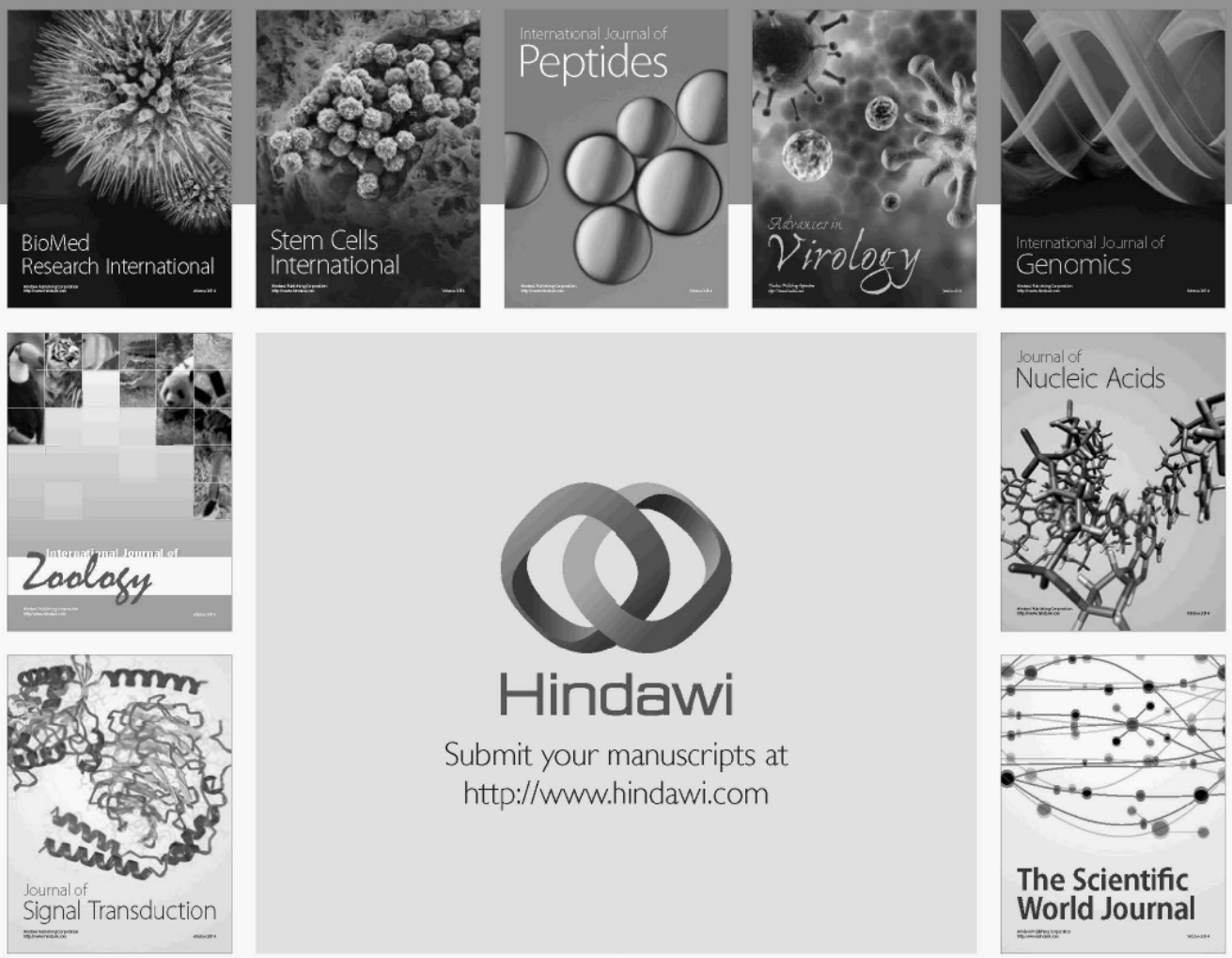
[143] U. Philipp, A. Vollmar, and O. Distl, "Evaluation of six candidate genes for dilated cardiomyopathy in Irish wolfhounds," *Animal Genetics*, vol. 39, no. 1, pp. 88–89, 2008.

[144] M. Lynne O'Sullivan, M. R. O'Grady, W. Glen Pyle, and J. F. Dawson, "Evaluation of 10 genes encoding cardiac proteins in Doberman Pinschers with dilated cardiomyopathy," *American Journal of Veterinary Research*, vol. 72, no. 7, pp. 932–938, 2011.

[145] P. Stabej, P. A. J. Leegwater, S. Imholz et al., "The canine sarcoglycan delta gene: BAC clone contig assembly, chromosome assignment and interrogation as a candidate gene for dilated cardiomyopathy in Dobermann dogs," *Cytogenetic and Genome Research*, vol. 111, no. 2, pp. 140–146, 2005.

[146] U. Philipp, A. Vollmar, and O. Distl, "Evaluation of the *Titin-Cap Gene* (*TCAP*) as candidate for dilated cardiomyopathy in Irish wolfhounds," *Animal Biotechnology*, vol. 19, no. 4, pp. 231–236, 2008.

[147] M. A. Oyama and S. Chittur, "Genomic expression patterns of cardiac tissues from dogs with dilated cardiomyopathy," *American Journal of Veterinary Research*, vol. 66, no. 7, pp. 1140–1155, 2005.



**From:** Darcy Adin <dbadin@ncsu.edu>
**To:** Jones, Jennifer L
**CC:** B6
**Sent:** 1/25/2019 10:01:01 PM
**Subject:** Sample?

Hi Jen,

I hope you are doing well - not sure how the partial government shutdown is impacting your area specifically?

B6 was able to collect fresh frozen myocardium from one of our presumed diet induced DCM cases and we are wondering if we should hang on to this in a -80 freezer or send you the sample for testing? This is an almost 2yr MI Yorkie mix that was diagnosed in April 2018 and was eating Castor and Pollux Organic GF Small Breed. The owners tried to change the diet to a grain based Royal canin diet but because of lack of interest he was changed to Primal (raw and grain free). He represented in September 2018 for CHF and was then changed to Fromm Adult Gold Small breed, grain-based supplemented with boiled chicken and rice. Progressive disease was noted at each exam echocardiographically with no improvement in systolic function. His whole blood taurine was B6 but he was still supplemented with taurine.

Thanks for your thoughts!
Take care
Darcy

**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** 'Darcy Adin'
**CC:** B6
**Sent:** 1/30/2019 4:37:05 PM
**Subject:** RE: Sample?

Hi Darcy,
Thank you for the kind words. Yes B6 d limited in my ability to respond to emails. I apologize for the delay.

We are definitely interested in the case. We'd just need a complaint submitted through the Safety Reporting Portal found here: https://www.safetyreporting.hhs.gov/

After you submit the report, please send me the ICSR number (confirmation of report submission). We can send you a box to collect the tissue. Was there also a full necropsy report with medical records you could share as well? Those can be attached to the report you submit.
Please let me know if you have questions.
Thank you again for your help,
Jen

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421

-----Original Message-----
From: Darcy Adin <dbadin@ncsu.edu>
Sent: Friday, January 25, 2019 5:01 PM
To: Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
Cc: B6
Subject: Sample?

Hi Jen,

I hope you are doing well - not sure how the partial government shutdown is impacting your area specifically?

B6 was able to collect fresh frozen myocardium from one of our presumed diet induced DCM cases and we are wondering if we should hang on to this in a -80 freezer or send you the sample for testing? This is an almost 2yr MI Yorkie mix that was diagnosed in April 2018 and was eating Castor and Pollux Organic GF Small Breed. The owners tried to change the diet to a grain based Royal canin diet but because of lack of interest he was changed to Primal (raw and grain free). He represented in September 2018 for CHF and was then changed to Fromm Adult Gold Small breed, grain-based supplemented with boiled chicken and rice. Progressive disease was noted at each exam echocardiographically with no improvement in systolic function. His whole blood taurine was B6 but he was still supplemented with taurine.

Thanks for your thoughts!
Take care
Darcy

**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** 'Freeman, Lisa'
**Sent:** 8/21/2018 2:45:53 PM
**Subject:** RE: updates

Thank you, Lisa.
We're going to send you the box this week with 7 whirl-pak bags. Each bag will be labelled for the dog and our internal identifier number (EON-XXXXXX). Please fill the bags with the respective food. I've calculated the return weight based on filling 7 bags full.

Also, I have the medical records for B6, but did you submit a pet food report for him? I'm wondering if I didn't see it on our end.

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Monday, August 20, 2018 6:18 PM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** updates

Hi Jen
I forgot to note on the report I submitted today that I have a food sample and UPC code for the Acana food that the 2 Dobies were eating.

Also, for B6 whose heart has improved significantly, I just got a sample from the owner who found some food remaining at her summer house – it is not fresh but I'm saving for you in case you want
Thanks
Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Board Certified Veterinary Nutritionist™
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** 'Freeman, Lisa'
**CC:** Reimschuessel, Renate
**Sent:** 7/20/2018 12:46:42 PM
**Subject:** RE: 800.267-FDA Case Investigation for B6 EON-358523)

Good morning Lisa,
Yes, we got the reports you previously submitted and recorded the information for our database. Will you please forward any medical records for:

- B6 are you able to send any updates on the Taurine testing or echocardiogram (if done?)
- B6 -Also was an autopsy done?

Thank you in advance and for your time to report all the cases!
Jen

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Friday, July 20, 2018 8:06 AM
**To:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Renata and Jennifer
That seems reasonable. I was never contacted about the other cases that I submitted. There was some confusion about the way I submitted them so I want to be sure you actually got them B6 B6 I'm sure you're all getting slammed with reports (and there will probably be even more coming now) but just wanted to check to be sure they got recorded.
Thanks
Lisa

**From:** Reimschuessel, Renate [mailto:Renate.Reimschuessel@fda.hhs.gov]
**Sent:** Friday, July 20, 2018 7:55 AM
**To:** Freeman, Lisa <Lisa.Freeman@tufts.edu>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Lisa
Thanks for gathering the information.
I think, since we are getting so many reports since our CVM update, we should pass on the B6 as it is not clear-cut.

I think Jen is more familiar with the B6 case, so I'll let her respond regarding that one.
Thank you again for all your work on this investigation.
rr

Renate Reimschuessel V.M.D. Ph.D. Director Vet-LIRN
*Phone 1- 240-402-5404*
Fax 301-210-4685

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Thursday, July 19, 2018 5:59 PM
**To:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Renate
In looking back through this case, I'm not sure this is a completely clear-cut one. The dog has degenerative mitral valve disease and CHF but also has reduced cardiac contractility so might be a combination.
Do you still want me to collect the info below?

Also, I have an update on B6 who died at home last week. I do have food from the owner if you want that.
Thanks
Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Sent:** Tuesday, July 17, 2018 11:48 AM
**To:** Freeman, Lisa <lisa.freeman@tufts.edu>
**Subject:** 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Dr. Freeman,
Thank you for submitting your consumer complaint to FDA. I'm sorry to hear about B6 illness.
As part of our investigation, we'd like to request:

- **Full Medical Records**
  - Please email (preferred) or fax (301-210-4685) a copy of B6 **entire** medical history (not just this event), including any referral diagnostics.
- **Phone interview** about B6 diet and environmental exposures
  - Please confirm permission to contact the owner.
  - The interview generally lasts 30 minutes.

I attached a copy of our Vet-LIRN network procedures. The procedures describe how Vet-LIRN operates and how veterinarians help with our case investigations.
**Please respond to this email so that we can initiate our investigation.**

Thank you kindly, especially for submitting multiple cases,
Dr. Reimschuessel

Renate Reimschuessel V.M.D. Ph.D.
Director: Vet-LIRN
*(Veterinary Laboratory Investigation and Response Network)*

Center For Veterinary Medicine, FDA,
8401 Muirkirk Road, Laurel, MD 20708
*Phone 1-240-402-5404* Fax 301-210-4685
*EMAIL :* renate.reimschuessel@fda.hhs.gov

Vet-LIRN

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

Phish-Pharm

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ToolsResources/Phish-Pharm/default.htm

Aquaculture

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ResearchAreas/ucm130892.htm

**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** 'Freeman, Lisa'
**Sent:** 8/3/2018 10:35:50 AM
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Thank you, Lisa. We will collect all the food you have. Can you please forward the best address for us to ship the box? You'll reuse the box to ship the food to us using the provided return label.
How much is the total weight and size of the food, collectively?

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Friday, August 03, 2018 5:23 AM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Hi Jen
I'm attaching records from B6 re: B6
She's also given permission for you to contact her.

B6

I still have food in my office from

B6

if you want any of that

Thanks
Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Board Certified Veterinary Nutritionist™
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Sent:** Friday, July 20, 2018 8:47 AM
**To:** Freeman, Lisa <lisa.freeman@tufts.edu>
**Cc:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Good morning Lisa,

Yes, we got the reports you previously submitted and recorded the information for our database. Will you please forward any medical records for:

- B6 are you able to send any updates on the Taurine testing or echocardiogram (if done?)
- B6 -Also was an autopsy done?

Thank you in advance and for your time to report all the cases!
Jen

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Friday, July 20, 2018 8:06 AM
**To:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Renata and Jennifer
That seems reasonable. I was never contacted about the other cases that I submitted. There was some confusion about the way I submitted them so I want to be sure you actually got them B6 B6 I'm sure you're all getting slammed with reports (and there will probably be even more coming now) but just wanted to check to be sure they got recorded.
Thanks
Lisa

**From:** Reimschuessel, Renate [mailto:Renate.Reimschuessel@fda.hhs.gov]
**Sent:** Friday, July 20, 2018 7:55 AM
**To:** Freeman, Lisa <Lisa.Freeman@tufts.edu>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Lisa
Thanks for gathering the information.
I think, since we are getting so many reports since our CVM update, we should pass on the B6 case as it is not clear-cut.

I think Jen is more familiar with the B6 case, so I'll let her respond regarding that one.
Thank you again for all your work on this investigation.
rr

Renate Reimschuessel V.M.D. Ph.D. Director Vet-LIRN
*Phone 1- 240-402-5404*
Fax 301-210-4685
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Thursday, July 19, 2018 5:59 PM
**To:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Renate

In looking back through this case, I'm not sure this is a completely clear-cut one. The dog has degenerative mitral valve disease and CHF but also has reduced cardiac contractility so might be a combination.
Do you still want me to collect the info below?

Also, I have an update on B6 who died at home last week. I do have food from the owner if you want that.
Thanks
Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Sent:** Tuesday, July 17, 2018 11:48 AM
**To:** Freeman, Lisa <lisa.freeman@tufts.edu>
**Subject:** 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Dr. Freeman,
Thank you for submitting your consumer complaint to FDA. I'm sorry to hear about B6 illness.
As part of our investigation, we'd like to request:

- **Full Medical Records**
  - Please email (preferred) or fax (301-210-4685) a copy o B6 **entire** medical history (not just this event), including any referral diagnostics.
- **Phone interview** about B6 diet and environmental exposures
  - Please confirm permission to contact the owner.
  - The interview generally lasts 30 minutes.

I attached a copy of our Vet-LIRN network procedures. The procedures describe how Vet-LIRN operates and how veterinarians help with our case investigations.
**Please respond to this email so that we can initiate our investigation.**

Thank you kindly, especially for submitting multiple cases,
Dr. Reimschuessel

Renate Reimschuessel V.M.D. Ph.D.
Director: Vet-LIRN
*(Veterinary Laboratory Investigation and Response Network)*
Center For Veterinary Medicine, FDA,
8401 Muirkirk Road, Laurel, MD 20708
*Phone 1-240-402-5404* Fax 301-210-4685
*EMAIL :* renate.reimschuessel@fda.hhs.gov

Vet-LIRN

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

Phish-Pharm

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ToolsResources/Phish-Pharm/default.htm

Aquaculture

**From:** Freeman, Lisa <Lisa.Freeman@tufts.edu>
**To:** Jones, Jennifer L
**Sent:** 8/3/2018 10:43:55 AM
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

One 30 pound bag and then about 3-4 pounds from the other 2 samples

My address is

Lisa Freeman
Department of Clinical Sciences
Tufts Cummings School of Veterinary Medicine
200 Westboro Road
North Grafton, MA 01536
508-887-4523

Thanks
Lisa

**From:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Sent:** Friday, August 03, 2018 6:36 AM
**To:** Freeman, Lisa <lisa.freeman@tufts.edu>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Thank you, Lisa. We will collect all the food you have. Can you please forward the best address for us to ship the box? You'll reuse the box to ship the food to us using the provided return label.
How much is the total weight and size of the food, collectively?

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Friday, August 03, 2018 5:23 AM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Hi Jen
I'm attaching records from B6 re: B6
She's also given permission for you to contact her.

B6

I still have food in my office from

B6

if you want any of that

Thanks
Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Board Certified Veterinary Nutritionist™
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Sent:** Friday, July 20, 2018 8:47 AM
**To:** Freeman, Lisa <lisa.freeman@tufts.edu>
**Cc:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Good morning Lisa,
Yes, we got the reports you previously submitted and recorded the information for our database. Will you please forward any medical records for:

- B6 are you able to send any updates on the Taurine testing or echocardiogram (if done?)
- B6 Also was an autopsy done?

Thank you in advance and for your time to report all the cases!
Jen

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Friday, July 20, 2018 8:06 AM
**To:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Renata and Jennifer
That seems reasonable. I was never contacted about the other cases that I submitted. There was some confusion about the way I submitted them so I want to be sure you actually got them B6 B6. I'm sure you're all getting slammed with reports (and there will probably be even more coming now) but just wanted to check to be sure they got recorded.
Thanks
Lisa

**From:** Reimschuessel, Renate [mailto:Renate.Reimschuessel@fda.hhs.gov]
**Sent:** Friday, July 20, 2018 7:55 AM
**To:** Freeman, Lisa <Lisa.Freeman@tufts.edu>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Lisa
Thanks for gathering the information.
I think, since we are getting so many reports since our CVM update, we should pass on the B6 case as it is

not clear-cut.

I think Jen is more familiar with the B6 case, so I'll let her respond regarding that one.
Thank you again for all your work on this investigation.
rr

Renate Reimschuessel V.M.D. Ph.D. Director Vet-LIRN
***Phone 1- 240-402-5404***
Fax 301-210-4685
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

**From:** Freeman, Lisa [mailto:Lisa.Freeman@tufts.edu]
**Sent:** Thursday, July 19, 2018 5:59 PM
**To:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** RE: 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Renate
In looking back through this case, I'm not sure this is a completely clear-cut one. The dog has degenerative mitral valve disease and CHF but also has reduced cardiac contractility so might be a combination.
Do you still want me to collect the info below?

Also, I have an update on B6 who died at home last week. I do have food from the owner if you want that.
Thanks
Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Sent:** Tuesday, July 17, 2018 11:48 AM
**To:** Freeman, Lisa <lisa.freeman@tufts.edu>
**Subject:** 800.267-FDA Case Investigation for B6 (EON-358523)

Dear Dr. Freeman,
Thank you for submitting your consumer complaint to FDA. I'm sorry to hear about B6 illness.
As part of our investigation, we'd like to request:

- **Full Medical Records**
  - Please email (preferred) or fax (301-210-4685) a copy of B6 **entire** medical history (not just this event), including any referral diagnostics.
- **Phone interview** about B6 diet and environmental exposures
  - Please confirm permission to contact the owner.
  - The interview generally lasts 30 minutes.

I attached a copy of our Vet-LIRN network procedures. The procedures describe how Vet-LIRN operates and how veterinarians help with our case investigations.
**Please respond to this email so that we can initiate our investigation.**

Thank you kindly, especially for submitting multiple cases,
Dr. Reimschuessel

Renate Reimschuessel V.M.D. Ph.D.
Director: Vet-LIRN
*(Veterinary Laboratory Investigation and Response Network)*
Center For Veterinary Medicine, FDA,
8401 Muirkirk Road, Laurel, MD 20708
***Phone 1- 240-402-5404*** Fax 301-210-4685
***EMAIL :*** renate.reimschuessel@fda.hhs.gov

Vet-LIRN

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

Phish-Pharm

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ToolsResources/Phish-Pharm/default.htm

Aquaculture

http://www.fda.gov/AnimalVeterinary/ScienceResearch/ResearchAreas/ucm130892.htm

| | |
|---|---|
| **From:** | Reimschuessel, Renate </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=4C00C47AE2794134B2906D6B9252FCF6-RREIMSCH> |
| **To:** | lisa.freeman@tufts.edu |
| **Sent:** | 7/17/2018 3:44:26 PM |
| **Subject:** | 800.267-FDA Case Investigation for B6 EON-358522) |
| **Attachments:** | 02-Vet-LIRN-NetworkProceduresVets-12.22.2015.pdf; 03-Vet-LIRN-Network ProceduresOwners-12.22.2015.pdf |

Dear Dr. Freeman,
Thank you for submitting your consumer complaint to FDA. I'm sorry to hear about B6 illness.
As part of our investigation, we'd like to request:

- **Full Medical Records**
  - Please email (preferred) or fax (301-210-4685) a copy of B6 **entire** medical history (not just this event), including any referral diagnostics.
- **Phone interview** about B6 diet and environmental exposures
  - Please confirm permission to contact the owner.
  - The interview generally lasts 30 minutes.

I attached a copy of our Vet-LIRN network procedures. The procedures describe how Vet-LIRN operates and how veterinarians help with our case investigations.
**Please respond to this email so that we can initiate our investigation.**
Thank you kindly,
Dr. Reimschuessel

Renate Reimschuessel V.M.D. Ph.D.
Director: Vet-LIRN
*(Veterinary Laboratory Investigation and Response Network)*
Center For Veterinary Medicine, FDA,
8401 Muirkirk Road, Laurel, MD 20708
*Phone 1-240-402-5404* Fax 301-210-4685
*EMAIL :* renate.reimschuessel@fda.hhs.gov

Vet-LIRN
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

Phish-Pharm
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ToolsResources/Phish-Pharm/default.htm

Aquaculture
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ResearchAreas/ucm130892.htm

# Network Procedures for Veterinary Laboratory Investigation and Response Network Case Investigations

## Network Procedures for Veterinarians

### 1. Introduction

The purpose of this Network Procedure is to facilitate basic interactions between the Vet-LIRN Program Office (VPO) and veterinarians participating in Vet-LIRN case investigations. General procedures such as information flow, sample handling procedures, submission of reports and billing for services are discussed. The focus of most Vet-LIRN case investigations is on diagnostic samples, although occasionally animal food samples will also be submitted. Animal food testing conducted after receiving a consumer complaint is typically handled by FDA's Office of Regulatory Affairs (ORA) Laboratories or accredited laboratories.

1.1 In the case of Vet-LIRN investigations, the government is the client.

1.1.1 The government is requesting assistance in its investigation, and is requesting tests or services to be performed by your clinic during this investigation.

1.1.2 The government will pay for these services.

1.1.3 The owner is helping with the government's investigation of a regulated product.

1.1.4 The goal of the investigation is to determine if the product is at fault and why.

1.1.5 The government's investigation may not provide a definitive diagnosis for the patient's illness.

### 2. Case Background – Consumer complaint

2.1 Vet-LIRN obtains information about the cases we investigate from 3 main sources,

2.1.1 Consumer complaints (cc) - obtained by FDA Consumer Complaint Coordinators by phone

2.1.2 Electronic consumer complaint submissions through FDA's Food Safety Reporting Portal, and

2.1.3 Vet-LIRN partner laboratories.

*NOTE: Generally, the information received in a consumer complaint* ***is not*** *kept confidential. In most cases, only protected personal information (such as names and addresses) is withheld in an effort to prevent the complaint from being traced back to the individual who submitted it.*

## 3. Communications

3.1 VPO will discuss the case with the referring veterinarian and or the owner.

3.2 VPO evaluates the case history and determines a need for follow up testing to determine if the food (or drug) is the cause of the illness or death.

3.3 VPO contacts the appropriate member laboratory(-ies) (chosen based on location and capabilities) and provides initial information

3.3.1 In some cases only partial history is available

3.3.2 Follow up information will be sent as it becomes available.

3.4 VPO proposes the tests to be conducted and prepares billing documents.

3.5 VPO makes arrangements with the veterinarian to obtain and ship samples.

3.5.1 VPO receives test results and forwards the results to the veterinarian who will then communicate the results to the owner.

## 4. Case history

4.1 A complete medical history is essential,

4.1.1 age, sex, breed, animal's ID/name,

4.1.2 other animals affected,

4.1.3 duration of problem, lesion distribution (diagrams or photos are welcome),

4.1.4 treatment of problem (especially dose and duration of therapy) and response to treatment.

4.1.5 concomitant drugs or dietary supplements administered (not used for treatment of the reaction, but administered for other reasons at the same time or within a short time of the problem occurrence).

4.2 Vet-LIRN Case Numbers:

4.2.1 Include Vet-LIRN case number in all correspondence.

4.2.2 E-mail: include the Vet-LIRN case number as the first part of the subject line. This will help archiving data for each case.

4.3 Electronic submission of medical records and laboratory results is preferred.

4.4 Histories can also be submitted by FAX to Vet-LIRN (301-210-4685).

4.5 Information about follow-up visits related to the investigation and additional laboratory reports should be provided as soon as possible. Phone calls are very useful for

discussing cases in depth, but should be followed up with the medical records and lab reports.

4.5.1 Due to time difference around the country, email communication is often the best way to assure information is transferred in a timely manner.

## 5. Services Requested by VPO

5.1 Services typically tests will fall into 3 categories:

5.1.1 Office Examination

5.1.2 Clinical laboratory samples

5.1.3 Pathology

5.2 Office Examination:

5.2.1 To evaluate the current status of the patient.

5.2.2 To obtain samples from the patient for further analysis (blood, urine, feces).

5.3 Clinical Laboratory Samples:

5.3.1 VPO may ask for repeat analysis of new samples to be run either by the veterinary hospital, or by its usual testing laboratory.

5.3.2 Typical tests include clinical hematology, microbial cultures, urinalysis, and fecal examination.

5.3.3 Additional testing may be requested and the samples sent to a Vet-LIRN network laboratory.

5.4 Pathology:

5.4.1 Either submit the entire carcass or conduct a routine necropsy examination. Record your findings in detail and submit. Histopathology and microbiological cultures as appropriate.

5.4.1.1 Describe all lesions – location, color, size, texture.

5.4.1.2 Culture lesions or intestinal contents as deemed appropriate based on the history.

5.4.1.3 Save tissues for histopathology- be sure to use 10:1 formalin to tissue mass.

5.4.2 Histopathology tissues (preserve in 10% neutral buffered formalin 10:1 ratio fixative to tissue):

5.4.2.1 thyroid, thymus, lung, heart, liver, spleen, adrenal, kidney, pancreas, stomach, duodenum, jejunum, ileum, colon, urinary bladder, skeletal muscle, brain.

5.4.2.2 Request a duplicate set of H&E for submission to VPO for archiving.

5.5 Toxicology:

5.5.1 Freeze and hold tissues if there is any indication that a toxic substance may be involved:

5.5.1.1 brain (for organophosphates and carbamates),

5.5.1.2 eyes, liver, kidney, brain, stomach content, fat,

5.5.1.3 if available, serum, EDTA blood, urine.

5.5.2 Following a review of histopathology, VPO may select tissues to be analyzed and request that tissues be sent to a Vet-LIRN laboratory.

5.5.3 When the case is closed by VPO, samples can be disposed of. When in doubt, please ask.

5.5.3.1 The animal's remains can be disposed of following the laboratories' customary procedures.

## 6. Sample submissions

6.1 Normally, VPO prefers that the veterinarian, not the pet owner submit samples.

6.2 Arrangements for transport should be made with the VPO (see additional shipping instructions).

6.3 A Vet-LIRN Sample Submission Form, given by VPO to the veterinarian, should be provided to the veterinarian and should accompany all samples being sent to our Vet-LIRN laboratory, listing the recommended tests.

6.4 A Shipping Inventory Sheet, given by VPO to the veterinarian, should also be provided by VPO and should be submitted with all samples. This form will be filled out and faxed to the VPO (301-210-4685) by the receiving Vet-LIRN laboratory.

6.5 Vet-LIRN case numbers should be provided by the VPO and should be included on all samples and reports.

6.5.1 Rarely, an owner will deliver a specimen or an animal for necropsy directly to the participating laboratory. Vet-LIRN should notify the lab to expect the owner if this happens and will provide appropriate forms.

## 7. Sample types that Vet-LIRN may request from the Veterinarian

7.1 Entire bodies (fresh or frozen)

7.2 Organs from necropsy (fresh, frozen or formalin fixed)

7.3 Clinical samples (serum, blood, urine, feces, biopsy samples, cultures)

7.4 Food samples (open bag products from home)

## 8. Reporting

8.1 All reports from Vet-LIRN testing labs are submitted to VPO.

8.2 VPO will forward reports to the veterinarian, who should discuss the results with the owner.

8.3 If appropriate, VPO will forward reports to the owner.

## 9. Communications with Owners

9.1 General:

9.1.1 VPO usually will have contacted the owner to request permission and assistance in the investigation.

9.1.2 Vet-LIRN's investigation is focused on determining if a regulated product is the cause of the animal's illness. The testing requested by Vet-LIRN may not provide a definitive diagnosis

9.1.3 VPO will provide testing results to the veterinarian for communication to the owner. This ensures that:

9.1.3.1 Owners can be counseled on the interpretation of the test results,

9.1.3.2 Appropriate medical follow-up care based on test results can be recommended by the owner's veterinarian.

## 10. Billing

10.1 Vet-LIRN VPO can only pay for services which were requested and approved by VPO. Vet-LIRN cannot pay for treatment, or for diagnostic testing outside of the scope of the investigation.

10.2 Procurement and Billing Process: The following process needs to be followed in order to adhere to government regulations.

10.2.1 The veterinarian must provide estimates so a Purchase Request can be prepared. Estimates should include items such as office visit(s), in-house diagnostic test costs, biopsy or pathology costs and additional charges such as potential shipping charges.

10.2.2 A billing contact must be provided: include name, address, telephone + fax numbers, and email.

10.2.3 Approved Purchase Request is required prior to beginning service.

10.2.4 Additional services may only be initiated after authorized by Vet-LIRN, but must first be approved by VPO with an additional Purchase Request.

10.2.5 Hospitals must provide an invoice to Vet-LIRN upon the completion of work before they can be paid. VPO is tax exempt. Taxes should be removed from all charges. The invoice must include the Vet-LIRN case number.

# Network Procedures for Veterinary Laboratory Investigation and Response Network Case Investigations

## Network Procedures for Owners

The purpose of this Network Procedure is to help you, the owner, understand how the Veterinary Laboratory Investigation and Response Network (Vet-LIRN) Program Office conducts case investigations (follow up to consumer complaints).

The following items are explained below:

- General Introduction
- Billing
- Step by Step Process
- Types of Services and Tests

## 1. General Introduction:

### 1.1. What is the goal of the case investigation?

The goal of the case investigation is to determine if the product is causing your pet's illness. Our case investigation MAY NOT provide a definitive diagnosis for your pet's illness, although we may rule out several other potential reasons for your pet's illness.

### 1.2. What is the focus of a case investigation?

Most case investigations focus on diagnostic samples (such as blood, urine or tissue from the pet), although we occasionally request and test pet food samples.

### 1.3. What is my veterinarian's role during the case investigation?

Your veterinarian helps our investigation into FDA- regulated products by providing information about your pet's medical history and by obtaining any diagnostic samples like blood, urine or tissue.

### 1.4. What will Vet-LIRN ask of me during a case investigation?

We may ask that your veterinarian perform certain tests or services or provide diagnostic samples to FDA or a Vet-LIRN cooperating laboratory.

### 1.5. Will Vet-LIRN pay for tests or services requested?

Yes, we will pay veterinarians or laboratories *for tests or services requested by Vet-LIRN* and approved through our government purchasing system. We cannot, however, reimburse owners for tests already performed or not specifically requested by Vet-LIRN. We recommend that you discuss with your veterinarian which tests and services will be billed to you and which will be covered by Vet-LIRN. For instance, Vet-LIRN may request that your veterinarian perform a urinalysis on your pet while he or she is hospitalized. Vet-LIRN will pay for the collection and testing of the sample, but would not cover the cost of your pet's stay in the hospital.

### 1.6. Is the information received in the consumer complaint confidential?

Generally, the information received in the consumer complaint is not kept confidential. In most cases, only protected personal information (such as names and addresses) is withheld in an effort to prevent the complaint from being traced back to the individual who submitted it.

## 2. Billing:

### 2.1. Will Vet-LIRN pay for bills related to the case investigation?

Vet-LIRN will cover the cost of services and testing that we specifically request. You should understand that Vet-LIRN *CANNOT* reimburse owners for any veterinary bills. Services *MUST* be pre-authorized and paid directly to the veterinarian.

### 2.2. Will Vet-LIRN pay for testing that was not requested by Vet-LIRN?

No, we will only pay for testing that we request and authorize.

### 2.3. Will Vet-LIRN pay for treatments or private cremation?

No, we cannot pay for treatment or cremation.

### 2.4. If I allow my veterinarian to submit my pet's body for testing, will I be able to have back his or her remains?

Each Vet-LIRN member laboratory has its own procedures for handling remains. Some Vet-LIRN member laboratories offer private cremation services for a fee payable directly to the laboratory. We advise you to discuss directly with the member laboratory the possibilities and costs for obtaining your pet's remains after examination are complete.

## 3. Step by Step Process:

**Vet-LIRN will do the following during a case investigation:**

3.1. Assign a case number which MUST be included in all correspondences
3.2. Discuss the case with you and your veterinarian
3.3. Request medical records from your veterinarian
3.4. Coordinate with your veterinarian and you to obtain and submit samples for testing
3.5. Provide results to your veterinarian who will discuss the results with you.

**Vet-LIRN requests that:**

3.6. Any follow-up veterinary visits related to the investigation are reported to Vet-LIRN
3.7. Additional laboratory reports are reported to Vet-LIRN by your veterinarian.

## 4. Types of Services and Tests:

### 4.1. What may a veterinary examination include once the case investigation is started?

A veterinary examination may include:

- an office visit and physical examination to assess your animals current health
- collection of clinical samples from your animal (blood, urine, feces).

### 4.2. Will your animal be tested more than once?

It is possible that Vet-LIRN may request additional tests or examinations depending on results from initial testing.

### 4.3. Will Vet-LIRN need to conduct a necropsy in the event of an animal death?

Yes, if you are willing, we may request that your veterinarian or another Vet-LIRN cooperating laboratory to conduct a necropsy to collect samples for testing. The samples collected may be tested right away or may be held for future testing or archiving. If the veterinarian completes the necropsy then the remains will be handled according the veterinarians normal procedures. If a Vet-LIRN cooperative laboratory completes the necropsy the remains are usually disposed of by that laboratory. Vet-LIRN cannot pay for private cremation. You are welcome to discuss normal procedures with the laboratory.

### 4.4. Will Vet-LIRN ask for a food sample?

Our main focus is on testing diagnostic tissue or fluid samples from the animal, but we may need to test the food. Please hold all food samples once the consumer complaint is submitted. If needed, we will make arrangements to collect the food.

### 4.5. What are some general tests that Vet-LIRN may request?

General tests that we may request include, but are not limited to:

- Hematology
- Microbial cultures
- Urinalysis
- Fecal examination
- Necropsy/Histology/Toxicology

### 4.6. Will I get results from Vet-LIRN requested tests?

Results of testing on your animal's diagnostic tissue or fluid samples will be forwarded to your veterinarian who will be asked to share the results with you.

# Network Procedures for Veterinary Laboratory Investigation and Response Network Case Investigations

## Network Procedures for Veterinarians

### 1. Introduction

The purpose of this Network Procedure is to facilitate basic interactions between the Vet-LIRN Program Office (VPO) and veterinarians participating in Vet-LIRN case investigations. General procedures such as information flow, sample handling procedures, submission of reports and billing for services are discussed. The focus of most Vet-LIRN case investigations is on diagnostic samples, although occasionally animal food samples will also be submitted. Animal food testing conducted after receiving a consumer complaint is typically handled by FDA's Office of Regulatory Affairs (ORA) Laboratories or accredited laboratories.

1.1 In the case of Vet-LIRN investigations, the government is the client.

1.1.1 The government is requesting assistance in its investigation, and is requesting tests or services to be performed by your clinic during this investigation.

1.1.2 The government will pay for these services.

1.1.3 The owner is helping with the government's investigation of a regulated product.

1.1.4 The goal of the investigation is to determine if the product is at fault and why.

1.1.5 The government's investigation may not provide a definitive diagnosis for the patient's illness.

### 2. Case Background – Consumer complaint

2.1 Vet-LIRN obtains information about the cases we investigate from 3 main sources,

2.1.1 Consumer complaints (cc) - obtained by FDA Consumer Complaint Coordinators by phone

2.1.2 Electronic consumer complaint submissions through FDA's Food Safety Reporting Portal, and

2.1.3 Vet-LIRN partner laboratories.

*NOTE: Generally, the information received in a consumer complaint* ***is not*** *kept confidential. In most cases, only protected personal information (such as names and addresses) is withheld in an effort to prevent the complaint from being traced back to the individual who submitted it.*

## 3. Communications

3.1 VPO will discuss the case with the referring veterinarian and or the owner.

3.2 VPO evaluates the case history and determines a need for follow up testing to determine if the food (or drug) is the cause of the illness or death.

3.3 VPO contacts the appropriate member laboratory(-ies) (chosen based on location and capabilities) and provides initial information

3.3.1 In some cases only partial history is available

3.3.2 Follow up information will be sent as it becomes available.

3.4 VPO proposes the tests to be conducted and prepares billing documents.

3.5 VPO makes arrangements with the veterinarian to obtain and ship samples.

3.5.1 VPO receives test results and forwards the results to the veterinarian who will then communicate the results to the owner.

## 4. Case history

4.1 A complete medical history is essential,

4.1.1 age, sex, breed, animal's ID/name,

4.1.2 other animals affected,

4.1.3 duration of problem, lesion distribution (diagrams or photos are welcome),

4.1.4 treatment of problem (especially dose and duration of therapy) and response to treatment.

4.1.5 concomitant drugs or dietary supplements administered (not used for treatment of the reaction, but administered for other reasons at the same time or within a short time of the problem occurrence).

4.2 Vet-LIRN Case Numbers:

4.2.1 Include Vet-LIRN case number in all correspondence.

4.2.2 E-mail: include the Vet-LIRN case number as the first part of the subject line. This will help archiving data for each case.

4.3 Electronic submission of medical records and laboratory results is preferred.

4.4 Histories can also be submitted by FAX to Vet-LIRN (301-210-4685).

4.5 Information about follow-up visits related to the investigation and additional laboratory reports should be provided as soon as possible. Phone calls are very useful for

discussing cases in depth, but should be followed up with the medical records and lab reports.

4.5.1 Due to time difference around the country, email communication is often the best way to assure information is transferred in a timely manner.

## 5. Services Requested by VPO

5.1 Services typically tests will fall into 3 categories:

5.1.1 Office Examination

5.1.2 Clinical laboratory samples

5.1.3 Pathology

5.2 Office Examination:

5.2.1 To evaluate the current status of the patient.

5.2.2 To obtain samples from the patient for further analysis (blood, urine, feces).

5.3 Clinical Laboratory Samples:

5.3.1 VPO may ask for repeat analysis of new samples to be run either by the veterinary hospital, or by its usual testing laboratory.

5.3.2 Typical tests include clinical hematology, microbial cultures, urinalysis, and fecal examination.

5.3.3 Additional testing may be requested and the samples sent to a Vet-LIRN network laboratory.

5.4 Pathology:

5.4.1 Either submit the entire carcass or conduct a routine necropsy examination. Record your findings in detail and submit. Histopathology and microbiological cultures as appropriate.

5.4.1.1 Describe all lesions – location, color, size, texture.

5.4.1.2 Culture lesions or intestinal contents as deemed appropriate based on the history.

5.4.1.3 Save tissues for histopathology- be sure to use 10:1 formalin to tissue mass.

5.4.2 Histopathology tissues (preserve in 10% neutral buffered formalin 10:1 ratio fixative to tissue):

5.4.2.1 thyroid, thymus, lung, heart, liver, spleen, adrenal, kidney, pancreas, stomach, duodenum, jejunum, ileum, colon, urinary bladder, skeletal muscle, brain.

5.4.2.2 Request a duplicate set of H&E for submission to VPO for archiving.

5.5 Toxicology:

5.5.1 Freeze and hold tissues if there is any indication that a toxic substance may be involved:

5.5.1.1 brain (for organophosphates and carbamates),

5.5.1.2 eyes, liver, kidney, brain, stomach content, fat,

5.5.1.3 if available, serum, EDTA blood, urine.

5.5.2 Following a review of histopathology, VPO may select tissues to be analyzed and request that tissues be sent to a Vet-LIRN laboratory.

5.5.3 When the case is closed by VPO, samples can be disposed of. When in doubt, please ask.

5.5.3.1 The animal's remains can be disposed of following the laboratories' customary procedures.

## 6. Sample submissions

6.1 Normally, VPO prefers that the veterinarian, not the pet owner submit samples.

6.2 Arrangements for transport should be made with the VPO (see additional shipping instructions).

6.3 A Vet-LIRN Sample Submission Form, given by VPO to the veterinarian, should be provided to the veterinarian and should accompany all samples being sent to our Vet-LIRN laboratory, listing the recommended tests.

6.4 A Shipping Inventory Sheet, given by VPO to the veterinarian, should also be provided by VPO and should be submitted with all samples. This form will be filled out and faxed to the VPO (301-210-4685) by the receiving Vet-LIRN laboratory.

6.5 Vet-LIRN case numbers should be provided by the VPO and should be included on all samples and reports.

6.5.1 Rarely, an owner will deliver a specimen or an animal for necropsy directly to the participating laboratory. Vet-LIRN should notify the lab to expect the owner if this happens and will provide appropriate forms.

## 7. Sample types that Vet-LIRN may request from the Veterinarian

7.1 Entire bodies (fresh or frozen)

7.2 Organs from necropsy (fresh, frozen or formalin fixed)

7.3 Clinical samples (serum, blood, urine, feces, biopsy samples, cultures)

7.4 Food samples (open bag products from home)

## 8. Reporting

8.1 All reports from Vet-LIRN testing labs are submitted to VPO.

8.2 VPO will forward reports to the veterinarian, who should discuss the results with the owner.

8.3 If appropriate, VPO will forward reports to the owner.

## 9. Communications with Owners

9.1 General:

9.1.1 VPO usually will have contacted the owner to request permission and assistance in the investigation.

9.1.2 Vet-LIRN's investigation is focused on determining if a regulated product is the cause of the animal's illness. The testing requested by Vet-LIRN may not provide a definitive diagnosis

9.1.3 VPO will provide testing results to the veterinarian for communication to the owner. This ensures that:

9.1.3.1 Owners can be counseled on the interpretation of the test results,

9.1.3.2 Appropriate medical follow-up care based on test results can be recommended by the owner's veterinarian.

## 10. Billing

10.1 Vet-LIRN VPO can only pay for services which were requested and approved by VPO. Vet-LIRN cannot pay for treatment, or for diagnostic testing outside of the scope of the investigation.

10.2 Procurement and Billing Process: The following process needs to be followed in order to adhere to government regulations.

10.2.1 The veterinarian must provide estimates so a Purchase Request can be prepared. Estimates should include items such as office visit(s), in-house diagnostic test costs, biopsy or pathology costs and additional charges such as potential shipping charges.

10.2.2 A billing contact must be provided: include name, address, telephone + fax numbers, and email.

10.2.3 Approved Purchase Request is required prior to beginning service.

10.2.4 Additional services may only be initiated after authorized by Vet-LIRN, but must first be approved by VPO with an additional Purchase Request.

10.2.5 Hospitals must provide an invoice to Vet-LIRN upon the completion of work before they can be paid. VPO is tax exempt. Taxes should be removed from all charges. The invoice must include the Vet-LIRN case number.

# Network Procedures for Veterinary Laboratory Investigation and Response Network Case Investigations

## Network Procedures for Owners

The purpose of this Network Procedure is to help you, the owner, understand how the Veterinary Laboratory Investigation and Response Network (Vet-LIRN) Program Office conducts case investigations (follow up to consumer complaints).

The following items are explained below:

- General Introduction
- Billing
- Step by Step Process
- Types of Services and Tests

## 1. General Introduction:

### 1.1. What is the goal of the case investigation?

The goal of the case investigation is to determine if the product is causing your pet's illness. Our case investigation MAY NOT provide a definitive diagnosis for your pet's illness, although we may rule out several other potential reasons for your pet's illness.

### 1.2. What is the focus of a case investigation?

Most case investigations focus on diagnostic samples (such as blood, urine or tissue from the pet), although we occasionally request and test pet food samples.

### 1.3. What is my veterinarian's role during the case investigation?

Your veterinarian helps our investigation into FDA- regulated products by providing information about your pet's medical history and by obtaining any diagnostic samples like blood, urine or tissue.

### 1.4. What will Vet-LIRN ask of me during a case investigation?

We may ask that your veterinarian perform certain tests or services or provide diagnostic samples to FDA or a Vet-LIRN cooperating laboratory.

### 1.5. Will Vet-LIRN pay for tests or services requested?

Yes, we will pay veterinarians or laboratories *for tests or services requested by Vet-LIRN* and approved through our government purchasing system. We cannot, however, reimburse owners for tests already performed or not specifically requested by Vet-LIRN. We recommend that you discuss with your veterinarian which tests and services will be billed to you and which will be covered by Vet-LIRN. For instance, Vet-LIRN may request that your veterinarian perform a urinalysis on your pet while he or she is hospitalized. Vet-LIRN will pay for the collection and testing of the sample, but would not cover the cost of your pet's stay in the hospital.

### 1.6. Is the information received in the consumer complaint confidential?

Generally, the information received in the consumer complaint is not kept confidential. In most cases, only protected personal information (such as names and addresses) is withheld in an effort to prevent the complaint from being traced back to the individual who submitted it.

## 2. Billing:

### 2.1. Will Vet-LIRN pay for bills related to the case investigation?

Vet-LIRN will cover the cost of services and testing that we specifically request. You should understand that Vet-LIRN *CANNOT* reimburse owners for any veterinary bills. Services *MUST* be pre-authorized and paid directly to the veterinarian.

### 2.2. Will Vet-LIRN pay for testing that was not requested by Vet-LIRN?

No, we will only pay for testing that we request and authorize.

### 2.3. Will Vet-LIRN pay for treatments or private cremation?

No, we cannot pay for treatment or cremation.

### 2.4. If I allow my veterinarian to submit my pet's body for testing, will I be able to have back his or her remains?

Each Vet-LIRN member laboratory has its own procedures for handling remains. Some Vet-LIRN member laboratories offer private cremation services for a fee payable directly to the laboratory. We advise you to discuss directly with the member laboratory the possibilities and costs for obtaining your pet's remains after examination are complete.

## 3. Step by Step Process:

**Vet-LIRN will do the following during a case investigation:**

3.1. Assign a case number which MUST be included in all correspondences
3.2. Discuss the case with you and your veterinarian
3.3. Request medical records from your veterinarian
3.4. Coordinate with your veterinarian and you to obtain and submit samples for testing
3.5. Provide results to your veterinarian who will discuss the results with you.

**Vet-LIRN requests that:**

3.6. Any follow-up veterinary visits related to the investigation are reported to Vet-LIRN
3.7. Additional laboratory reports are reported to Vet-LIRN by your veterinarian.

## 4. Types of Services and Tests:

### 4.1. What may a veterinary examination include once the case investigation is started?

A veterinary examination may include:

- an office visit and physical examination to assess your animals current health
- collection of clinical samples from your animal (blood, urine, feces).

### 4.2. Will your animal be tested more than once?

It is possible that Vet-LIRN may request additional tests or examinations depending on results from initial testing.

### 4.3. Will Vet-LIRN need to conduct a necropsy in the event of an animal death?

Yes, if you are willing, we may request that your veterinarian or another Vet-LIRN cooperating laboratory to conduct a necropsy to collect samples for testing. The samples collected may be tested right away or may be held for future testing or archiving. If the veterinarian completes the necropsy then the remains will be handled according the veterinarians normal procedures. If a Vet-LIRN cooperative laboratory completes the necropsy the remains are usually disposed of by that laboratory. Vet-LIRN cannot pay for private cremation. You are welcome to discuss normal procedures with the laboratory.

### 4.4. Will Vet-LIRN ask for a food sample?

Our main focus is on testing diagnostic tissue or fluid samples from the animal, but we may need to test the food. Please hold all food samples once the consumer complaint is submitted. If needed, we will make arrangements to collect the food.

### 4.5. What are some general tests that Vet-LIRN may request?

General tests that we may request include, but are not limited to:

- Hematology
- Microbial cultures
- Urinalysis
- Fecal examination
- Necropsy/Histology/Toxicology

### 4.6. Will I get results from Vet-LIRN requested tests?

Results of testing on your animal's diagnostic tissue or fluid samples will be forwarded to your veterinarian who will be asked to share the results with you.

**From:** Joshua A Stern <jstern@ucdavis.edu>
**To:** Jones, Jennifer L; Reimschuessel, Renate
**Sent:** 10/19/2018 5:11:29 PM
**Subject:** DCM question

HI Jennifer & Renate -
I'm giving a presentation at the ACVIM Cardiology conference (all board certified vet cardiologists will be the audience) and I'm giving an update on our work in golden retrievers. I will however be highlighting that goldens are a bit different and may not represent the problem so accurately.

I was hoping I could get a tiny bit of general info from you to update my slides. I'm happy to chat by phone if that is easier.

Here a few questions - that I would love to know if you are able to answer:

1.) how many confirmed cases are now part of your investigation (dogs, vs. cats)
2.) How many of your confirmed cases have measured low taurine levels
3.) Can you give me any idea of breed distribution (how many goldens, what are the top breeds, etc)
4.) Have all of the companies that manufacture diets fed to the cases been notified of the case being reported? If possible roughly how many diets are found in your case investigation (more than 20, more than 50, more than 100, etc)

I really appreciate your help if possible. The cardiologists would love to have a tiny bit more info and I'm in a position to pass this on to a captive audience tomorrow afternoon.

Also I'll be reviewing the recommendations for reporting and necropsy info too - so this will hopefully give the investigation a bit of a boos.

Best

Josh

Joshua Stern, DVM, PhD, DACVIM (Cardiology)
Associate Professor of Cardiology
Department of Medicine & Epidemiology
University of California Davis; CCAH Room 258
B6 (530) 752.2475 office
jstern@ucdavis.edu

Associate Editor - Journal of Veterinary Cardiology
www.journals.elsevier.com/journal-of-veterinary-cardiology

**From:** Joshua A Stern <jstern@ucdavis.edu>
**To:** Reimschuessel, Renate
**CC:** B6
**Sent:** 9/19/2018 4:24:14 PM
**Subject:** Necropsy on nutritionally mediate DCM case

Hi - I was contacted about a dog with DCM that was eating a suspect diet and was euthanized. I advised on necropsy procedure ant the clinician did this and collected samples. They now need to make contact with you about the process. They are CC'd on this email.

B6

Best

Josh

Joshua Stern, DVM, PhD, DACVIM (Cardiology)
Associate Professor of Cardiology
Department of Medicine & Epidemiology
University of California Davis; CCAH Room 258
B6 (530) 752.2475 office
jstern@ucdavis.edu

Associate Editor - Journal of Veterinary Cardiology
www.journals.elsevier.com/journal-of-veterinary-cardiology

**Patient History Report:** B6

**Clinic:**
B6

**Client:**
B6

Home Phone: B6
Work Phone1: B6 xCell
ID: B6 File #: 9819

**Patient:** B6
ID: B6
Tag: Chip: no
Species: Canine, Great Dane
Sex: male/neutered
Age: B6 DOB: B6
Weight: 67.2 Kg
Color: Merle
Last visit: B6
**Referred By:**

NO RDVM
NO RDVM
Tel: / Fax:

**Medical Record Entries:**
B6 4:18 PM

**Examination Recheck (Brief)** - PATIENT WAS SEEN IN: B6

PRESENTING COMPLAINT: Tachycardia

HISTORY:
B6 4y MI Great Dane, presented as a transfer for evaluation of tachycardia. For the past week, B6 has been intermittently coughing/vomiting clear liquid that occasionally contains kibble. MO has been intermittently feeding him kibble by dropping it over him while he's in a kennel and thought these episodes were secondary to him breathing in kibble occasionally. Today, B6 was much more lethargic than usual and was having difficulty getting up and down stairs. He was brought to an rDVM, where an irregular, fast heart rhythm was auscultated and he was transferred to B6 for continued evaluation.
B6 has a history of B6 MO reports no other health issues, no current medications.
B6 is fed a grain-free diet (Halo).

SUBJECTIVE:
QAR, anxious, clinically euhydrated

OBJECTIVE:

B6

PAIN ASSESSMENT: 0/5

DIAGNOSTICS COMPLETED:
ECG: tachycardia, concern for absent/buried P waves, irregular R-R interval
TFAST: Pleural fluid, minimal left ventricular contractility, enlarged left atrium
AFAST: Peritoneal fluid

ASSESSMENT:

Concern for DCM +/- a-fib
Peritoneal and pleural effusion

CLIENT COMMUNICATION:
Informed owner of concern for DCM. Strongly recommend a cardiology consultation. Offered treatment here, but ultimately will need follow up with cardiology. Discussed risk of sudden cardiac arrest. We've been seeing an association between grain-free diets and DCM, so may discuss submitting blood for a taurine level. If taurine deficient, may be able to reverse illness. There is a high likelihood that this is genetic, which will require lifetime treatment with medications. MO elected to transfer to Tufts for further workup.

FINANCIAL COMMUNICATION:
B6 for workup here

B6

rDVM COMMUNICATION (minimum of every 72hrs or after major event):
Initial rDVM or most recent contac B6

PLAN:
Diagnostic Pending or Planned:
Therapeutic/Monitoring Plan:

B6

DISCHARGE PLAN:
Transfer to Tufts Grafton, IVC in place

Attending Clinician: B6, DVM B6 DVM)

B6

B6

B6

# B6

# B6

B6

B6

# B6

# B6

**Lab Results:**

**11/30/2017**

| Assay Name | Value | Ref. Range | Units |
|---|---|---|---|
| GLU | B6 | 74-143 | mg/dL |
| CREA | | 0.5-1.8 | mg/dL |
| BUN | | 7-27 | mg/dL |
| BUN/CR | | 00000-00000 | |
| PHOS | | 2.5-6.8 | mg/dL |
| Ca | | 7.9-12.0 | mg/dL |
| TP | | 5.2-8.2 | g/dL |
| ALB | | 2.3-4.0 | g/dL |
| GLOB | | 2.5-4.5 | g/dL |
| ALB/GL | | 00000-00000 | |
| ALT | | 10-125 | U/L |
| ALKP | | 23-212 | U/L |
| GGT | | 0-11 | U/L |
| TBIL | | 0.0-0.9 | mg/dL |
| CHOL | | 110-320 | mg/dL |

B6

**1/23/2016**

| Assay Name | Value | Ref. Range | Units |
|---|---|---|---|
| BUN | B6 | 7-27 | mg/dL |
| CREA | | 0.5-1.8 | mg/dL |
| BUN/CR | | 00000-00000 | |

B6

**1/23/2016**

| Assay Name | Value | Ref. Range | Units |
|---|---|---|---|

cit-PT B6 11.0-17.0 second B6

9/21/2015

| Assay Name | Value | Ref. Range | Units |
|---|---|---|---|
| Ova and Parasite | B6 | | |
| | B6 | | |
| Giardia (ELISA) | B6 | | |

**From:** Jones, Jennifer L </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=0F6CA12EAA9348959A4CBB1E829AF244-JENNIFER.JO>
**To:** Peloquin, Sarah; Rotstein, David; Queen, Jackie L; Carey, Lauren; Palmer, Lee Anne
**CC:** Nemser, Sarah; Ceric, Olgica; Reimschuessel, Renate
**Sent:** 8/28/2018 12:37:37 PM
**Subject:** RE: 800.267 EON-358522 B6 Halo GF

Owner does have food. I don't believe the necropsy was done at Tufts but worth asking the owner if done at the rDVM.

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Peloquin, Sarah
**Sent:** Tuesday, August 28, 2018 8:32 AM
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>; Queen, Jackie L <Jackie.Queen@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Nemser, Sarah <Sarah.Nemser@fda.hhs.gov>; Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** 800.267 EON-358522 B6 Halo GF

Interview +/- food pending
Will ask if necropsy was performed

B6 – 4 yr MN Great Dane
No rDVM mrx per O
h/o B6 no other existing health conditions per cardio mrx; eats GF Halo

B6 ER visit for tachycardia; coughing, vomiting, and leth x1 week; PE: irregular heart rhythm, intermittent dropped pulses, harsh bilat BV sounds; EKG à tachycardia, absent/buried p waves, irregular RR interval; tFAST pleural fluid, minimal LV contract, incr LA size; aFAST peritoneal fluid à transferred to Tufts
B6 Tufts: HR 210-230, grade 2/6 murmur, weak pulses; echo à severe dilated LV w/ marked decr contract, thin LV walls, mod dilation LA, mild thicken mitral valve, trace pleural effusion, mild ascites, dilated hep vessels, FS 4.51% à DCM with secondary CHF, A-fib; WB tau B6 ; started B6
BID
B6 recheck at Tufts; not eating well, lethargic; gallop, murmur 2/6; echo à large volume ascites, no pleural/pericard effusion; EKG à a-fib, HR 220, rare VPCs; abdominocentesis removed 3.2 L straw fluid; BW showed elevated kidney values; added B6
Died at home B6

**From:** Rotstein, David
**Sent:** Monday, July 9, 2018 9:13 AM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Nemser, Sarah <Sarah.Nemser@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Queen, Jackie L <Jackie.Queen@fda.hhs.gov>
**Subject:** more DCMFW: Halo grain-free dry food (exact variety unknown): Lisa Freeman - EON-358522

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
**240-506-6763 (BB)**




**From:** PFR Event [mailto:pfreventcreation@fda.hhs.gov]
**Sent:** Monday, July 09, 2018 9:00 AM
**To:** Cleary, Michael * <Michael.Cleary@fda.hhs.gov>; HQ Pet Food Report Notification <HQPetFoodReportNotification@fda.hhs.gov>; B6
**Subject:** Halo grain-free dry food (exact variety unknown): Lisa Freeman - EON-358522

A PFR Report has been received and PFR Event [EON-358522] has been created in the EON System.

A "PDF" report by name "2051557-report.pdf" is attached to this email notification for your reference.

Below is the summary of the report:

**EON Key:** EON-358522
**ICSR #:** 2051557
**EON Title:** PFR Event created for Halo grain-free dry food (exact variety unknown); 2051557

| | | | |
|---|---|---|---|
| **AE Date** | B6 | **Number Fed/Exposed** | |
| **Best By Date** | | **Number Reacted** | 1 |
| **Animal Species** | Dog | **Outcome to Date** | Stable |
| **Breed** | Great Dane | | |
| **Age** | B6 Years | | |
| **District Involved** | PFR-New England DO | | |

**Product information**
**Individual Case Safety Report Number:** 2051557
**Product Group:** Pet Food

**Product Name:** Halo grain-free dry food (exact variety unknown)
**Description:** DCM and CHF Taurine not measured
**Submission Type:** Initial
**Report Type:** Adverse Event (a symptom, reaction or disease associated with the product)
**Outcome of reaction/event at the time of last observation:** Stable
**Number of Animals Reacted With Product:** 1

| Product Name | Lot Number or ID | Best By Date |
|---|---|---|
| Halo grain-free dry food (exact variety unknown) | | |

**Sender information**
Lisa Freeman
200 Westboro Rd
North Grafton, MA 01536
USA

**Owner information**
B6
USA

To view this PFR Event, please click the link below:
https://eon.fda.gov/eon//browse/EON-358522

To view the PFR Event Report, please click the link below:
https://eon.fda.gov/eon//EventCustomDetailsAction!viewReport.jspa?decorator=none&e=0&issueType=12&issueId=375146

==========

This email and attached document are being provided to you in your capacity as a Commissioned Official with the U.S. Department of Health and Human Services as authorized by law. You are being provided with this information pursuant to your signed Acceptance of Commission.

This email message is intended for the exclusive use of the recipient(s) named above. It may contain information that is protected, privileged, or confidential. Any dissemination, distribution, or copying is strictly prohibited.

The information is provided as part of the Federal-State Integration initiative. As a Commissioned Official and state government official, you are reminded of your obligation to protect non-public information, including trade secret and confidential commercial information that you receive from the U.S. Food and Drug Administration from further disclosure. The information in the report is intended for situational awareness and should not be shared or acted upon independently. Any and all actions regarding this information should be coordinated through your local district FDA office.

Failure to adhere to the above provisions could result in removal from the approved distribution list. If you think

you received this email in error, please send an email to FDAReportableFoods@fda.hhs.gov immediately.

**From:** Peloquin, Sarah </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=8607F880DF2B494AA639E6D9A3874132-SARAH.PELOQ>
**To:** Rotstein, David; Queen, Jackie L; Carey, Lauren; Palmer, Lee Anne
**CC:** Jones, Jennifer L; Nemser, Sarah; Ceric, Olgica; Reimschuessel, Renate
**Sent:** 9/6/2018 4:35:42 PM
**Subject:** RE: 800.267 EON-358522 B6 Halo GF
**Attachments:** EON-358522 owner interview 9.5.18.pdf

Interview attached, food pending
No necropsy performed

**From:** Peloquin, Sarah
**Sent:** Tuesday, August 28, 2018 8:31 AM
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>; Queen, Jackie L <Jackie.Queen@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Nemser, Sarah <Sarah.Nemser@fda.hhs.gov>; Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** 800.267 EON-358522 B6 Halo GF

Interview +/- food pending
Will ask if necropsy was performed

B6 y – 4 yr MN Great Dane
No rDVM mrx per O
h/o B6 no other existing health conditions per cardio mrx; eats GF Halo

B6 : ER visit for tachycardia; coughing, vomiting, and leth x1 week; PE: irregular heart rhythm, intermittent dropped pulses, harsh bilat BV sounds; EKG à tachycardia, absent/buried p waves, irregular RR interval; tFAST pleural fluid, minimal LV contract, incr LA size; aFAST peritoneal fluid à transferred to Tufts
B6 Tufts: HR 210-230, grade 2/6 murmur, weak pulses; echo à severe dilated LV w/ marked decr contract, thin LV walls, mod dilation LA, mild thicken mitral valve, trace pleural effusion, mild ascites, dilated hep vessels, FS 4.51% à DCM with secondary CHF, A-fib; WB tau B6 ; started B6
BID
B6 recheck at Tufts; not eating well, lethargic; gallop, murmur 2/6; echo à large volume ascites, no pleural/pericard effusion; EKG à a-fib, HR 220, rare VPCs; abdominocentesis removed 3.2 L straw fluid; BW showed elevated kidney values; added B6
Died at home B6

**From:** Rotstein, David
**Sent:** Monday, July 9, 2018 9:13 AM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Nemser, Sarah <Sarah.Nemser@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Queen, Jackie L <Jackie.Queen@fda.hhs.gov>
**Subject:** more DCMFW: Halo grain-free dry food (exact variety unknown): Lisa Freeman - EON-358522

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
**240-506-6763 (BB)**

U.S. FOOD & DRUG ADMINISTRATION



**From:** PFR Event [mailto:pfreventcreation@fda.hhs.gov]
**Sent:** Monday, July 09, 2018 9:00 AM
**To:** Cleary, Michael * <Michael.Cleary@fda.hhs.gov>; HQ Pet Food Report Notification <HQPetFoodReportNotification@fda.hhs.gov>; B6
**Subject:** Halo grain-free dry food (exact variety unknown): Lisa Freeman - EON-358522

A PFR Report has been received and PFR Event [EON-358522] has been created in the EON System.

A "PDF" report by name "2051557-report.pdf" is attached to this email notification for your reference.

Below is the summary of the report:

**EON Key:** EON-358522
**ICSR #:** 2051557
**EON Title:** PFR Event created for Halo grain-free dry food (exact variety unknown); 2051557

| | | | |
|---|---|---|---|
| **AE Date** | B6 | **Number Fed/Exposed** | |
| **Best By Date** | | **Number Reacted** | 1 |
| **Animal Species** | Dog | **Outcome to Date** | Stable |
| **Breed** | Great Dane | | |
| **Age** | B6 Years | | |
| **District Involved** | PFR-New England DO | | |

**Product information**
**Individual Case Safety Report Number:** 2051557
**Product Group:** Pet Food
**Product Name:** Halo grain-free dry food (exact variety unknown)
**Description:** DCM and CHF Taurine not measured
**Submission Type:** Initial
**Report Type:** Adverse Event (a symptom, reaction or disease associated with the product)
**Outcome of reaction/event at the time of last observation:** Stable

**Number of Animals Reacted With Product:** 1

| Product Name | Lot Number or ID | Best By Date |
|---|---|---|
| Halo grain-free dry food (exact variety unknown) | | |

**Sender information**
Lisa Freeman
200 Westboro Rd
North Grafton, MA 01536
USA

**Owner information**
B6
USA

To view this PFR Event, please click the link below:
https://eon.fda.gov/eon//browse/EON-358522

To view the PFR Event Report, please click the link below:
https://eon.fda.gov/eon//EventCustomDetailsAction!viewReport.jspa?decorator=none&e=0&issueType=12&issueId=375146

==========

**Follow-up Case Information Uniform Data Entry Form**
**Vet-LIRN**

Date (mm/dd/yy): 9/5/2018

EON/CC Number: 358,522

## PATIENT INFORMATION

Pet Name: B6

(•) Dog ( ) Cat

Breed: Great Dane

Age in years (if < 6 months, put 0.5): 4

Gender:

( ) M (•) MN ( ) F ( ) FS

This form serves as a Uniform Data Entry Form to capture additional case specific information not clear from the Consumer Complaint or Medical Records in a standardized manner. Because each follow-up interview made with owners features questions tailored specifically to the case, each box of information contained in this Uniform Data Entry Form may not be completed.

## HISTORY-Additional Comments from Owner

Owner's Description of What Happened: Started 2 weeks before B6 he toppled over after laying down once, started coughing constantly. No signs before at all--a week before he started coughing he was still running around the yard and playing. O googled it and thought it could have been related to ear infections, but lasted 2 full days so

Any Health Problems Prior to the Event (e.g. allergies, surgeries): Repeated B6

Sensitive GI tract (e.g. stomach upset when switching foods, eats a lot of grass) [ ] Yes

Changes to the pet's diet prior to illness [ ] Yes

Date Diet Change:

## CLINICAL INFORMATION--Additional Comments from Owner on What Happened

Appetite [ ] Increased [X] Decreased

Vomiting [ ] Yes

Diarrhea [ ] Yes

Duration of Diarrhea (days):

Blood in Feces [ ] Fresh,Red [ ] Coffee Ground [ ] Black,Tarry

Water Consumption [ ] Increased [ ] Decreased

Urination [ ] Increased [ ] Decreased

Lethargy [X] Yes

Other:

## MEDICATIONS-Taken Prior to the Event and Mentioned by Owner

List medications mentioned by owner (e.g. NSAIDs, steroids, heartworm/flea prevention, antibiotics, etc.): B6

List probiotics, vitamins, or supplements mentioned by owner: None



Continued other side

**Follow-up Case Information Uniform Data Entry Form**
**Vet-LIRN**

EON/CC Number: 358,522

Owner: B6 Pet's Name: B6

DIET-Any other foods the owner mentions were given to the animal during this period. (check all that apply)

☒ Commercial Dry — Product Use as Part of Diet: ☒ Primary ☐ Secondary ☐ Occasional
List Product Label Name: Fromm puppy food (O unsure if GF); ~1 year old switched and tried different foods but not long on each, settled on Halo Surf and Turf GF (filled his bowl, didn't measure--estimated around 2-3 dog

☐ Commercial Wet-Canned — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional
List Product Label Name:

☐ Commercial Wet-Pouch — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional
List Product Label Name:

☐ Commercial-Raw — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional
List Product Label Name:

☐ Homemade-Raw — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional
Describe Product Type:

☐ Homemade-Cooked — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional
Describe Product Type:

☒ Table Scraps/Human Food (as an occasional contribution to diet) — Describe Product Type(s): Boiled egg, piece of cheese, would sneak butter off the counter

☒ Pet Treat Products — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☒ Occasional

| | | | | |
|---|---|---|---|---|
| ☒ Commercial | Product Label Name/Lot: Green Briar kennel club variety dog biscuits | Date first fed: since puppy | | |
| | How Product Administered: 0-5 per day | Date last fed: until death | | |
| ☐ Rawhides or Pig Ears | Product Label Name/Lot: | Date first fed: | | |
| | How Product Administered: | Date last fed: | | |
| ☒ Marrow Bones | Product Label Name/Lot: Cow marrow bones | Date first fed: since adopted | | |
| | How Product Administered: Once every 4-5 months | Date last fed: June 1st | | |
| ☐ Chicken Jerky | Product Label Name/Lot: | Date first fed: | | |
| | How Product Administered: | Date last fed: | | |
| ☒ Duck Jerky | Product Label Name/Lot: O's dad makes own duck jerky, probably had some | Date first fed: | | |
| | How Product Administered: | Date last fed: | | |
| ☐ Sweet Potato Jerky or Treats | Product Label Name/Lot: | Date first fed: | | |
| | How Product Administered: | Date last fed: | | |



Continued other side

**Follow-up Case Information Uniform Data Entry Form**
**Vet-LIRN**

EON/CC Number: 358,522

Owner: B6

Pet's Name: B6

DIET-*continued*-Any other foods the owner mentions were given to the animal during this period. (check all that apply)

☐ Other Treats

Product Label Name/Lot: | Date first fed

How Product Administered: | Date last fed

ENVIRONMENTAL EXPOSURES-Environmental Exposures Mentioned by the Owner Potentially Affecting the Animal's Overall State of Health Prior to the Event . (check all that apply)

| | | | | | | |
|---|---|---|---|---|---|---|
| ☐ Indoor | ☐ Outdoor | ☒ Indoor & Outdoor | ☐ Carrion | ☐ Rodents | ☐ Grapes or Raisins | ☐ Nuts |
| ☐ Plants | ☒ Trash | ☐ Hunt | ☒ Pet Shows | ☐ Sporting Events | ☒ Pet Recreation Facilities | |
| ☐ Livestock | ☐ Poultry | ☐ Reptiles | ☐ Pet Birds | ☐ Small Mammals | ☐ Untreated Surface Water | |
| ☐ Anti-freeze | ☐ Mushrooms | ☐ Heavy Metals | ☒ Ticks | ☐ Urban | ☒ Suburban | ☒ Rural |

Comments:

Indoor and outdoor dog--mostly indoor but went outside in fenced-in kennel. Also huge fenced-in yard, but went off leash outside the yard, usually supervised but not always. Went on leashed walks on beaches, etc. Would eat grass but no other plants. Did like to get into the trash but just played with it, didn't really eat it (last time probably 3 years ago). Dog training class as a puppy and used to hang out with dogs from that class. Would go on park walks with other dogs. Did a show-and-go in his first year (like a mini dog show).

Farm around they would walk by but didn't interact with animals. There is a cat in the house, 1 other dog chocolate lab B6 FS 5 yr (did NOT eat the GF food, eats Purina).

Pulled a tick off of him once or twice when trying to find a good f/t product, then used seresto collar. Farms but quiet town (mix of suburban-rural).

HOUSEHOLD-Signalment of Additional Animals Given the Product mentioned by the owner.

Animal 1 ☐ Reacted

Animal 2 ☐ Reacted

Animal 3 ☐ Reacted

Comments

Submit

**Follow-up Case Information Uniform Data Entry Form**
**Vet-LIRN**

Date (mm/dd/yy): 9/28/2018

EON/CC Number: 365,002

## PATIENT INFORMATION

Pet Name: B6

(•) Dog ( ) Cat

Breed: Great Dane

Age in years (if < 6 months, put 0.5): 6

Gender:

( ) M (•) MN ( ) F ( ) FS

This form serves as a Uniform Data Entry Form to capture additional case specific information not clear from the Consumer Complaint or Medical Records in a standardized manner. Because each follow-up interview made with owners features questions tailored specifically to the case, each box of information contained in this Uniform Data Entry Form may not be completed.

## HISTORY-Additional Comments from Owner

Owner's Description of What Happened: In February O was leaving town, night before he started a hacking cough, pretty constant. Took to vet and they told them to go to a cardiologist. Weren't able to get right in, so took to B6 Quick progression, started coughing once on that Thursday, then Saturday morning took to vet. No exercise

Any Health Problems Prior to the Event (e.g. allergies, surgeries): B6

Sensitive GI tract (e.g. stomach upset when switching foods, eats a lot of grass) ☐ Yes

Changes to the pet's diet prior to illness ☐ Yes

Date Diet Change:

## CLINICAL INFORMATION--Additional Comments from Owner on What Happened

Appetite ☐ Increased ☐ Decreased

Vomiting ☐ Yes

Diarrhea ☐ Yes

Duration of Diarrhea (days):

Blood in Feces ☐ Fresh,Red ☐ Coffee Ground ☐ Black,Tarry

Water Consumption ☐ Increased ☐ Decreased

Urination ☐ Increased ☐ Decreased

Lethargy ☐ Yes

Other:

## MEDICATIONS-Taken Prior to the Event and Mentioned by Owner

List medications mentioned by owner (e.g. NSAIDs, steroids, heartworm/flea prevention, antibiotics, etc.): B6

List probiotics, vitamins, or supplements mentioned by owner: Salmon oil 2 pumps daily since allergy issues when younger



Continued other side

**Follow-up Case Information Uniform Data Entry Form**
**Vet-LIRN**

EON/CC Number: 365,002

Owner: B6

Pet's Name: B6

DIET-Any other foods the owner mentions were given to the animal during this period. (check all that apply)

☒ Commercial Dry — Product Use as Part of Diet: ☒ Primary ☐ Secondary ☐ Occasional

List Product Label Name: Blue Seal active adult as a puppy - 14 mos; Nature's Variety Instinct Raw Boost chicken 14 mos-December 2017; Rachael Ray Nutrish Chicken/Veggies December 2017 to this past Monday; just

☐ Commercial Wet-Canned — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

List Product Label Name:

☐ Commercial Wet-Pouch — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

List Product Label Name:

☐ Commercial-Raw — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

List Product Label Name:

☐ Homemade-Raw — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

Describe Product Type:

☒ Homemade-Cooked — Product Use as Part of Diet: ☐ Primary ☒ Secondary ☐ Occasional

Describe Product Type: Used to get cooked chicken half cup every meal; just low sodium chicken broth now

☒ Table Scraps/Human Food (as an occasional contribution to diet) — Describe Product Type(s): pizza crusts occasionally, popcorn occasionally

☒ Pet Treat Products — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

| | | | | |
|---|---|---|---|---|
| ☐ Commercial | Product Label Name/Lot: | | Date first fed | |
| | How Product Administered: | | Date last fed | |
| ☐ Rawhides or Pig Ears | Product Label Name/Lot: | | Date first fed | |
| | How Product Administered: | | Date last fed | |
| ☐ Marrow Bones | Product Label Name/Lot: | | Date first fed | |
| | How Product Administered: | | Date last fed | |
| ☐ Chicken Jerky | Product Label Name/Lot: | | Date first fed | |
| | How Product Administered: | | Date last fed | |
| ☐ Duck Jerky | Product Label Name/Lot: | | Date first fed | |
| | How Product Administered: | | Date last fed | |
| ☐ Sweet Potato Jerky or Treats | Product Label Name/Lot: | | Date first fed | |
| | How Product Administered: | | Date last fed | |



Continued other side

**Follow-up Case Information Uniform Data Entry Form**
**Vet-LIRN**

EON/CC Number: 365,002

Owner: B6

Pet's Name: B6

DIET-*continued*-Any other foods the owner mentions were given to the animal during this period. (check all that apply)

☒ Other Treats

Product Label Name/Lot: Occasional pumpkin homemade dog biscuit

Date first fed

How Product Administered: Really don't get treats, just ice cubes

Date last fed

ENVIRONMENTAL EXPOSURES-Environmental Exposures Mentioned by the Owner Potentially Affecting the Animal's Overall State of Health Prior to the Event . (check all that apply)

| | | | | | | |
|---|---|---|---|---|---|---|
| ☒ Indoor | ☐ Outdoor | ☐ Indoor & Outdoor | ☒ Carrion | ☐ Rodents | ☐ Grapes or Raisins | ☐ Nuts |
| ☐ Plants | ☐ Trash | ☐ Hunt | ☐ Pet Shows | ☐ Sporting Events | ☐ Pet Recreation Facilities | |
| ☐ Livestock | ☐ Poultry | ☐ Reptiles | ☐ Pet Birds | ☐ Small Mammals | ☒ Untreated Surface Water | |
| ☐ Anti-freeze | ☒ Mushrooms | ☐ Heavy Metals | ☒ Ticks | ☐ Urban | ☐ Suburban | ☒ Rural |

Comments:

Indoor dog, goes outside to go to the bathroom in a yard; 90% of the time he's supervised but not fenced-in. Doesn't run off. Have gotten into deer poop and brought home an occasional deer leg. Never seen him with a rodent. Eats grass outside but no other plants. Never gotten into trash.

Farms around but no access to them. Cat in the house (indoor), on non-GF food. B6 plays in and drinks from stream and pond. Mushrooms in the yard but he hasn't eaten them. Has pulled ticks off but it has been awhile.

No trauma, neoplasia, hyperthermia, irradiation, electric shock; no access to human drugs/chemo agents/alcohol; no recent anesthesia (only when neutered/gastropexy); no lily-of-the-valley, gossypol, buttercup, japanese yew, black locust, foxglove. Not hypothyroid

HOUSEHOLD-Signalment of Additional Animals Given the Product mentioned by the owner.

Animal 1 ☐ Reacted

Animal 2 ☐ Reacted

Animal 3 ☐ Reacted

Comments

Submit

Asian-Aust. J. Anim. Sci.
Vol. 21, No. 3 : 434 - 442
March 2008
www.ajas.info

# The Optimum Methionine to Methionine Plus Cystine Ratio for Growing Pigs Determined Using Plasma Urea Nitrogen and Nitrogen Balance

**Shiyan Qiao, Xiangshu Piao, Zhanyu Feng, Yuhua Ding*, Longyao Yue and P. A. Thacker**
Ministry of Agriculture Feed Industry Center, China Agricultural University
No. 2 Yuanmingyuan West Road, Beijing 100094, China

**ABSTRACT :** The objective of this study was to determine the optimum ratio of methionine to methionine plus cystine for growing pigs. A nitrogen balance trial was conducted using a total of 21 barrows (Large White×Landrace) over two replicates. The initial body weight was 20.36±1.22 kg (mean±SD) in the first replicate and 23.54±1.02 kg (mean±SD) in the second. For each replicate, the 21 pigs were randomly assigned to one of seven dietary treatments with three observations per treatment. The diets included a methionine and cystine-deficient basal diet with all other essential nutrients meeting nutrient requirements and six diets formulated with graded levels of DL-methionine (0.00, 0.03, 0.06, 0.10, 0.13, 0.16%) and L-Cystine·HCl·$H_2O$ (0.19, 0.15, 0.11, 0.07, 0.04, 0.00%). This resulted in ratios of methionine to methionine plus cystine of 41.3, 29.6, 35.3, 41.2, 46.0, 51.6 and 57.5%. Each experimental period lasted 12 days consisting of a seven-day adaptation period followed by a five-day total collection of urine and feces. During the collection period, pigs were fed 900 g/day for the first replicate and 1,200 g/day for the second replicate. The feed was provided in three equal portions at 0800, 1500, and 2200 h daily. Pigs had *ad libitum* access to water after feeding. There was a linear ($p<0.01$) and quadratic ($p<0.01$) effect on daily gain and feed conversion as the ratio of methionine to methionine plus cystine increased. Pigs receiving the diets providing a methionine to methionine plus cystine ratio of 51.6% had the best daily gain and feed conversion. Plasma urea nitrogen was also lowest for this treatment. Nitrogen retention increased ($p<0.01$) as the relative proportion of methionine increased up to 51.6% and then a downward trend occurred at 57.5%. The quadratic regression model, as well as one- and two- slope regression line models, were used to determine the optimum ratio of methionine to methionine plus cystine. Eliminating the 35.3% methionine to methionine plus cystine treatment resulted in $R^2$ values in excess of 0.92. The optimal ratio of methionine to methionine plus cystine was estimated to be 54.15% for nitrogen retention and 56.72% for plasma urea nitrogen. (**Key Words :** Pigs, Methionine, Cystine, Ratio, Nitrogen Retention, Plasma Urea Nitrogen)

## INTRODUCTION

The sulfur containing amino acids methionine and cystine are often the third or fourth limiting amino acids in practical diets fed to growing pigs (Russell et al., 1983). Methionine is essential for normal growth as it cannot be synthesized in the body, but cystine can be converted from methionine as needed, hence it is considered dispensable. As a result, the amount of methionine needed in the diet depends on the amount of cystine also present (Chung and Baker, 1992; Yang et al., 1997; Zimmermann et al., 2005). The absolute amounts of methionine and cystine are important but so is the ratio between methionine and cystine. Therefore, nutritionists need to consider not only methionine but also methionine plus cystine requirements when formulating pig diets.

Previous studies with growing pigs have shown that the minimum methionine to methionine plus cystine ratio ranged between 30 and 70% (Wang and Fuller, 1989; Fuller et al., 1989). Part of this variability is due to differences in response criteria (i.e., nitrogen balance vs. growth performance), the bioavailability of the amino acids in the basal diet, and weight of pigs used in the experiments. In a nitrogen balance study with growing gilts (40-80 kg) fed varying ratios of methionine to cystine diets, Reijmers et al. (2002) found the minimum methionine to methionine plus cystine ratio at which protein deposition was maximized was 55%. This value is within the range of values reported in the literature (NRC 1998, Roth and Kirchgessner, 1989).

There is very limited data about the required methionine to methionine plus cystine ratio for maximal protein

* Corresponding Author: Yuhua Ding. Tel: +86-10-6273-3588, Fax: +86-10-6273-3688, E-mail: Dingyh@mafic.ac.cn
Received August 9, 2006; Accepted January 3, 2007

**Table 1.** Ingredient composition of experimental diets formulated to determine the effects of various methionine to methionine plus cystine ratios on pig performance and nitrogen balance (% as fed)

| Ingredients | Methionine to methionine plus cystine ratio | | | | | | |
|---|---|---|---|---|---|---|---|
| | 41.3 | 29.6 | 35.3 | 41.2 | 46.0 | 51.6 | 57.5 |
| Corn | 39.90 | 39.90 | 39.90 | 39.90 | 39.90 | 39.90 | 39.90 |
| Field peas | 24.79 | 24.79 | 24.79 | 24.79 | 24.79 | 24.79 | 24.79 |
| Peanut meal | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Corn starch | 8.90 | 8.71 | 8.72 | 8.73 | 8.73 | 8.73 | 8.74 |
| Wheat barn | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| Sucrose | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Dicalcium phosphate | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 |
| Limestone | 0.83 | 0.83 | 0.83 | 0.83 | 0.83 | 0.83 | 0.83 |
| Vitamin and mineral premix[a] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| NaCl | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 |
| Choline chloride (50%) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Soybean oil | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| L-lysine HCl (98.5%) | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 |
| L-threonine (99%) | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| L-valine (99.5%) | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| L-isoleucine (99%) | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| L-tryptophan (98%) | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 |
| DL-methionine (99%) | - | - | 0.03 | 0.06 | 0.10 | 0.13 | 0.16 |
| L-cystine·HCl·$H_2O$[b] (99.1%) | - | 0.19 | 0.15 | 0.11 | 0.07 | 0.04 | - |

[a] Provided per kilogram of complete feed: vitamin A, 5,512 IU; vitamin $D_3$, 2,200 IU; vitamin E, 66.1 IU; riboflavin, 5.5 mg; D-pantothenic acid, 13.8 mg; niacin, 30.3 mg; vitamin $B_{12}$, 27.6 μg; Mn, 100 mg; Fe, 100 mg; Cu, 234 mg; Zn, 100 mg; I, 0.3 mg; Se, 0.3 mg; Co, 1.0 mg.

[b] 1 kg of L-cystine·HCl·H2O (99.1%) contained 0.851 kg of L-cystine.

deposition in 20 to 30 kg growing pigs. Therefore, the objective of the current study was to establish the optimum dietary ratio of methionine to methionine plus cystine for growing pigs using the nitrogen balance technique and plasma urea nitrogen.

## MATERIALS AND METHODS

### Animals and diets

A nitrogen balance trial was conducted in the Metabolism Laboratory of the Animal Science and Technology College located on the campus of China Agriculture University (Beijing, China). The trial, conducted in two replicates, utilized 21 barrows (Large White×Landrace) obtained from the Haudu Group (Beijing, China). The initial bodyweight of the pigs averaged 20.36±1.22 kg in the first replicate and 23.54±1.02 kg in the second replicate. In each replicate, the 21 pigs were randomly allocated to one of seven different dietary treatments with three observations per treatment. The basal diet was formulated to meet the requirements for all amino acids except methionine and cystine (NRC, 1998). All other nutrients were formulated to meet or exceed requirements (NRC, 1998). Batches of each feed ingredient were obtained before the start of the study, sampled and analyzed in order to adjust the nutrient composition of the diets.

The content of methionine and cystine in the basal diet was determined to be 0.19 and 0.27%, respectively. Crystalline DL-methionine (0.0, 0.03, 0.06, 0.10, 0.13 and 0.16%) and L-Cystine·HCl·$H_2O$ (0.19, 0.15, 0.11, 0.07, 0.04 and 0.0%) were added to the basal diet by replacing corn starch resulting in seven treatments with ratios of methionine to methionine plus cystine ranging from 29.6 to 57.5% (41.3, 29.6, 35.3, 41.2, 46.0, 51.6 and 57.55%). The ingredient composition of all the diets is presented in Table 1.

For all experimental diets, the vitamin-trace mineral mix and synthetic amino acids were premixed with 10 kg corn before addition to the mixer. A basal mix was manufactured and aliquots of this mix were used to manufacture the final feed.

### Experimental procedures

Each replicate consisted of a seven day adjustment period followed by a five day total collection of feces and urine. The pigs were kept in individual metabolic crates and separate collection of feces and urine was accomplished by fitting adhesive feces collection bags onto the back of pigs (Van Kleef et al., 1994). Each stainless steel crate (0.6×0.3 ×0.5 m) was equipped with plastic slotted flooring and contained a 0.25 $m^3$ round bottom single feeder at the front. The temperature and humidity of the room were controlled within the range of 22 to 25°C and 55 to 70%, using the environmental control system.

The daily ration was divided into three feedings per day, with approximately one third of the ration being fed at

**Table 2.** Chemical analysis for experimental diets formulated to determine the effects of various methionine to methionine plus cystine ratios on pig performance and nitrogen balance (% as fed)[a]

| | Methionine to methionine plus cystine ratio | | | | | | |
|---|---|---|---|---|---|---|---|
| | 41.3 | 29.6 | 35.3 | 41.2 | 46.0 | 51.6 | 57.5 |
| Chemical analysis | | | | | | | |
| Dry matter | 89.62 | 90.10 | 88.98 | 88.28 | 89.20 | 88.94 | 90.12 |
| Ash | 4.83 | 4.93 | 5.01 | 4.81 | 5.01 | 4.81 | 47.56 |
| Crude protein | 14.92 | 15.02 | 14.82 | 14.71 | 15.01 | 14.68 | 14.08 |
| Crude fibre | 2.28 | 2.51 | 2.36 | 2.29 | 2.37 | 2.89 | 2.40 |
| Ether extract | 3.45 | 3.68 | 3.81 | 3.69 | 3.76 | 3.72 | 3.68 |
| Analyzed amino acids | | | | | | | |
| Arginine | 1.18 | 1.21 | 1.17 | 1.11 | 1.15 | 1.17 | 1.18 |
| Cystine | 0.27 | 0.45 | 0.42 | 0.37 | 0.34 | 0.30 | 0.27 |
| Histidine | 0.34 | 0.36 | 0.36 | 0.35 | 0.37 | 0.35 | 0.36 |
| Isoleucine | 0.63 | 0.62 | 0.65 | 0.63 | 0.63 | 0.62 | 0.63 |
| Leucine | 1.07 | 1.11 | 1.10 | 1.08 | 1.09 | 1.09 | 1.11 |
| Lysine | 1.03 | 1.10 | 1.07 | 1.10 | 1.02 | 1.06 | 1.06 |
| Methionine | 0.19 | 0.19 | 0.23 | 0.26 | 0.29 | 0.32 | 0.38 |
| Phenylalanine | 0.68 | 0.67 | 0.69 | 0.68 | 0.66 | 0.67 | 0.68 |
| Threonine | 0.68 | 0.68 | 0.69 | 0.68 | 0.70 | 0.70 | 0.65 |
| Tryptophan | 0.23 | 0.26 | 0.20 | 0.22 | 0.22 | 0.23 | 0.21 |
| Valine | 0.82 | 0.78 | 0.78 | 0.76 | 0.74 | 0.73 | 0.79 |

8,000, 1,500 and 2,200 h. The daily feed allowance of the experimental animals was adjusted according to the feed intake observed in the last three days of the acclimation period. This was the amount of feed that pigs could consume within 20 minutes based on our observations.

From d 4 until the end of the 12-d experimental period, the same amount of feed was fed which exceeded 2.6 times the pig's maintenance energy requirements. This energy intake has been shown not to limit protein deposition (Möhn et al., 2000; De Lange et al., 2001). The feeding rate ranged from 4% to 5.5% of body weight (900 g/d/pig for the first replicate and 1,200 g/d/pig for the second replicate). In the collection period, the wasted feed for each pig was collected, dried and recorded on a dry matter basis.

The animals were weighed at the start of every quantitative feeding period and again at the termination of the trial. Weighing was conduced at 0800 to 0900 h with no feed available. After feeding, water was provided *ad libitum* in the feeding trough.

**Sample collection**

Feces were collected in the morning, afternoon and evening for five consecutive days taking care to avoid contamination with urine. The total weight of the raw feces for each pig was recorded daily. After collection, feces were placed into labeled plastic bags and frozen at approximately -20°C. At the end of each trial, each pig's daily samples were combined into a single composite sample. From that, a 5% sub-sample was preserved for laboratory analysis. Sub-samples were dried to a constant weight in a forced-air oven at 65°C, equilibrated at room temperature for 24 h, and ground through a 0.45 mm mesh screen.

The urine of individual pigs was collected in plastic containers containing 50 ml of 6 N HCl to maintain the pH of the urine below 3. The total amount of urine excreted by each pig was measured once a day at approximately 1,530 h and recorded on a daily basis. After being filtered through glass wool, a fixed proportion of the urine from each pig was preserved in screw-capped polyethylene containers and frozen at approximately -20°C. When the collection for all five days was completed, each pig's daily samples were thawed and combined into a single sample. A 100 ml composite sample was obtained and then frozen until needed for nitrogen analysis.

At the end of each replicate, 7 ml of blood was collected from the jugular vein of each pig using heparinized vacutainer tubes (Greiner Bio-One Company), approximately 1 h after feeding. All blood samples were chilled and then centrifuged at 3,000×g for 15 min at 4°C within 1 h after collection (Ciji 800 Model Centrifuge, Surgical Instrument Factory, Shanghai, China). An aliquot of plasma was stored at -20°C until analyzed for plasma urea nitrogen.

**Chemical analysis**

Samples of the feed ingredients were collected before the diets were manufactured, while samples of complete feeds were collected at the start of the trial for analyses. The chemical composition and the amino acid content of all ingredients was analyzed in duplicate in the laboratory of the Ministry of Feed Industry Center (Beijing, China). Moisture, crude protein, crude fiber, ether extract and ash were determined following standard methods (AOAC, 1995).

**Table 3.** Performance and plasma urea nitrogen for growing pigs fed varying ratios of methionine to methionine plus cystine

| | Methionine to methionine plus cystine ratio | | | | | | | SEM[a] | Linear | Quadratic |
|---|---|---|---|---|---|---|---|---|---|---|
| | 41.3 | 29.6 | 35.3 | 41.2 | 46.0 | 51.6 | 57.5 | | | |
| Weight gain (g/day) | 368 | 375 | 375 | 397 | 395 | 422 | 422 | 36.92 | 0.01 | 0.01 |
| Feed intake (g/day) | 924 | 923 | 923 | 927 | 925 | 924 | 927 | 59.36 | 0.96 | 0.99 |
| Feed conversion | 2.56 | 2.47 | 2.51 | 2.33 | 2.39 | 2.20 | 2.22 | 0.27 | 0.01 | 0.01 |
| Plasma urea nitrogen (mg/dl) | 12.50 | 12.33 | 12.67 | 11.83 | 10.17 | 9.33 | 10.17 | 1.96 | 0.14 | 0.33 |

[a] SEM = Standard error of the mean.

**Table 4.** Nitrogen balance response for growing pigs fed varying ratios of methionine to methionine plus cystine

| | Methionine to methionine plus cystine ratio | | | | | | | SEM[a] | Linear | Quadratic |
|---|---|---|---|---|---|---|---|---|---|---|
| | 41.3 | 29.6 | 35.3 | 41.2 | 46.0 | 51.6 | 57.5 | | | |
| Nitrogen intake (g/day) | 24.79 | 24.73 | 26.11 | 25.60 | 26.14 | 26.16 | 26.07 | 1.65 | 0.58 | 0.80 |
| Fecal nitrogen (g/day) | 3.12 | 3.56 | 3.54 | 3.43 | 3.00 | 3.19 | 2.81 | 0.48 | 0.18 | 0.41 |
| Urinary nitrogen (g/day) | 9.62 | 8.85 | 10.49 | 7.88 | 7.66 | 6.91 | 7.88 | 1.29 | 0.17 | 0.37 |
| Retained nitrogen (g/day) | 12.05 | 12.32 | 12.09 | 14.29 | 15.49 | 16.05 | 15.38 | 1.23 | 0.01 | 0.01 |
| Nitrogen retained (%) | 48.61 | 51.07 | 46.64 | 56.61 | 60.00 | 62.05 | 59.57 | 4.53 | 0.02 | 0.05 |
| Nitrogen digestibility (%) | 88.74 | 86.01 | 86.62 | 86.76 | 88.48 | 88.09 | 89.30 | 1.38 | 0.04 | 0.13 |

[a] SEM = Standard error of the mean.

The amino acid content of the diets was determined by High Performance Liquid Chromatography (Hitachi L-8800 Amino Acid Analyzer, Tokyo, Japan). All samples were hydrolyzed for 24 h at 110°C with 6 N HCl prior to analysis. Sulfur-containing amino acids were analyzed after cold formic acid oxidation for 16 h before acid hydrolysis. Tryptophan was determined after alkaline hydrolysis (4 N NaOH) for 22 h at 110°C. The chemical composition of the diets is listed in Table 2.

Plasma urea nitrogen was determined on a fully automatic Biochemical Analyzer (Technicon RA 1000) and by enzymatic UV test (Ureaza method/GLDH) based on the report of Kerschner and Ziegenhorn (1985). A urea kit produced by Zhong Sheng Beikong Bio-technology and Science Inc. (Beijing, China) was used for this analysis. Fecal and urinary nitrogen were analyzed with a semi-automatic analyzer (Kjeltec$^{TM}$ 2100 Distillation Unit) by the Kjeldahl method (AOAC, 1990).

**Statistical analysis**

Data from the two replicates were analyzed using the General Linear Model (GLM) procedure of the SAS statistical package (SAS, 2002) using the pig as the experimental unit. The experimental data were subjected to analysis of variance using a model that included the effect of diet and the two replicates. The results were considered significant if $p<0.05$.

The optimal ratio between methionine and methionine plus cystine of the growing pigs was estimated with a quadratic regression model as well as one- and two- slope regression line models (Coma et al., 1995a) using nitrogen retention and plasma urea nitrogen as the dependent variables regressed against dietary level of methionine to methionine plus cystine ratio. The appropriate GLM and NLIN procedures of SAS (2002) were used for these estimates. The applied quadratic model was:

$$Y = b_0+b_1X+b_{11}X^2$$

Where Y = the response parameter (nitrogen retention, plasma urea nitrogen) and $X_1$ = the ratio of methionine to methionine plus cystine $b_0$, $b_1$, $b_{11}$ = the coefficients of the equation. The ratio at which the response reached 95% of the maximum response was estimated as the optimal value.

The regression of the one-slope and two-slope models used in the present experiment are described as follows:

$$Y = L+U\ (R-X_{LR});$$

$$Y = L+U\ (R-X_{LR})+V(X_{GR}-R)$$

Where L = the ordinate; R = the abscissa of the breakpoint (the estimated requirement). $X_{LR}$ means X less than R; $X_{GR}$ means X greater than R. U = the slope of the line at X<R, and V = the slope of the line at X>R. By definition, $(R-X_{LR})$ is zero when X greater than R, and $(X_{GR}-R)$ is zero when X less than R. The ratio at which the breakpoint was achieved was estimated as the optimal value (Robbins et al., 1979; Coma et al., 1995a). The mean square error (MSE) and the coefficient of determination ($R^2$) were used to assess the goodness of fit for the different models (Coma et al., 1995a).

## RESULTS

The results showed no significant replicate×treatment interaction ($p>0.05$) for any of the studied variables. Therefore, data from the two replicates were pooled for analysis.

Since the level of feed intake was controlled, feed intake

**Table 5.** Asymptotic characteristics of plasma urea nitrogen and nitrogen retention responses to relative proportions of methionine to methionine plus cystine

| Variable | Model | Requirement | $R^2$ | MSE |
|---|---|---|---|---|
| Nitrogen retention | Quadratic | 54.15 | 0.99 | 0.095 |
| | One-slope broken line | 51.24 | 0.96 | 0.192 |
| | Two-slope broken line | 53.96 | 0.98 | 0.159 |
| Plasma urea nitrogen | Quadratic | 56.17 | 0.92 | 0.563 |
| | One-slope broken line | 53.94 | 0.94 | 0.233 |
| | Two-slope broken line | 56.72 | 0.99 | 0.113 |

was similar among all dietary treatments. As the relative proportion of methionine to methionine plus cystine increased from 29.6 to 57.5%, average daily gain and feed conversion improved linearly ($p = 0.01$) and quadratically ($p = 0.01$). The poorest weight gain and feed utilization was observed for pigs fed the basal diet. The best daily gain and feed conversion was observed for pigs fed the diet in which the methionine to methionine plus cystine ratio was 51.6%. For plasma urea nitrogen, the lowest and highest values occurred for pigs fed the 51.6 and 35.3% methionine to methionine plus cystine ratio diets (Table 3).

Increasing the relative proportion of methionine to methionine plus cystine resulted in a significant linear ($p = 0.02$) and quadratic ($p = 0.05$) increase in nitrogen retention (Table 4). Nitrogen digestibility increased linearly ($p = 0.04$) with increased proportions of methionine to methionine plus cystine.

Three statistical models were fitted to the nitrogen retention and plasma urea nitrogen data (Tables 3 and 4). Based on the nitrogen retention response to the ratio of methionine to methionine plus cystine, three regression equations were obtained using the quadratic regression, one- and two- slope regression models, respectively:

$$Y = -18.19+1.20X-0.011X^2$$

$$Y = 15.72-0.26\times(51.24-X_{LR})$$

$$Y = 16.42-0.26\times(53.96-X_{LR})-0.10\times(X_{GR} - 53.96)$$

Based on the corresponding equations, the optimal ratios of methionine to methionine plus cystine were determined to be 54.15, 51.24 and 53.96%, respectively.

When plasma urea nitrogen was considered as the dependent variable, the optimal ratios of methionine to methionine plus cystine were estimated to be 56.17, 53.94 and 56.72%. The corresponding regression equations were listed as followed:

$$Y = 33.951-0.816\ X-0.0069\ X^2$$

$$Y = 9.75+0.19\times(53.94-X_{LR})$$

$$Y = 9.22+0.19\times(56.72-X_{LR})-0.13\times(X_{GR}-56.72)$$

## DISCUSSION

In the present study, the optimal ratio of methionine to methionine plus cystine was estimated in 20 to 30 kg growing pigs using the nitrogen balance technique. Based on our design, the six test diets contained varying levels of methionine and cystine but the total content of methionine plus cystine was similar across treatments and was close to the value recommended by the NRC (1998). Moreover, all other essential nutrients, especially energy and other amino acids were designed to be at or above requirement (NRC, 1998).

For growing animals, amino acids are basically used for protein accretion and maintenance and deposited protein relies on the level of the first limiting amino acid. Assuming our dietary formulation was accurate and the methionine plus cystine content of the test diets did not exceed the requirements of growing pigs, whole-body protein synthesis should theoretically occur at a level determined by the optimal ratio of methionine to cystine. If the dietary ratio of methionine to cystine is below the optimal value, more cystine and less methionine will be consumed. Protein synthesis will be determined by the level of dietary methionine, which leads to less protein deposition. When the ratio in the test diets is above the value, methionine will be in relative excess and cystine will be in relative deficiency. However, the deficiency in cystine can be overcome by conversion from methionine via the trans-sulfuration pathway. In fact, cysteine (1/2 cystine) is the genuine element used to incorporate into protein. Cystine (the dimmer form of cysteine), is produced when cysteine is in solution (Lewis, 2003). Because of the molecular weight difference between methionine and cysteine, the efficiency of methionine in meeting the biological need for cysteine on a weight basis is 80% (Chung and Baker, 1992). Thus, an excess of methionine is not sufficient to make up for a deficiency in cystine in this condition. Protein synthesis will also be reduced due to the low cystine intake. From this, it can be concluded that increasing the relative proportion of methionine will result in greater protein synthesis until the optimal ratio of methionine to cystine is attained, and subsequently, when the relative proportion of methionine is above its optimal value, protein synthesis will be reduced with further increases in methionine intake.

Because of conversion from methionine, a deficiency of cystine will lead to relatively less of a change in protein synthesis than a deficiency of methionine in the presence of a constant methionine plus cystine content. This has been confirmed by Roth and Kirchgessner (1989). In a similar experiment for 30 to 60 and 60 to 90 kg pigs, they found that pigs with a predominant proportion of methionine obtained higher performance than those with a predominant proportion of cystine. Here, the parameters of performance reflect the status of protein synthesis. According to the current experimental design and statistical analysis, when the optimal ratio of methionine to cystine is fed, maximal protein synthesis occurs.

The status of protein synthesis can be measured using biological response criteria such as growth, nitrogen retention and plasma urea. In fact, an inherent relationship exists between several criteria. Nitrogen retention is a direct indicator of protein synthesis. Here, protein deposition (synthesis) can be calculated as nitrogen retained×100/16 (Möhn et al., 2000). For young pigs with minimal fat deposition, growth is almost directly proportional to lean tissue deposition which primarily relies on protein deposition.

When protein synthesis is limited due to an unsuitable ratio of methionine to cystine, excess amino acids (including methionine or cystine) are catabolized to their metabolic end-products which for all amino acids include bicarbonate and ammonia. Ammonia enters the nitrogen pool of the body and is excreted primarily as urea in mammals. The status of urea in the body is therefore reflected by plasma urea. The measurement of these excreta provides an indirect and inverse measurement of changes in protein synthesis. As the relative proportion of methionine increases towards its optimal value, more protein is synthesized which leads to increased nitrogen retention, improved animal performance and decreased plasma urea. The inverse changes of nitrogen retention, animal performance and plasma urea occur when there is a continuous increase in the relative proportion of methionine from its optimal value. The inverse relationship between plasma urea (nitrogen) and lean growth (growth for young pigs) was also detected in previous reports published by Coma et al. (1995b).

So, based on maximal nitrogen retention or minimal plasma urea, the optimal ratio of methionine to cystine can be determined for the growing pigs in the present study. This estimation can be conducted by applying suitable statistical modeling techniques to the chosen biological response. We observed nitrogen retention and plasma urea nitrogen exhibited an anticipative change tendency from 35.3 to 57.5% methionine to methionine plus cystine (Tables 3 and 4). However, nitrogen retention and plasma urea nitrogen in the 29.6% treatment were superior to those in the 35.3% methionine to methionine plus cystine treatments, which meant that an increase in the relative proportion of methionine towards its optimal value caused a decrease in those variables. The reason for this is not known.

The quadratic and broken-line regression analyses were used to determine the relation between methionine and cystine in our study. For nitrogen retention and plasma urea nitrogen, when the values generated from the treatment for 29.6% of methionine to methionine plus cystine were removed, those models fitted the data very well.

Using the quadratic regression, as well as the one- and two- slope broken-line regression models, the required ratios of methionine to methionine plus cystine were estimated to be 54.15, 51.24 and 53.96% for nitrogen retention. However, when considering the plasma urea nitrogen variable, using the corresponding statistical models, the required ratios of methionine to methionine plus cystine were estimated to be 56.17, 53.94 and 56.72%. Obviously, the plasma urea nitrogen assay resulted in higher values than the nitrogen retention assay using the corresponding statistical models.

These differences may be attributed to an imbalance of electrolytes in the diets, where the chloride existing in the crystalline cystine (L-Cystine·HCl·$H_2O$) would tend to decrease the cation:anion ratio. Several reports in pigs have indicated that a diet with excess anion or chloride resulted in markedly lower plasma urea nitrogen concentrations (Slagle and Zimmerman, 1979; Honeyfield et al., 1985). However, total nitrogen excretion in pigs was found to be constant, although the excess cation intake resulted in significantly greater urea excretion (Cai et al. 1992). This was explained by Welbourne et al. (1986) who suggested that with the maintenance of acid-base balance in the body, urea was isochronously synthesized with ammonia production so that nitrogen excretion remained constant. Thus, the addition of various levels of L-Cystine-HC1 in the test diets influenced plasma urea nitrogen but not nitrogen retention, which may have produced the difference between the estimated results from the two variables. So the result from the nitrogen retention assay is more reasonable and acceptable.

For the nitrogen retention response, we found that the quadratic regression model had lower MSE and higher $R^2$ than either of the broken-line regression models (Figures 1-3). So the nitrogen retention response is better described by the quadratic regression model than by the broken-line regression models. Therefore, the determined value 54.15% is considered to be the estimated required ratio methionine to methionine plus cystine according to the nitrogen retention response. Similarly, for the plasma urea nitrogen response, we observed that the two-slope broken-line regression model tended to fit the data better than the other two regression models. The determined value 56.72% is

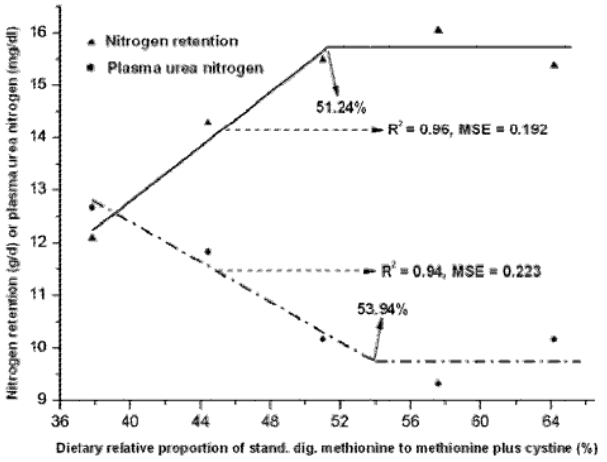



**Figure 1.** Use of the one-slope, broken-line regression model to describe the responses of nitrogen retention and plasma urea nitrogen to the proportion of methionine to methionine plus cystine in 20-50 kg growing pigs.

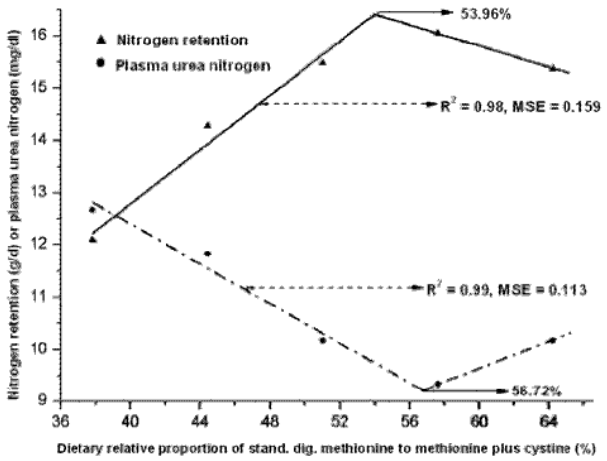



**Figure 2.** Use of the two-slope, broken-line regression model to describe the responses of nitrogen retention and plasma urea nitrogen to the relative proportion of methionine to methionine plus cystine in 20-50 kg growing pigs.

considered to be the estimated required ratio when the plasma urea nitrogen response was considered (Figures 1-3).

Referring to the dietary amino acid requirements for 20 to 50 kg growing pigs (NRC 1998), the recommended methionine and methionine plus cystine levels are 0.25% and 0.54%, respectively. Therefore, the ratio of methionine to methionine plus cystine is 46.29%. Obviously, our determined value is higher than this value. However, in a recent nitrogen balance experiment for 40 to 80 kg growing gilts, Reijmers et al. (2002) found the ratio of digestible methionine to methionine plus cystine for maximal body protein deposition was 55%. This value is very close to our evaluation of 54.15% based on maximal nitrogen retention.

These results agree with the studies conducted by

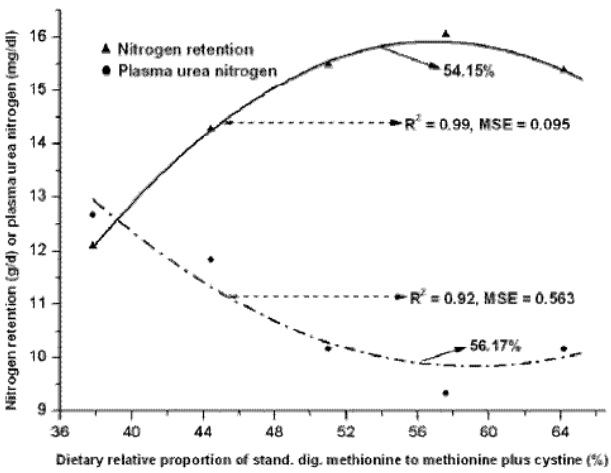



**Figure 3.** Use of the quadratic model to describe the responses of nitrogen retention and plasma urea nitrogen to the relative proportion of methionine to methionine plus cystine in 20-50 kg growing pigs.

Schutte et al. (1991) and Chung and Baker (1992), who indicated in growing pigs (respectively 20 to 50 kg and 10 to 20 kg) that methionine should contribute more than 50% of the total methionine and cystine requirement. In a previous growth assay for 30 to 60 kg and 60 to 90 kg pigs, Roth and Kirchgessner (1989) found that the ratio of methionine to methionine plus cystine, at maximal weight gain or feed efficiency, was more than 55%. This is somewhat higher than either of our estimated values. Several factors may have contributed to the differences in the relative proportion of methionine to methionine plus cystine estimates of growing pigs in the above studies including: 1) use of a different experimental design, i.e. nitrogen balance vs. growth assay, 2) use of different response criteria, i.e., nitrogen retention, plasma urea nitrogen or performance, 3) differing methionine plus cystine content employed in the diets, In addition, young animals use more amino acids for protein accretion than for maintenance compared with older ones. Protein accretion in pigs requires a greater proportion of methionine (Fuller et al., 1989; Mahan and Shields, 1998), while maintenance in pigs requires a greater proportion of cystine (Fuller et al., 1989; NRC, 1998). So the estimated result may also be influenced by age of pig.

For the present experiment, there are some additional factors which may have affected our results. In our design, the test diets were provided with constant levels of methionine plus cystine and varying levels of methionine and cystine. Lewis (2003) indicated that the molecular weight of methionine (149) is greater than that of cysteine (121), and equal weights of these two amino acids provide only 81% as many moles of methionine as cysteine (121/149 = 0.81). Thus, on a weight basis, increasing the

methionine to cystine ratio provides a decreasing number of moles of sulfur containing amino acids. In our study, the content of methionine plus cystine was not constant when expressed on a molar basis, which possibly influenced the results to some extent. However, we found that previous experiments also ignored this effect (Roth and Kirchgessner, 1989; Reijmers et al., 2002).

In practical swine diets containing sufficient amounts of the sulfur amino acids, generally cystine is more in excess than methionine. A high cystine intake increased the requirement of methionine plus cystine in pigs, but there was no evidence that excess cystine interferes with methionine (Lewis, 2003). When low protein diets are used in young pigs, perhaps methionine will be lacking. In this case, methionine should be added to meet its requirement, even though methionine plus cystine may appear to be adequate.

## CONCLUSION

In the present nitrogen balance trial with an equal feed intake, nitrogen retention and plasma urea nitrogen variables were used to determine the optimum methionine to methionine plus cystine ratio. The data from the two variables were analyzed to fit a quadratic regression, as well as one- and two- slope regression models. By comparing the estimated results from three regression models, the two most precise values, 54.15 and 56.72%, were concluded to be the optimal relative proportion of methionine for nitrogen retention and plasma urea nitrogen responses, respectively. Due to the influence of added crystalline cystine on plasma urea nitrogen, the value 54.15% estimated by nitrogen retention assay, was considered to be the more reasonable result.

## REFERENCES

AOAC. 1995. Official Methods of Analysis, 16th ed. Association of Official Analysis Chemists, Washington, DC.

Cai, Y. J., R. C. Ewan and D. R. Zimmerman. 1992. Effects of dietary energy, protein and potassium levels on plasma urea nitrogen and free amino acids in finishing pigs. J. Anim. Sci. 70(Suppl. 1):236(Abstr.).

Chung, T. K. and D. H. Baker. 1992. Maximal portion of the young pig's sulfur amino acid requirement that can be furnished by cystine. J. Anim. Sci. 70:1182-1187.

Coma, J., D. Carrion and D. R. Zimmerman. 1995a. Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. J. Anim. Sci. 73:472-481.

Coma, J., D. R. Zimmerman and D. Carrion. 1995b. Relationship of rate of lean tissue growth and other factors to concentration of urea in plasma of pigs. J. Anim. Sci. 73: 3649-3656.

De Lange, C. F. M., A. M. Gillis and G. J. Simpson. 2001. Influence of threonine intake on whole-body protein deposition and threonine utilization in growing pigs fed purified diets. J. Anim. Sci. 79:3087-3095.

Fuller, M. F., R. McWilliam, T. C. Wang and L. R. Giles. 1989. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. Br. J. Nutr. 64:255-267.

Honeyfield, D. C., J. A. Froseth and R. J. Barke. 1985. Dietary sodium and chloride levels for growing-finishing pigs. J. Anim. Sci. 60:691-698.

Kerschner, L. and J. Ziegenhorn. 1985. Urea. In: Methods in Enzymatic Analysis. 3rd edn. (Ed. H. U. Bergmeyer). Vol. 8, Verlag Chemie, Weinheim, pp. 444-453.

Lewis, A. J. 2003. Methionine-cystine relationships in pig nutrition. In: Amino Acids in Animal Nutrition., 2nd edn. (Ed. J. P. F. D'Mello) vol. 8, CAB International, Wallingford, UK pp. 143-155.

Mahan, D. C. and R. G. Jr. Shields. 1998. Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. J. Anim. Sci. 76:513-521.

Möhn, S., A. M. Gillis, P. J. Moughan and C. F. M. de Lange. 2000. Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. J. Anim. Sci. 78:1510-1519.

NRC. 1998. Nutrient Requirements of Swine, 10th ed. National Academic Press, Washington, DC.

Reijmers, A. T. H., A. M. Gillis and C. F. M. de Lang. 2002. Optimum dietary methionine to methionine+cystine ratio for growing pigs. In: Amino Acids in Animal Nutrition (Ed. M. Pack, J. Ficklen, M. Rademachen, A. Lemme, S. Mack, D. Hohlen, J. Fonlaine and A. Petoi), CORAL SANIVET, Bucharest pp. 453-457.

Robbins, K. B., H. W. Norton and D. H. Baker. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710-1714.

Roth, F. X. and M. Kirchgesser. 1989. Influence of the methionine:cysteine relationship in the feed on the performance of growing pigs. J. Anim. Physiol. Anim. Nutr. 61:265-274.

Russell, L. E., G. L. Cromwell and T. S. Stahly. 1983. Tryptophan, threonine, isoleucine and methionine supplementation of a 12% protein, lysine-supplemented, corn-soybean meal diet for growing pigs. J. Anim. Sci. 56:1115-1123.

SAS. 2002. SAS/STAT at User's Guide (Release 9.2.0.1.0 Ed.). SAS Inst. Inc., Cary, NC.

Schutte, J. B., M. W. Bosch, J. de Jong, E. J. van Weerden and F. Koch. 1991. Factors affecting the requirement of dietary sulphur-containing amino acids of young pigs. Netherland J. Agric. Sci. 39:91-101.

Slagle, S. P. and D. R. Zimmerman. 1979. Evaluation of a yeast single cell protein with young pigs. J. Anim. Sci. 49:1252-1260.

Van Kleef, D. J., K. Deuring and P. van Leeuwen. 1994. A new method of faeces collection in the pig. Lab. Anim. 28:78-79.

Wang, T. C. and M. F. Fuller. 1989. The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. Br. J. Nutr. 62:77-89.

Welbourne, T. C., V. Phromphetcharat, G. Givens and S. Joshi. 1986. Regulation of interorganal glutamine flow in metabolic acidosis. Am. J. Physiol. 250(4 Pt 1):E457-E463.

Yang, C. J., D. W. Lee, I. B. Chung, Y. M. Cho, I. S. Shin, B. J. Chae, J. H. Kim and In K. Han. 1997. Developing model equation to subdivide methionine+cystine requirements into requirement for growth and maintenance in pigs. Asian-Aust. J. Anim. Sci. 10(1):86-97.

Zimmermann, B., R. Mosenthin, M. Rademacher, P. B. Lynch and E. Esteve-Garcia. 2005. Comparative studies on the relative efficacy of DL-methionine and liquid mechionine hydroxy analogue in growing pigs. Asian-Aust. J. Anim. Sci. 18(7):1003-1010.

**From:** PFR Event <pfreventcreation@fda.hhs.gov>

**To:** Cleary, Michael *; HQ Pet Food Report Notification; B6

**Sent:** 12/4/2018 10:17:32 PM

**Subject:** Earthborn Meadow Feast dry: Lisa Freeman - EON-372804

**Attachments:** 2059619-report.pdf; 2059619-attachments.zip

A PFR Report has been received and PFR Event [EON-372804] has been created in the EON System.

A "PDF" report by name "2059619-report.pdf" is attached to this email notification for your reference. Please note that all documents received in the report are compressed into a zip file by name "2059619-attachments.zip" and is attached to this email notification.

Below is the summary of the report:

**EON Key:** EON-372804
**ICSR #:** 2059619
**EON Title:** PFR Event created for Earthborn Meadow Feast dry; 2059619

| | | | |
|---|---|---|---|
| **AE Date** | 11/21/2018 | **Number Fed/Exposed** | 5 |
| **Best By Date** | | **Number Reacted** | 4 |
| **Animal Species** | Dog | **Outcome to Date** | Stable |
| **Breed** | Boxer (German Boxer) | | |
| **Age** | 3 Years | | |
| **District Involved** | PFR-New England DO | | |

**Product information**
**Individual Case Safety Report Number:** 2059619
**Product Group:** Pet Food
**Product Name:** Earthborn Meadow Feast dry
**Description:** Littermate diagnosed with reduced cardiac contractility Eating BEG diet (Earthborn) so screened all housemates B6 within normal limits but elevated NT-proBNP and cardiac troponin I Taurine pending Owner changing diet and will recheck in 3 months
**Submission Type:** Initial
**Report Type:** Adverse Event (a symptom, reaction or disease associated with the product)
**Outcome of reaction/event at the time of last observation:** Stable

**Number of Animals Treated With Product:** 5
**Number of Animals Reacted With Product:** 4

| Product Name | Lot Number or ID | Best By Date |
|---|---|---|
| Earthborn Meadow Feast dry | | |

**Sender information**
Lisa Freeman
200 Westboro Rd
North Grafton, MA 01536
USA

**Owner information**

B6

To view this PFR Event, please click the link below:
https://eon.fda.gov/eon//browse/EON-372804

To view the PFR Event Report, please click the link below:
https://eon.fda.gov/eon//EventCustomDetailsAction!viewReport.jspa?decorator=none&e=0&issueType=12&issueId=389773

==========

B6

**All Medical Records**

Client:
Address: B6

Patient: B6
Breed: Boxer
DOB: B6

Species: Canine
Sex: Female

**Referring Information**

B6

Client:
Patien: B6

**Initial Complaint:**
Scanned Record

**SOAP Text Nov 20 2018 12:24PM - Clinician, Unassigned FHSA**

**Initial Complaint:**
DCM study

**SOAP Text Nov 20 2018 1:12PM -** B6

**Disposition/Recommendations**

B6

AT TUFTS UNIVERSITY

Client: B6
Veterinarian:
Patient ID: B6
Visit ID:

| Patient: | B6 |
|---|---|
| Species: | Canine |
| Breed: | Boxer |
| Sex: | Female |
| Age: | B6 Years Old |

## Lab Results Report

**11/20/2018 5:45:57 PM** **Accession ID:** B6

| Test | Results | Reference Range | Units |
|---|---|---|---|
| Troponin I Research - FHSA | B6 | 0 - 0.08 | mg/dl |



B6

stringsoft

Printed Tuesday, December 04, 2018

Client: B6
Patient:

**IDEXX BNP - 11/20/2018**

IDEXX Reference Laboratories

B6

IDEXX VetConnect 1-888-433-9967

Clien B6
Patie
Species: CANINE
Breed: BOXER
Gender: FEMALE
Age: 3Y

Date: 11/20/2018
Requisition #: 354628
Accession #: 2302872168
Ordered b B6

TUFTS UNIVERSITY
200 WESTBORO RD
NORTH GRAFTON, Massachusetts 01536
508-839-5395

Account #88933

**CARDIOPET proBNP - CANINE**

| Test | Result | Reference Range | Low | Normal | High |
|---|---|---|---|---|---|
| CARDIOPET proBN - CANINE | B6 | 0 - 900 pmol/L | HIGH | B6 | |

B6

Please note: Complete interpretive comments for all concentrations of Cardiopet proBNP are available in the online directory of services. Serum specimens received at room temperature may have decreased NT-proBNP concentrations.

Page 1 of 1

Client: B6
Patient:

**Gastrointestinal Lab Texas A&M 11/20/18**


**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: clinpath@tufts.edu**


**GI Lab Assigned Clinic ID: 11405**

B6
Tufts University-Clinical Pathology Lab
Attn: B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: B6
Owner Name:
Species: Canine
Date Received: Nov 27, 2018

**Tufts University-Clinical Pathology Lab**
**Tracking Number: 1811200085**

**GI Lab Accession:** B6

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | B6 | ≤0.06 | 11/27/18 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

B6 Canine
11/20/2018 1:14 PM
CARDIAC TROPONIN/TEXGI
SST


**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

Client: Patient: **B6**

**Gastrointestinal Lab** **B6**

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Important Notices:**

Ongoing studies

**Cobalamin Supplementation Study-** Dogs and cats with cobalamin deficiency with normal PLI, and either normal or low(consistent with EPI) TLI to compare the efficacy of oral vs parenteral cobalamin supplementation. Contact Dr. Chang at chchang@cvm.tamu.edu for further information.

**Chronic Pancreatitis with Uncontrolled Diabetes Mellitus-** Seeking dogs with chronic pancreatitis and uncontrolled diabetes mellitus for enrollment into a drug trial(medication provided at no cost). Contact Dr. Sue Yee Lim at slim@cvm.tamu.edu or Dr. Sina Marsilio at smarsilio@cvm.tamu.edu

**Dogs with Primary Hyperlipidemia-** Prescription diet naïve dogs newly diagnosed with primary hyperlipidemia are eligible to be enrolled in a dietary trial. Contact Dr. Lawrence at ylawrence@cvm.tamu.edu for more information.

**Dogs with Chronic Pancreatitis-**Dogs with chronic pancreatitis (cPLi >400µg/L) and hypertriglyceridemia (>300 mg/dl) are eligible to be enrolled in a dietary trial. Contact Dr. Lawrence at ylawrence@cvm.tamu.edu

**Chronic enteropathies in dogs-**Please fill out this brief form http://tinyurl.com/ibd-enroll to see if your patient qualifies.

**Feline Chronic Pancreatitis-** Cats with chronic pancreatitis for more than 2 weeks and fPLI >10 µg/L are eligible for enrollment into a treatment trial investigating the efficacy of prednisolone or cyclosporine. Please contact Dr. Yamkate for further information at pyamkate@cvm.tamu.edu.

We can not accept packages that are marked "Bill Receiver"

Use our preprinted shipping labels to save on shipping. Call 979-862-2861 for assistance. The GI Lab is not here to accept packages on the weekend. Samples may be compromised if you ship for arrival on Saturday or Sunday or if shipped via US Mail.

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864

Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

Client: B6
Patient:

**Diet hx**

## CARDIOLOGY DIET HISTORY FORM

Please answer the following questions about your pet

Pet's name: B6 Owner's nam B6 Today's date: 20NOV 2018

1. How would you assess your pet's appetite? (mark the point on the line below that best represents your pet's appetite)
   *Example:* ***Poor*** _____|_____ ***Excellent***

   **Poor** _____|_____ **Excellent**

2. Have you noticed a change in your pet's appetite over the last 1-2 weeks? (check all that apply)
   ☑ Eats about the same amount as usual ☐ Eats less than usual ☐ Eats more than usual
   ☐ Seems to prefer different foods than usual ☐ Other_____

3. Over the last few weeks, has your pet (check one)
   ☐ Lost weight ☐ Gained weight ☑ Stayed about the same weight ☐ Don't know

4. Please list below ALL pet foods, people food, treats, snack, dental chews, rawhides, and any other food item that your pet currently eats. Please include the brand, specific product, and flavor so we know exactly what you pet is eating.

   *Examples are shown in the table – please provide enough detail that we could go to the store and buy the exact same food.*

| Food (include specific product and flavor) | Form | Amount | How often? | Fed since |
|---|---|---|---|---|
| *Nutro Grain Free Chicken, Lentil, & Sweet Potato Adult* | *dry* | *1 ½ cup* | *2x/day* | *Jan 2018* |
| *85% lean hamburger* | *microwaved* | *3 oz* | *1x/week* | *Jan 2015* |
| *Pupperoni original beef flavor* | *treat* | *½* | *1x/day* | *Aug 2015* |
| *Rawhide* | *treat* | *6 inch twist* | *1x/week* | *Dec 2015* |
| EARTHBORN - MEADOW FEAST | DRY | ~1 1/2 C | 2X DAY | FEB 2016 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Any additional diet information can be listed on the back of this sheet*

5. Do you give any dietary supplements to your pet (for example: vitamins, glucosamine, fatty acids, or any other supplements)? ☐ Yes ☐ No If yes, please list which ones and give brands and amounts:

| | | Brand/Concentration | Amount per day |
|---|---|---|---|
| Taurine | ☐ Yes ☐ No | | |
| Carnitine | ☐ Yes ☐ No | | |
| Antioxidants | ☐ Yes ☐ No | | |
| Multivitamin | ☐ Yes ☐ No | | |
| Fish oil | ☐ Yes ☐ No | | |
| Coenzyme Q10 | ☐ Yes ☐ No | | |
| Other (please list): | | | |
| *Example: Vitamin C* | | *Nature's Bounty* | *500 mg tablets – 1 per day* |
| SUPPLEMENT | | NUPRO - DOG SUPPLEMENT | 1 SCOOP (~1 TBSP) 1-2 x day |
| | | | |
| | | | |
| | | | |
| | | | |

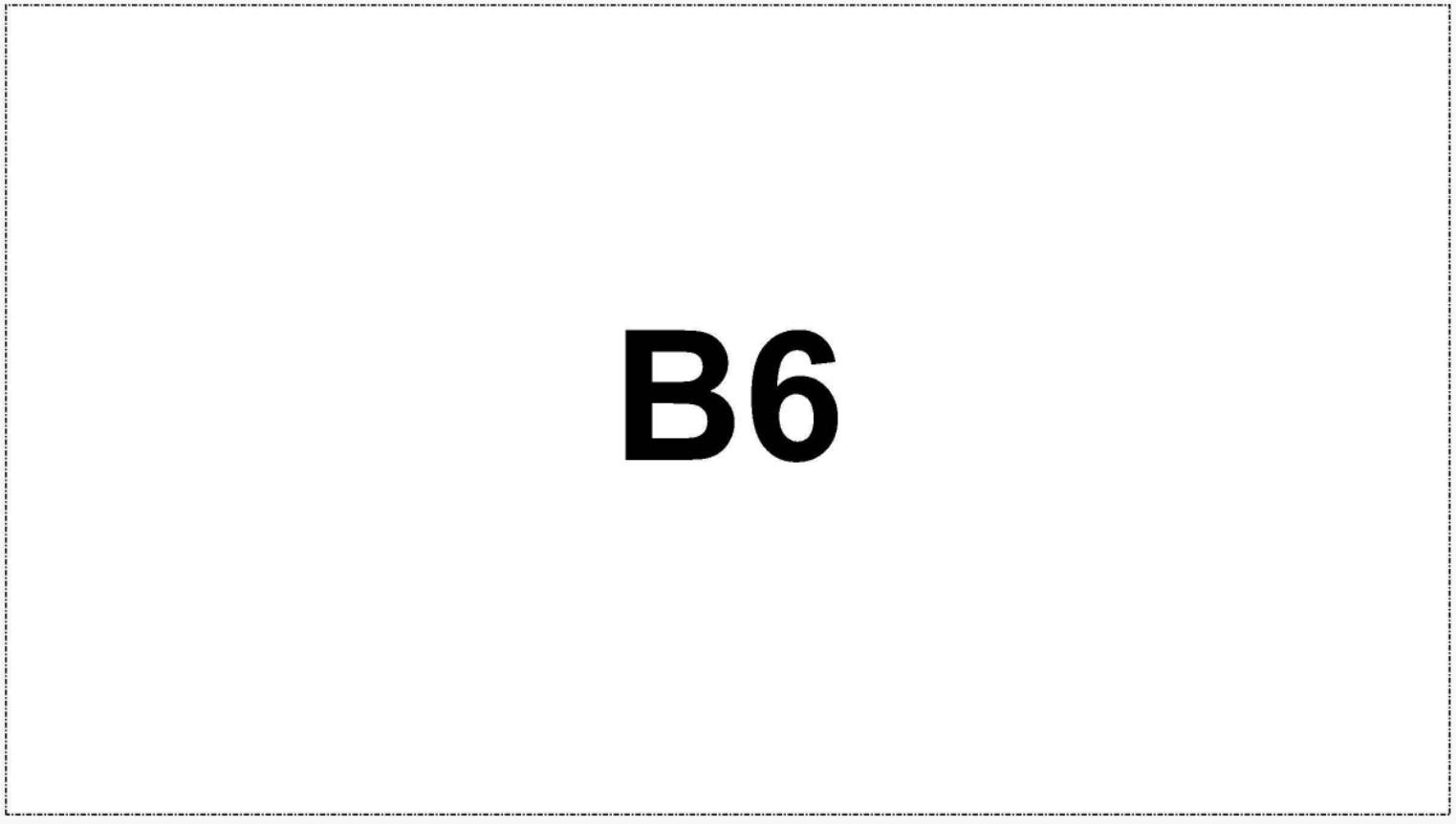
6. How do you administer pills to your pet?
   ☐ I do not give any medications
   ☐ I put them directly in my pet's mouth without food
   ☐ I put them in my pet's dog/cat food
   ☐ I put them in a Pill Pocket or similar product
   ☑ I put them in foods (list foods): bologna or cheese

Client: B6
Patient:

**ECG from Cardio**

B6

11/20/2018 2:45:12 PM
Tufts University
Tufts Cummings School of Vet Med
Cardiology



Client:
Patient: B6

**ECG from Cardio**

B6

11/20/2018 2:45:12 PM
Tufts University
Tufts Cummings School of Vet Med
Cardiology

Client: B6
Patient:

**Patient History**

| | |
|---|---|
| 09/27/2018 02:02 PM | Appointment |
| 11/14/2018 05:13 PM | Appointment |
| 11/19/2018 06:05 PM | Appointment |
| 11/20/2018 12:38 PM | UserForm |
| 11/20/2018 01:10 PM | UserForm |
| 11/20/2018 04:05 PM | Purchase |
| 11/20/2018 04:05 PM | Purchase |
| 11/20/2018 04:06 PM | Purchase |
| 11/20/2018 04:56 PM | Treatment |
| 11/20/2018 05:46 PM | Labwork |
| 11/20/2018 05:46 PM | Purchase |
| 11/21/2018 11:19 AM | UserForm |
| 11/26/2018 11:33 AM | Email |

B6

Cummings
Veterinary Medical Center
AT TUFTS UNIVERSITY

Foster Hospital for Small Animals
55 Willard Street
North Grafton, MA 01536
Telephone (508) 839-5395
Fax (508) 839-7951
http://vetmed.tufts.edu/

B6

B6 Female
Canine Boxer Fawn
345628

11/22/2018

Dear Dr B6

Thank you for referri B6 with their pe B6

If you have any questions, or concerns, please contact us at 508-887-4988.

Thank you,

B6 VM, DACVIM (Cardiology)

## Report Details - EON-380743

| | | | |
|---|---|---|---|
| **ICSR:** | 2063134 | | |
| **Type Of Submission:** | Initial | | |
| **Report Version:** | FPSR.FDA.PETF.V.V1 | | |
| **Type Of Report:** | Adverse Event (a symptom, reaction or disease associated with the product) | | |
| **Reporting Type:** | Voluntary | | |
| **Report Submission Date:** | 2019-02-25 07:58:43 EST | | |
| **Reported Problem:** | **Problem Description:** | Housemate (half sister, B6 - (ICSR) of 2063133) diagnosed with DCM and CHF so screened by RDVM for BNP which was elevated. Evaluated at Tufts 2/1/19. ARVC/diet-induced DCM with ventricular arrhythmia. Diet changed to Royal Canin Early Cardiac and will re-evaluate in 3 months I have diet sample. 3 other dogs in household (1 had normal BNP, other 2 not yet evaluated) | |
| | **Date Problem Started:** | 02/01/2019 | |
| | **Concurrent Medical Problem:** | Yes | |
| | **Pre Existing Conditions:** | Spinal trauma as puppy | |
| | **Outcome to Date:** | Stable | |
| **Product Information:** | **Product Name:** | Wellness CORE Grain-Free Ocean Whitefish dry Wellness Core grain free turkey, chicken liver, and turkey liver formula canned Wellness Core Hearty Cuts grain-free in gravy chicken and turkey recipe | |
| | **Product Type:** | Pet Food | |
| | **Lot Number:** | | |
| | **Product Use Information:** | **Description:** | Please see diet history for more info (and refer to B6 B6 diet history for more complete info - all dogs eat same diets) |
| | **Manufacturer /Distributor Information:** | | |
| | **Purchase Location Information:** | | |
| **Animal Information:** | **Name:** | B6 | |
| | **Type Of Species:** | Dog | |
| | **Type Of Breed:** | Bulldog | |
| | **Gender:** | Male | |
| | **Reproductive Status:** | Neutered | |
| | **Weight:** | 22.1 Kilogram | |
| | **Age:** | 8 Years | |
| | **Assessment of Prior Health:** | Good | |
| | **Number of Animals Given the Product:** | 6 | |
| | **Number of Animals Reacted:** | 3 | |
| | **Owner Information:** | **Owner Information provided:** | Yes |
| | | **Contact:** | **Name:** B6<br>**Phone:**<br>**Email:** |
| | | **Address:** | B6<br>United States |
| | **Healthcare Professional** | **Practice Name:** | Tufts Cummings School of Veterinary Medicine |

| | | | | |
|---|---|---|---|---|
| | **Information:** | **Contact:** | **Name:** | Lisa Freeman |
| | | | **Phone:** | (508) 887-4523 |
| | | | **Email:** | lisa.freeman@tufts.edu |
| | | **Address:** | 200 Westboro Rd<br>North Grafton<br>Massachusetts<br>01536<br>United States | |
| **Sender Information:** | **Name:** | Lisa Freeman | | |
| | **Address:** | 200 Westboro Rd<br>North Grafton<br>Massachusetts<br>01536<br>United States | | |
| | **Contact:** | **Phone:** | 5088874523 | |
| | | **Email:** | lisa.freeman@tufts.edu | |
| | **Permission To Contact Sender:** | Yes | | |
| | **Preferred Method Of Contact:** | Email | | |
| **Additional Documents:** | | | | |
| | **Attachment:** | rpt_medical_record_preview.pdf | | |
| | **Description:** | Medical record | | |
| | **Type:** | Medical Records | | |

**From:** Nemser, Sarah </O=FDA/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS /CN=SARAH.YACHETTI>
**To:** CVM Vet-LRN-OR
**Sent:** 2/12/2015 8:07:27 PM
**Subject:** FW: EON-196802 - Blue Buffalo Freedom Grain Free Adult Lamb [B6]

Olga,

Can I punt this one to you? I am currently working on another necropsy follow up case.

I started gathering up all the documents here: A:\6-CASES\!-PENDING\EON-196802-[B6]-Blue Buffao-potentialnec

Looks like the vet is out of office tomorrow, but Dave got her cell phone.

Thanks,
Sarah

Sarah Nemser M.S.

tel: 240-402-0892
**sarah.nemser@fda.hhs.gov**

---

**From:** Carey, Lauren
**Sent:** Thursday, February 12, 2015 12:59 PM
**To:** Palmer, Lee Anne; Rotstein, David; Reimschuessel, Renate; CVM Vet-LRN-OR; Queen, Jackie L
**Subject:** RE: EON-196802 - Blue Buffalo Freedom Grain Free Adult Lamb [B6]

Yay! A lucky save.

---

**From:** Palmer, Lee Anne
**Sent:** Thursday, February 12, 2015 12:58 PM
**To:** Rotstein, David; Reimschuessel, Renate; Carey, Lauren; CVM Vet-LRN-OR; Queen, Jackie L
**Subject:** RE: EON-196802 - Blue Buffalo Freedom Grain Free Adult Lamb: [B6]

Good catch and quick action everyone, thank you!

---

**From:** Rotstein, David
**Sent:** Thursday, February 12, 2015 12:57 PM
**To:** Reimschuessel, Renate; Carey, Lauren; CVM Vet-LRN-OR; Palmer, Lee Anne; Queen, Jackie L
**Subject:** RE: EON-196802 - Blue Buffalo Freedom Grain Free Adult Lamb: [B6]
**Importance:** High

The body will be back in the vet clinic tomorrow!

[B6] will be in today, but is off tomorrow. She has an appt in the AM, but gave me her personal cell [B6]

Office number [B6]

Email i [B6]

We are set!

I'll send MRx as soon as I get them.

thanks

dave

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-276-9213 (Office and Fax)
240-506-6763 (BB)


---

**From:** Reimschuessel, Renate
**Sent:** Thursday, February 12, 2015 12:00 PM
**To:** Rotstein, David; Carey, Lauren; CVM Vet-LRN-OR; Palmer, Lee Anne; Queen, Jackie L
**Subject:** RE: EON-196802 - Blue Buffalo Freedom Grain Free Adult Lamb: B6

Thanks for your rapid response.
Bodies are so often disposed of before we even have a chance at trying to help out.

Renate Reimschuessel V.M.D. Ph.D. Vet-LIRN
*Phone/Fax- 1-301-210-4024* – Will change soon to *1-240-402-5404*
Fax 301-210-4685
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

---

**From:** Rotstein, David
**Sent:** Thursday, February 12, 2015 11:58 AM
**To:** Carey, Lauren; Reimschuessel, Renate; CVM Vet-LRN-OR; Palmer, Lee Anne; Queen, Jackie L
**Subject:** RE: EON-196802 - Blue Buffalo Freedom Grain Free Adult Lamb: B6

Body is at crematorium. She is going to check into availability.

MRx will be faxed.

No known exposures. No overt systemic mineralization observed with x-rays.

d.

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-276-9213 (Office and Fax)
240-506-6763 (BB)


---

**From:** Carey, Lauren
**Sent:** Thursday, February 12, 2015 11:56 AM
**To:** Rotstein, David; Reimschuessel, Renate; CVM Vet-LRN-OR; Palmer, Lee Anne; Queen, Jackie L

**Subject:** RE:EON-196802 - Blue Buffalo Freedom Grain Free Adult Lamb: B6

Thanks! Edited subject line to show EON #. Whoops! Sorry.

---

**From:** Rotstein, David
**Sent:** Thursday, February 12, 2015 11:48 AM
**To:** Reimschuessel, Renate; Carey, Lauren; CVM Vet-LRN-OR; Palmer, Lee Anne; Queen, Jackie L
**Subject:** RE: Blue Buffalo Freedom Grain Free Adult Lamb: B6

Calling the vet right now...

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-276-9213 (Office and Fax)
240-506-6763 (BB)


---

**From:** Reimschuessel, Renate
**Sent:** Thursday, February 12, 2015 11:47 AM
**To:** Carey, Lauren; CVM Vet-LRN-OR; Palmer, Lee Anne; Queen, Jackie L; Rotstein, David
**Subject:** RE: Blue Buffalo Freedom Grain Free Adult Lamb B6

Suggest getting medical records and Contacting owner to find out if they want a necropsy
Vet-LIRN will be happy to arrange one it the owners are willing.

Renate Reimschuessel V.M.D. Ph.D. Vet-LIRN
*Phone/Fax- 1-301-210-4024* – Will change soon to *1-240-402-5404*
Fax 301-210-4685
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

---

**From:** Carey, Lauren
**Sent:** Thursday, February 12, 2015 11:44 AM
**To:** CVM Vet-LRN-OR; Palmer, Lee Anne; Queen, Jackie L; Rotstein, David
**Subject:** FW: Blue Buffalo Freedom Grain Free Adult Lamb: B6

14 mo Shiba Inu euthanized for renal failure within 3 weeks of starting new food/new bag – Blue Buffalo Blue Freedom Adult Lamb. Dog is neutered so possibly pre-neuter bloodwork to compare?

Any interest in follow up?

---

**From:** PFR Event [mailto:pfreventcreation@fda.hhs.gov]
**Sent:** Thursday, February 12, 2015 10:48 AM
**To:** nancy.powell@doveltech.com; HQ Pet Food Report Notification; olivia.kasik@doveltech.com; usha.gulati@doveltech.com
**Subject:** Blue Buffalo Freedom Grain Free Adult Lamb: B6

A PFR Report has been received and PFR Event [EON-196802] has been created in the EON System

A "PDF" report by name "1037940-report.pdf" is attached to this email notification for your reference. Please note that all documents received in the report are compressed into a zip file by name "1037940-attachments.zip"

and is attached to this email notification.

Below is the summary of the report

**EON Key:** EON-196802
**EON Title:** PFR Event created for Blue Buffalo Freedom Grain Free Adult Lamb; 1037940

To view this PFR Event, please click the link below:
https://eon.fda.gov/eon//browse/EON-196802

To view the PFR Event Report, please click the link below:
https://eon.fda.gov/eon//EventCustomDetailsAction!viewReport.jspa?decorator=none&e=0&issueType=12&issueId=209321

**Product information**
**Individual Case Safety Report Number:** 1037940
**Product Group:** Pet Food
**Product Name:** Blue Buffalo Freedom Grain Free Adult Lamb
**Description:** On January 17, 2015 we introduced our 14-month old Shiba Inu to Blue Buffalo Freedom Grain Free Adult Lamb. Two and a half weeks later on the night of February 4, 2015 he started vomiting and by B6 he was diagnosed with profound kidney failure. We put him to sleep the next day. B6 B6 The only change in his otherwise predictable routine was the switch to this new formula of the Blue Freedom dog food line. Prior to the switch he would finish his breakfast before lunch and eat his dinner immediately. After the switch he wouldn?t touch his breakfast until after his midday walk and didn?t always finish his dinner. We thought perhaps he didn?t like the taste of lamb but now believe that he was putting off eating as long as possible because something was wrong. And there were behavioral changes in the days before the physical symptoms manifested. He started destroying furniture he had previously ignored and began gnawing holes in the wall. He became unusually restless the two mornings prior to his vomiting episode. We thought he was being rambunctious but in hindsight he was agitated. He was sick. This course of events is suggestive of a gradual poisoning instead of a singular event on the day of symptom onset, which is what the veterinarian is assuming.
**Submission Type:** Initial
**Report Type:** Adverse Event (a symptom, reaction or disease associated with the product)
**Outcome of reaction/event at the time of last observation:** Died Euthanized
**Number of Animals Treated With Product:** 1
**Number of Animals Reacted With Product:** 1

**Sender information**

B6

==========

This email and attached document are being provided to you in your capacity as a Commissioned Official with the U.S. Department of Health and Human Services as authorized by law. You are being provided with this information pursuant to your signed Acceptance of Commission.

This email message is intended for the exclusive use of the recipient(s) named above. It may contain information that is protected, privileged, or confidential. Any dissemination, distribution, or copying is strictly prohibited.

The information is provided as part of the Federal-State Integration initiative. As a Commissioned Official and state government official, you are reminded of your obligation to protect non-public information, including trade secret and confidential commercial information that you receive from the U.S. Food and Drug Administration from further disclosure. The information in the report is intended for situational awareness and should not be shared or acted upon independently. Any and all actions regarding this information should be coordinated through your local district FDA office.

Failure to adhere to the above provisions could result in removal from the approved distribution list. If you think you received this email in error, please send an email to FDAReportableFoods@fda.hhs.gov immediately.

**From:** Freeman, Lisa <Lisa.Freeman@tufts.edu>
**To:** Jones, Jennifer L
**Sent:** 3/10/2019 6:48:25 PM
**Subject:** FW: GILab Results
**Attachments:** RptAccPrint.pdf

Hi Jen,
Troponin results on a bunch of the dogs that I've already reported. The 4 McIntosh boxers are recheck values – interesting that all decreased at least a little. We'll re-evaluated them in 3 months. Will also forward their BNPs and echoes
Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Board Certified Veterinary Nutritionist™
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Tufts Veterinary Cardiology Service
**Sent:** Friday, March 08, 2019 10:40 AM
**To:** Freeman, Lisa <Lisa.Freeman@tufts.edu>; B6
**Subject:** FW: GILab Results

I can put into SS.

**From:** Clinical Pathology Lab <clinpath@tufts.edu>
**Sent:** Friday, March 8, 2019 8:28 AM
**To:** Tufts Veterinary Cardiology Service <cardiovet@tufts.edu>; B6 B6
**Subject:** FW: GILab Results

Forwarding Troponin Results that I think were sent through Cardio service.

B6

Clinical Pathology Laboratory

B6

---

**From:** gilab@cvm.tamu.edu [gilab@cvm.tamu.edu]
**Sent:** Wednesday, March 06, 2019 6:21 PM
**To:** Clinical Pathology Lab
**Cc** B6
**Subject:** GILab Results

Greetings:

Please see the attachment for updated results for your patient(s).

To obtain results faster, you can also login to our website at http://vetmed.tamu.edu/gilab/service/clinic-login to view results immediately when they become available.
Your username is B6

Thank you for using the GI Lab

The GI Lab - Promoting gastrointestinal health in companion animals
(979) 862 2861; FAX (979) 862 2864; http://vetmed.tamu.edu/gilab

==========

Accession: B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.111 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Result is within the upper limit of the reference interval. This can be normal in aging dogs,however if clinical signs are present,additional diagnostic workup is recommended. Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

==========

Accession: B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.208 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:

**********

==========

Accession # B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.627 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

==========

Accession B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 1.373 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

==========

Accession B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.505 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

==========

Accession B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.08 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Result is within the upper limit of the reference interval. This can be normal in aging dogs,however if clinical signs are present,additional diagnostic workup is recommended. Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

==========

Accession B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.375 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly

higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

==========

Accession: B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.304 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

==========

Accession: B6
Patient: B6

==========

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.385 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

Accession B6
Patient B6

**********

Ultra-Sensitive Troponin I Fasting

Timing: Fasting
Result: 0.182 ng/mL
Range: 0 - 0.06
Interpretation: Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**********

Report Comments:
**********

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

Dr. Freeman
Tufts University-Clinical Pathology Lab
Attn B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936

Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab**
**Tracking Number: 337144**

**GI Lab Accession: B6**

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Result is within the upper limit of the reference interval. This can be normal in aging dogs,however if clinical signs are present,additional diagnostic workup is recommended. Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

Dr. B6
Tufts University-Clinical Pathology Lab
Attn: B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** B6

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

Dr. Freeman
Tufts University-Clinical Pathology Lab
B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession: B6**

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: Cardiovet@tufts.edu OR clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

Dr. **B6**
Tufts University-Clinical Pathology Lab
Attn: **B6**
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** **B6**

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: Cardiovet@tufts.edu OR clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

Dr. B6
Tufts University-Clinical Pathology Lab
Attn: B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: B6
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** B6

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

Dr. Freeman
Tufts University-Clinical Pathology Lab
**B6**
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** **B6**

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Result is within the upper limit of the reference interval. This can be normal in aging dogs,however if clinical signs are present,additional diagnostic workup is recommended. Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

Gastrointestinal Laboratory
Dr. J.M. Steiner
Department of Small Animal Clinical Sciences
Texas A&M University
4474 TAMU
College Station, TX 77843-4474

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

B6
Tufts University-Clinical Pathology Lab
B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936

Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** B6

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

B6
Tufts University-Clinical Pathology Lab
B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** B6

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

Gastrointestinal Laboratory
Dr. J.M. Steiner
Department of Small Animal Clinical Sciences
Texas A&M University
4474 TAMU
College Station, TX 77843-4474

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

B6
Tufts University-Clinical Pathology Lab
B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** B6

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

Gastrointestinal Laboratory
Dr. J.M. Steiner
Department of Small Animal Clinical Sciences
Texas A&M University
4474 TAMU
College Station, TX 77843-4474

**Website User ID: clinpath@tufts.edu**

**GI Lab Assigned Clinic ID: 11405**

B6
Tufts University-Clinical Pathology Lab
B6
200 Westboro Road
North Grafton, MA 01536
USA

**Phone:** 508 887 4669
**Fax:** 9 508 839 7936
Animal Name: **B6**
Owner Name:
Species: Canine
Date Received: Mar 06, 2019

**Tufts University-Clinical Pathology Lab Tracking Number:**

**GI Lab Accession:** B6

| Test | Result | Reference Interval | Assay Date |
|---|---|---|---|
| **Ultra-Sensitive Troponin I Fasting** | **B6** | ≤0.06 | 03/06/19 |

Interpretation: Increased troponin I value. If clinical signs of heart disease are present,additional diagnostic work-up is recommended Patients who are being supplemented with biotin may exhibit a slightly higher ultra-sensitive troponin result (10% or lower); however, the ability of the assay to detect serial increases or decreases of ultra-sensitive troponin is maintained.

**Comments:**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**Gastrointestinal Laboratory**
**Dr. J.M. Steiner**
**Department of Small Animal Clinical Sciences**
**Texas A&M University**
**4474 TAMU**
**College Station, TX 77843-4474**

**Important Notices:**

Internal Medicine Conference

**Join us for a unique continuing education event in Phuket, Thailand Oct 7th - 11th, 2019. For details see** http://texasimconference.tamu.edu

Ongoing studies

**Cobalamin Supplementation Study-** Dogs and cats with cobalamin deficiency with normal PLI, and either normal or low(consistent with EPI) TLI to compare the efficacy of oral vs parenteral cobalamin supplementation. Contact Dr. Chang at chchang@cvm.tamu.edu for further information.

**Chronic Pancreatitis with Uncontrolled Diabetes Mellitus-** Seeking dogs with chronic pancreatitis and uncontrolled diabetes mellitus for enrollment into a drug trial(medication provided at no cost). Contact Dr. Sue Yee Lim at slim@cvm.tamu.edu or Dr. Sina Marsilio at smarsilio@cvm.tamu.edu

**Dogs with Primary Hyperlipidemia-** Prescription diet naïve dogs newly diagnosed with primary hyperlipidemia are eligible to be enrolled in a dietary trial. Contact Dr. Lawrence at ylawrence@cvm.tamu.edu for more information.

**Dogs with Chronic Pancreatitis-**Dogs with chronic pancreatitis (cPLi >400µg/L) and hypertriglyceridemia (>300 mg/dl) are eligible to be enrolled in a dietary trial. Contact Dr. Lawrence at ylawrence@cvm.tamu.edu

**Chronic enteropathies in dogs-**Please fill out this brief form http://tinyurl.com/ibd-enroll to see if your patient qualifies.

**Feline Chronic Pancreatitis-** Cats with chronic pancreatitis for more than 2 weeks and fPLI >10 µg/L are eligible for enrollment into a treatment trial investigating the efficacy of prednisolone or cyclosporine. Please contact Dr. Yamkate for further information at pyamkate@cvm.tamu.edu.

We can not accept packages that are marked "Bill Receiver"

**Use our preprinted shipping labels to save on shipping. Call 979-862-2861 for assistance. The GI Lab is not here to accept packages on the weekend. Samples may be compromised if you ship for arrival on Saturday or Sunday or if shipped via US Mail.**

**GI Lab Contact Information**

Phone: (979) 862-2861
Fax: (979) 862-2864
Email: gilab@cvm.tamu.edu
vetmed.tamu.edu/gilab

**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** 'Reimschuessel, Renate (Renate.Reimschuessel@fda.hhs.gov)'; Peloquin, Sarah; Ceric, Olgica
**CC:** 'Guag, Jake * (Jake.Guag@fda.hhs.gov)'
**Sent:** 2/21/2019 7:59:20 PM
**Subject:** FW: Now live: DCM Investigation Update
**Attachments:** DCM Plan_Feb2019.docx

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Norris, Anne
**Sent:** Tuesday, February 19, 2019 10:07 AM
**To:** Solomon, Steven M <Steven.Solomon@fda.hhs.gov>; Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>; Moxley, Shera <Shera.Moxley@fda.hhs.gov>; Flynn, William T <William.Flynn@fda.hhs.gov>; Murphy, Jeanette <Jenny.Murphy@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Allen, Mary <Mary.Allen@fda.hhs.gov>; Edwards, David <David.Edwards@fda.hhs.gov>; Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Burkholder, William <William.Burkholder@fda.hhs.gov>; Benton, Denise <Denise.Benton@fda.hhs.gov>; Goddard, Kristina <Kristina.Goddard@fda.hhs.gov>; Dewitt, Susan J <Susan.Dewitt@fda.hhs.gov>; Alvey, Laura - CVM <Laura.Alvey@fda.hhs.gov>; Stamper, Carmela <Carmela.Stamper@fda.hhs.gov>; Smith-Collier, Chandra E <Chandra.Smith-Collier@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
**Subject:** Now live: DCM Investigation Update

Good morning,

The long-awaited investigative update about the potential link between certain diets and canine DCM is now live. Outreach may now begin (plan is attached). Social media and an email to our CVM subscribers will go out around 11:00 am EST.

Links:
CVM Update: https://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm630991.htm
Investigation home page: http://www.fda.gov/AnimalVeterinary/NewsEvents/ucm630993.htm
Vet-LIRN page: https://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm630738.htm
Web Q&A: https://www.fda.gov/AnimalVeterinary/ResourcesforYou/AnimalHealthLiteracy/ucm616279.htm

Thanks so much to everyone for their hard work and patience! And thank you on behalf of the people who will be emailing and calling who might be too preoccupied to express their gratitude.

Anne

**Anne Norris**
*Strategic Initiatives*

**Office of the Director**
**Center for Veterinary Medicine**
**U.S. Food & Drug Administration**
O: 240-402-0132
M: 240-704-0579

Anne.Norris@fda.hhs.gov

**From:** Jones, Jennifer L </O=FDA/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=JENNIFER.JONESAA8>
**To:** Rotstein, David; Palmer, Lee Anne; Queen, Jackie L; Carey, Lauren; Reimschuessel, Renate; Ceric, Olgica
**Sent:** 8/22/2017 1:20:55 PM
**Subject:** RE: Taste of the Wild Sierra Mountain Canine Formula with Roasted Lamb: B6 - EON-331085

I'll see if lot/BB available

Jennifer Jones, DVM
Veterinary Medical Officer

U.S. FOOD & DRUG ADMINISTRATION

Vet-LIRN

---

**From:** Rotstein, David
**Sent:** Tuesday, August 22, 2017 9:18 AM
**To:** Palmer, Lee Anne; Queen, Jackie L; Carey, Lauren; Jones, Jennifer L; Reimschuessel, Renate; Ceric, Olgica
**Subject:** FW: Taste of the Wild Sierra Mountain Canine Formula with Roasted Lamb: B6 - EON-331085

The lot looks like the UPC code, but this may be one to follow-up with animal and open product testing (VL) if they have a lot code.

d.

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
**240-506-6763 (BB)**

U.S. FOOD & DRUG ADMINISTRATION



---

**From:** PFR Event [mailto:pfreventcreation@fda.hhs.gov]
**Sent:** Tuesday, August 22, 2017 9:16 AM
**To:** Cleary, Michael *; HQ Pet Food Report Notification; B6
**Subject:** Taste of the Wild Sierra Mountain Canine Formula with Roasted Lamb: B6 - EON-331085

A PFR Report has been received and PFR Event [EON-331085] has been created in the EON System.

A "PDF" report by name "2024905-report.pdf" is attached to this email notification for your reference.

Below is the summary of the report:

**EON Key:** EON-331085
**ICSR #:** 2024905
**EON Title:** PFR Event created for Taste of the Wild Sierra Mountain Canine Formula with Roasted Lamb a grain-free diet; 2024905

| | | | |
|---|---|---|---|
| **AE Date** | 08/14/2017 | **Number Fed/Exposed** | 1 |
| **Best By Date** | 06/01/2018 | **Number Reacted** | 1 |
| **Animal Species** | Dog | **Outcome to Date** | Better/Improved/Recovering |
| **Breed** | Dachshund - Miniature | | |
| **Age** | 9 Years | | |
| **District Involved** | PFR-Atlanta DO | | |

**Product information**
**Individual Case Safety Report Number:** 2024905
**Product Group:** Pet Food
**Product Name:** Taste of the Wild Sierra Mountain Canine Formula with Roasted Lamb, a grain-free diet
**Description:** After about 2 weeks of mixing in Taste of the Wild Lamb, she became sick with lethargy, diarrhea, wouldn't eat or walk. B6 vet diagnosed bacterial intestinal infection and thinks illness may have resulted from this food.
**Submission Type:** Initial
**Report Type:** Both
**Outcome of reaction/event at the time of last observation:** Better/Improved/Recovering
**Number of Animals Treated With Product:** 1
**Number of Animals Reacted With Product:** 1

| Product Name | Lot Number or ID | Best By Date |
|---|---|---|
| Taste of the Wild Sierra Mountain Canine Formula with Roasted Lamb, a grain-free diet | 717933014:45 | 06/01/2018 |

**Sender information**

B6

USA

To view this PFR Event, please click the link below:
https://eon.fda.gov/eon//browse/EON-331085

To view the PFR Event Report, please click the link below:
https://eon.fda.gov/eon//EventCustomDetailsAction!viewReport.jspa?decorator=none&e=0&issueType=12&issueId=346606

==========

**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** 'Freeman, Lisa'
**Sent:** 3/4/2019 12:13:16 PM
**Subject:** RE: taurine results for B6

Thanks, Lisa!

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Freeman, Lisa <Lisa.Freeman@tufts.edu>
**Sent:** Friday, March 01, 2019 4:51 PM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** FW: taurine results for B6

FYI
Weird – 3 bulldogs from same household and on same diet
B6 – DCM and CHF had B6 plasma and B6 WB
B6 – with ARVC and arrhythmias had B6
B6 – this most recent one (likely ARVC) was B6

Lisa

Lisa M. Freeman, DVM, PhD, DACVN
Board Certified Veterinary Nutritionist™
Professor
Cummings School of Veterinary Medicine
Friedman School of Nutrition Science and Policy
Tufts Clinical and Translational Science Institute
Tufts University
www.petfoodology.org

**From:** Jones, Jennifer L </o=FDA/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=Jennifer.Jonesaa8>
**To:** B6 Nemser, Sarah
**CC:** Freeman, Lisa*
**Sent:** 3/30/2016 2:14:14 PM
**Subject:** RE: UPS Ship Notification, Tracking Number 1ZA420F40194711273

Samples were received in good condition. Thank you, Tina.

Take care,
Jennifer

Jennifer Jones, DVM
Veterinary Medical Officer

---

**From** B6
**Sent:** Tuesday, March 29, 2016 2:52 PM
**To:** Nemser, Sarah; Jones, Jennifer L
**Cc:** Freeman, Lisa*
**Subject:** RE: UPS Ship Notification, Tracking Number 1ZA420F40194711273

Sounds good, Sarah.

Thank you!

---

**From:** Nemser, Sarah
**Sent:** Tuesday, March 29, 2016 1:50 PM
**To** B6 Jones, Jennifer L
**Cc:** Freeman, Lisa*
**Subject:** RE: UPS Ship Notification, Tracking Number 1ZA420F40194711273

Tina,

Thanks for sending along.

Jen will let you know when the samples arrive tomorrow.

Sarah

**Sarah Nemser M.S.**
**tel: 240-402-0892**
**fax: 301-210-4685**
**sarah.nemser@fda.hhs.gov**

---

**From:** B6
**Sent:** Tuesday, March 29, 2016 2:49 PM
**To:** Jones, Jennifer L
**Cc:** Nemser, Sarah; Freeman, Lisa*
**Subject:** FW: UPS Ship Notification, Tracking Number 1ZA420F40194711273

Dr. Jones,

Here is the tracking information for the samples you will be receiving for Sarah.

Have a great day.

Kind regards,

B6

---

**From:** UPS Quantum View [mailto:pkginfo@ups.com]
**Sent:** Tuesday, March 29, 2016 1:21 PM
**To:** B6
**Subject:** UPS Ship Notification, Tracking Number 1ZA420F40194711273

**You have a package coming.**

| | |
|---|---|
| **Scheduled Delivery Date:** | Wednesday, 03/30/2016 |

This message was sent to you at the request of NCTR-50 to notify you that the shipment information below has been transmitted to UPS. The physical package may or may not have actually been tendered to UPS for shipment. To verify the actual transit status of your shipment, click on the tracking link below.

# Shipment Details

| | |
|---|---|
| **From:** | NCTR-50 |
| **Tracking Number:** | 1ZA420F40194711273 |
| **Ship To:** | Center for Veterinary Medicine<br>Sarah Nemser<br>8401 Muirkirk Road<br>G202, HFV520<br>LAUREL, MD 207082482<br>US |
| **Number of Packages:** | 1 |
| **Scheduled Delivery:** | 03/30/2016 |
| **Hazardous Materials:** | Yes |
| **Weight:** | 24.0 LBS |
| **Reference Number 1:** | NCTR |
| **Reference Number 2:** | Pathology |

Get the UPS My Choice app for Facebook

Download the UPS mobile app



**UPS Privacy Notice**

**Contact UPS**

**From:** B6
**To:** Jones, Jennifer L
**Sent:** 6/14/2019 12:54:46 PM
**Subject:** Re[2]: 800.267-cc-297-FDA Case Investigation for B6 (cc-297)

Thank you Jennifer. I did receive an email from Jake.

Also, I believe that I read I should get a copy of the pathology results. Is this correct? I am interested in the results from B6

Thank you again.
I appreciate the work you all are doing for this.

B6

B6

------ Original Message ------
From: "Jones, Jennifer L" <Jennifer.Jones@fda.hhs.gov>
To: B6
Cc: "Peloquin, Sarah" <Sarah.Peloquin@fda.hhs.gov>; "Guag, Jake" <Jake.Guag@fda.hhs.gov>
Sent: 6/13/2019 11:01:47 AM
Subject: RE: 800.267-cc-297-FDA Case Investigation for B6 (cc-297)

Thank you, Dr. B6
We'll ship the box, and it should arrive before close of business Monday. Jake will send you a copy of the tracking when it ships.

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** B6
**Sent:** Tuesday, June 11, 2019 9:22 AM

**To:** Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>
**Cc:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** Re: 800.267-FDA Case Investigation for B6 (cc-297)

Good morning,
Weights for the samples from B6 are as follows:
Frozen tissue is 6ounces
Refrigerated samples (urine and small intestinal fluid) 2ounces
Formalin fixed samples 2 pounds 6 ounces

Thank you

B6

B6

------ Original Message ------
From: "Peloquin, Sarah" <Sarah.Peloquin@fda.hhs.gov>
To: "B6
Cc: "Jones, Jennifer L" <Jennifer.Jones@fda.hhs.gov>
Sent: B6 11:59:32 AM
Subject: 800.267-FDA Case Investigation for B6 (cc-297)

Good morning Dr. B6,

I'm filling in on the DCM case investigation for Dr. Jennifer Jones this week. Dr. Lisa Freeman informed me that B6 will be euthanized—I'm sorry to hear this. If you are willing, please collect the same samples from B6 as you did for B6 (i.e. intact heart in formalin; and if possible, fixed/frozen tissues as described in the rapid necropsy document).

Dr. Jones will return at the end of next week, and we will send you boxes then for shipment.

Please send me the approximate weights of the samples, as you did for B6

Let me know if you have any additional questions.

Thank you!
Dr. Sarah Peloquin

**Sarah K. Peloquin, DVM**

*Veterinary Medical Officer*

U.S. Food & Drug Administration
Center for Veterinary Medicine
Veterinary Laboratory Investigation and Response Network (Vet-LIRN)
tel: 240-402-1218
fax: 301-210-4685
e-mail: sarah.peloquin@fda.hhs.gov


---

| | |
|---|---|
| **From:** | Norris, Anne </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=891982B43D804C9396555BAF36C73DE1-ANNE.NORRIS> |
| **To:** | Hartogensis, Martine; Schell, Timothy; Jones, Jennifer L; Palmer, Lee Anne; Carey, Lauren; Burkholder, William; Rotstein, David; Conway, Charlotte; DeLancey, Siobhan |
| **Sent:** | 4/16/2019 10:47:51 AM |
| **Subject:** | TAPF: What you should know about Grains in your pet's food |

For awareness…

# What you should know about Grains in your pet's food

April 15, 2019

in Pet Food Ingredients


Grains in pet foods come with risks; a different set of risks than the current grain-free pet food potential risk to heart disease.

The FDA alerted pet owners to a current investigation into the "Potential Connection Between Diet and Canine Heart Disease" in July 2018. Though no scientific reason for the link has been released, the FDA states "*The U.S. Food and Drug Administration is alerting pet owners and veterinary professionals about reports of canine dilated cardiomyopathy (DCM) in dogs eating certain pet foods containing peas, lentils, other legume seeds, or potatoes as main ingredients. High levels of legumes or potatoes appear to be more common in diets labeled as "grain-free," but it is not yet known how these ingredients are linked to cases of DCM.*"

Because of this current risk of grain-free pet food, many pet owners are being urged to return to grain-included diets. But…grain-included pet foods come with another set of risks.

## The risk is mycotoxins.

High levels of mycotoxins are deadly, and even low levels consumed over time are linked to liver disease, kidney disease and cancer.

"Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi (molds). These low molecular weight compounds are naturally occurring and practically unavoidable. They can enter our food chain either directly from plant-based food components contaminated with mycotoxins or by indirect contamination from the growth of toxigenic fungi on food. Mycotoxins can accumulate in maturing corn, cereals, soybeans, sorghum, peanuts, and other food and feed crops in the field and in grain during transportation."

*Source: https: www.ncbi.nlm.nih.gov pmc articles PMC5486318*

Abstract from "*Mycotoxins in pet food: a review on worldwide prevalence and preventative strategies*":

Mycotoxins contaminate cereal grains worldwide, and their presence in pet food has been a potential health threat to companion animals. Aflatoxins, ochratoxin A, and Fusarium mycotoxins have been found in both raw ingredients and final products of pet food around the globe. Grain processing, sampling error, analytical methods, conjugated mycotoxins, storage conditions, and synergistic interactions are common challenges faced by the pet food industry.

From the full paper "Mycotoxins and the pet food industry: Toxicological evidence and risk assessment":

Mycotoxin contamination in pet food poses a serious health threat to pets, causing an emotional and economical concern to the pet owners. Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins and fusaric acid have been found in the ingredients and final products of pet food, resulting in both acute toxicity and chronic health problems in pets. Toxicological interaction among mycotoxins as a natural mixture further complicates the issue.

Aflatoxins have been the most common cause of acute mycotoxin outbreaks in commercial dog food. Corn is the usual source of aflatoxins in these cases. These available reports of acute mycotoxicosis, however, cannot provide the whole picture of the mycotoxin problem associated with pet foods since only a small number of food poisoning cases are published. Veterinarians, furthermore, often overlooked mycotoxins as the cause of chronic diseases such as liver and kidney fibrosis, infections resulting from immunosuppression and cancer. These findings suggest that mycotoxin contamination in pet food poses a serious health threat to pet species.

From the same paper as above, effects of different types of mycotoxins in pets are explained in more detail.

**Aflatoxins.** The primary clinical effects in aflatoxicosis are related to hepatic damage in all species studied. In acute aflatoxicosis, dogs exposed to >0.5–1 mg aflatoxin/kg body weight (BW) typically die within days, showing enlarged livers, disseminated intravascular coagulation and internal hemorrhaging. Sub-acute aflatoxicosis (0.5–1 mg aflatoxin/kg pet food) is characterized by anorexia, lethargy, jaundice, intravascular coagulation and death in 2–3 weeks. Similar hepatotoxic effects can also be produced by chronic aflatoxin exposure with 0.05–0.3 mg aflatoxin/kg pet food over 6–8 weeks.

In addition to their hepatotoxic properties, aflatoxins are also carcinogenic. The binding of DNA causes genotoxicity and mutation in cells. The chronic carcinogenic dose of aflatoxins is much lower than the acute dose. Since aflatoxins are both acute and chronic hepatotoxins and carcinogens, the actual number of dogs

affected by aflatoxins would be far more than the total number reported in acute poisoning cases.

**Ochratoxins.** Ochratoxins are a group of potent renal mycotoxins that widely contaminate the agricultural commodities, such as corn, wheat, oats and dried beans, in temperate regions.

Upon absorption, ochratoxins enter the circulatory system, bind tightly to serum proteins and accumulate in the kidneys, where they disrupt protein synthesis and other pathways in proximal tubular cells. OTA is also known to bind with DNA molecules and induce renal tumors in animal models, although its carcinogenic mechanism remains controversial.

Clinical symptoms of the OTA poisoning included anorexia, weight loss, vomiting, tenesmus, bloody diarrhea, increased body temperature, tonsillitis, dehydration, and prostration.

**Trichothecenes.** Trichothecenes are a family of *Fusarium* mycotoxins commonly found in corn, wheat, barley, as well as oats worldwide.
Trichothecenes are potent irritants and inhibitors of protein and DNA synthesis which interferes with cellular metabolic activities, ultimately leading to cell death.

**Zearalenone.** Zearalenone is an estrogenic *Fusarium* mycotoxin found in various cereal crops, most frequently in corn.

Both male and female dogs are affected by zearalenone toxicity. Some recent studies suggested that low levels of zearalenone exposure can also produce significant toxic effects.

**Fumonisins.** Fumonisins are found in corn throughout the world with more than 15 homologues isolated. Once they enter the blood circulation, fumonisins damage numerous organs in all species studied.

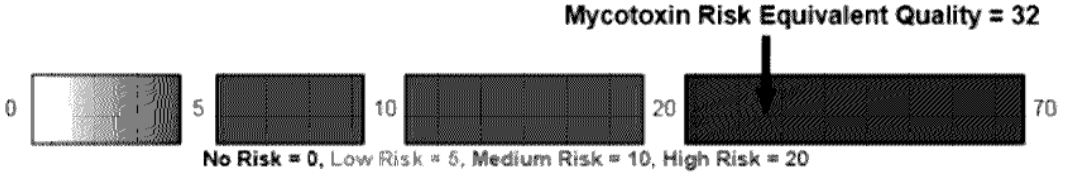
Mycotoxins also have a synergistic effect when 2 or more are found in a pet food. "***The toxicity of a particular mycotoxin, therefore, depends on not only its own concentration but also the presence of other mycotoxins***."

As example of dangerous synergy when multiple mycotoxins are present in a pet food is results from our consumer funded pet food testing performed in 2015.


**Sample ID:** INTI-11
**Species:** Dog

**Sample Description:** Purina Beneful Original Dog Food Dry

**Sample Comments:** Contains multiple mycotoxins. Zearalenone is at caution and can impact reproduction causing irregular heats, psuedopregnancy, poor sexual development, and abortions. Type B Trichothecenes are at low risk but Fusaric Acid is present and can act synergistically to magnify the impact on food intake, growth, gut health, liver function and immune response. The REQ is at high risk due to the number (10) and the levels of mycotoxins present




| Lab Results | | Comparison | | |
|---|---|---|---|---|
| **Toxins** | **Amount, PPB** | **Low Risk** | **Medium Risk** | **High Risk** |
| Aflatoxin (B1) | 0 | 5 | 10 | 20 |
| Aflatoxin (B1, B2, G1, G2) | 0 | 5 | 10 | 20 |
| Ochratoxins (A,B) | 0 | 10 | 20 | 30 |
| Type B Trichothecenes * | 104.59 | 100 | 250 | 500 |
| Type A Trichothecenes ** | 0 | 20 | 30 | 40 |
| Fumonisins (B1, B2, B3) | 353.73 | 500 | 1000 | 1500 |
| Zearalenone Group | 54.74 | 25 | 50 | 75 |
| Fusaric Acid | 216.21 | 1000 | 2000 | 3000 |
| Penicillium Mycotoxins *** | 28.21 | 40 | 70 | 100 |
| Aspergillus Mycotoxins **** | 7.26 | 40 | 60 | 80 |
| Ergot Toxins | 4.13 | 250 | 500 | 1000 |
| **Risk Equivalent Quality** | **32** | **5** | **10** | **20** |

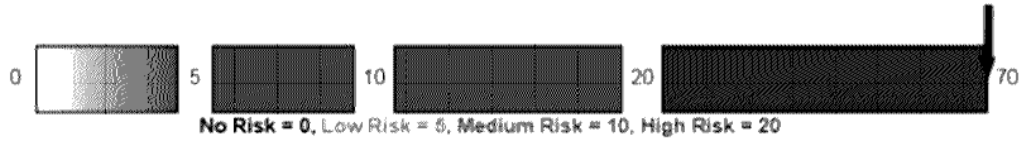
The above sampled Purina Beneful Dog Food contained 10 different types of mycotoxins. Each mycotoxin was below FDA's maximum allowed level individually (FDA has not established maximum levels for multiple mycotoxins present in pet foods), however based on industry based science our scientists classified this pet food has High Risk "*due to number (10) and the levels of mycotoxins present.*"

**Sample ID:** INTI-04
**Species:** Cat

**Sample Description:** Big Heart Meow Mix Tender Centers Salmon & Turkey Flavors Dry

**Sample Comments:** Contains multiple mycotoxins. Other Aspergillis is at high risk and these can cause tremors and convulsion, bloody diarrhea and lower immune response. Fumonisins are at caution and can lower food intake, cause digestive disorder, and impact liver function lowering immune response. Zearalenone is approaching caution and can impact reproduction and sexual maturity and at high levels can cause abortions. The REQ is at high risk due to the number (7) and the levels of mycotoxins present.

**Mycotoxin Risk Equivalent Quality = 70**



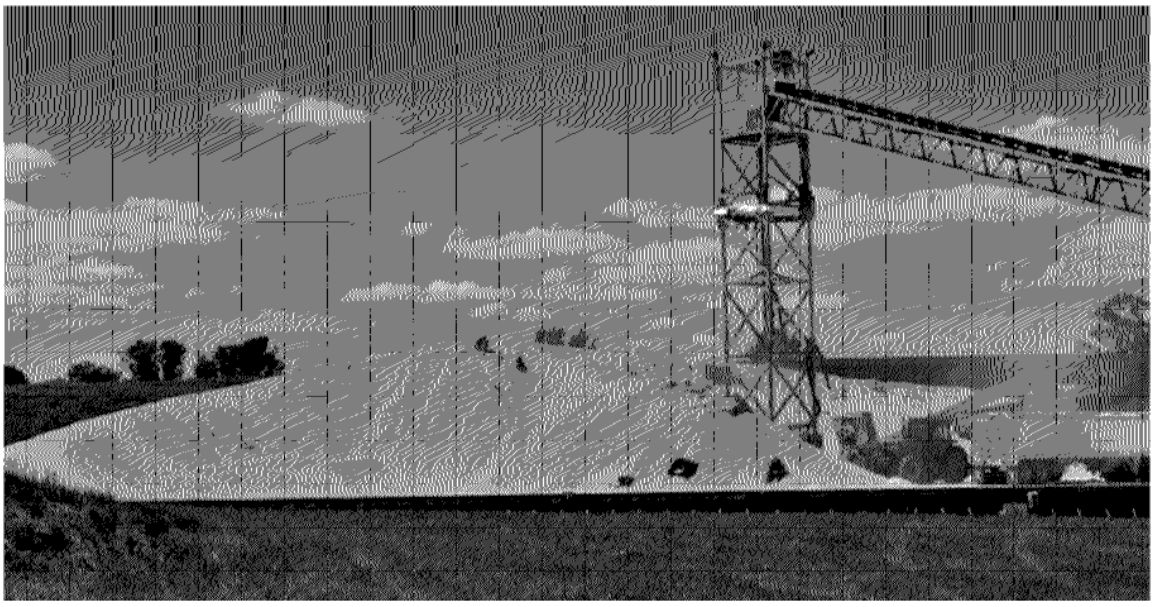

**No Risk = 0,** Low Risk = 5, **Medium Risk = 10, High Risk = 20**

| Lab Results | | Comparison | | |
|---|---|---|---|---|
| **Toxins** | **Amount, PPB** | **Low Risk** | **Medium Risk** | **High Risk** |
| Aflatoxin (B1) | 0 | 5 | 10 | 20 |
| Aflatoxin (B1, B2, G1, G2) | 0 | 5 | 10 | 20 |
| Ochratoxins (A,B) | 0 | 10 | 20 | 30 |
| Type B Trichothecenes * | 92.8 | 100 | 250 | 500 |
| Type A Trichothecenes ** | 0 | 20 | 30 | 40 |
| Fumonisins (B1, B2, B3) | 749.19 | 500 | 1000 | 1500 |
| Zearalenone Group | 24.57 | 25 | 50 | 75 |
| Fusaric Acid | 133.15 | 1000 | 2000 | 3000 |
| Penicillium Mycotoxins *** | 20.17 | 40 | 70 | 100 |
| Aspergillus Mycotoxins **** | 178.83 | 40 | 60 | 80 |
| Ergot Toxins | 0 | 250 | 500 | 1000 |
| **Risk Equivalent Quality** | **70** | **5** | **10** | **20** |

And again, this time with Big Heart Meow Mix cat food, our results showed all individual mycotoxins were below FDA allowed maximum. However, when the collective effect of mycotoxins were considered per scientific evidence, this pet food rated "*at high risk due to number (7) and the levels of mycotoxins present.*"

Another issue to consider with grain-included feed grade pet foods is the same consideration for all feed grade ingredients – the 'feed' system of regulation. Feed and feed ingredients are openly allowed by FDA to utilize what the food industry considers as waste. As example, the FDA allows "*the diversion of moisture-damaged grain as well as other food materials for animal feed use.*"

Below is an image of feed grade corn, stored in the middle of a field in Iowa (provided by a Iowa pet owner). Notice the grain is not under cover or protected from weather, insects or animals. This 'storage' of grain is openly allowed in feed.



Because mycotoxins are such a significant threat to all animals, each year various groups "survey" the grain productions for mycotoxin contaminations. Below is one of those survey's for grain production in the U.S. last year.

Adisseo's 2018 Mycotoxin Survey found that almost half of corn and silage samples taken in the U.S. were contaminated with zearalenone (ZEN) and more than 70 percent were contaminated with deoxynivalenol (DON), making the 2018 U.S. corn harvest of medium quality in terms of mycotoxin contamination.

The results showed that 49 percent of silage samples and 20 percent of corn samples were contaminated with ZEN.

Eighty percent of silage samples and 73 percent of corn samples were contaminated with DON.

Only 3 percent of samples had low levels of AfB1.

Several samples had two to four mycotoxins detected, which could lead to synergistic interactions. Forty-nine percent of samples were contaminated with two mycotoxins; 23 percent with three mycotoxins.

Another mycotoxin survey from Biomin.net states:

Our April update from the annual Biomin® PROcheck mycotoxin survey in corn harvested in 2018 includes 572 samples from 31 states.

**Corn**

98% of ground corn samples are positive for at least one mycotoxin, vs 89% in 2017

72% of samples have more than one mycotoxin, vs 47% in 2017

Aflatoxin prevalence in dry corn increased to 10% vs 4% in 2017, with average contamination levels increasing

over four fold.

Deoxynivalenol (vomitoxin) prevalence rose to 76% vs 70% in 2017, with average contamination level increasing by 44%

Fumonisin prevalence jumped to 79% vs 52% in 2017, with average contamination levels increasing by 45%

Zearalenone prevalence increased to 45% vs 25% in 2017, with average contamination levels remaining steady.

Grains in pet food come with mycotoxin risks. Grain-free pet foods are currently linked to cases of heart disease in dogs. **What are pet owners to do?**

There is no easy answer to this question. The FDA states: "*It's important to note that the reports* (cases of diet-related DCM reported to FDA) *include dogs that have eaten grain-free and grain containing foods, and also include vegetarian or vegan formulations. They also include all forms of diets: kibble, canned, raw and home-cooked. This is why we do not think these cases can be explained simply by whether or not they contain grains, or by brand or manufacturer*."

**Personal opinion:** No one has publicly provided the cause confirming the link between grain-free pet foods and canine heart disease. The unknown is frightening. Dogs have become sick and many have died linked to grain-free pet food. However, the other side of grain-free pet food is grain-included pet food that – as evidenced by decades of science – come with mycotoxin risks.

Would I give my own pets a grain-free commercial pet food? Yes, I would – and I do. My pets consume a human grade (made under constant USDA inspection) grain-free raw pet food – this is half of their diet (2 dogs, 5 cats).

Would I give my own pets grain? Yes, I would – and I do in a rotation of home prepared cooked foods which makes up the other half of my pets diet. Grain is not included with every batch of pet food I make, but it is included in the recipes I make. Significant to me, the grains I provide my pets in their rotation of recipes are human edible (human grade) grains purchased from my grocery store (though almost all are organic).

Would I give my own pets a feed grade grain-free or grain-included pet food? No, I would not.

And lastly, though I am certain it will be perceived by some as an attack, the intent of this post is to provide pet owners with scientific information about the risk of grains. It is one of several over many years that TruthaboutPetFood.com has published on the risk of grains. It is our belief that pet owners are fully capable of making their own pet food decisions when provided with all sides of an issue. Mycotoxin risk in pet food is definitely another side that needs to be well known.

**Anne Norris**
*Strategic Initiatives*

**Office of the Director**
**Center for Veterinary Medicine**

**U.S. Food & Drug Administration**
O: 240-402-0132
M: 240-704-0579
Anne.Norris@fda.hhs.gov


**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** Norris, Anne
**Sent:** 12/17/2018 11:07:32 PM
**Subject:** updated DCM-VetLIRN
**Attachments:** 800.267-draft-Dec 2018 Web Update-v3.docx

**Jennifer L. A. Jones, DVM**
Veterinary Medical Officer
U.S. Food & Drug Administration
Center for Veterinary Medicine
Office of Research
Veterinary Laboratory Investigation and Response Network (Vet-LIRN)
8401 Muirkirk Road, G704
Laurel, Maryland 20708
new tel: 240-402-5421
fax: 301-210-4685
e-mail: *jennifer.jones@fda.hhs.gov*
Web: http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

# Withheld in Full as B5

**From:** Hartogensis, Martine </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=02DF91D554D34B948FC58433D0E42073-MHARTOGE>
**To:** Rotstein, David; Palmer, Lee Anne; Jones, Jennifer L; Queen, Jackie L
**CC:** Rotstein, David
**Sent:** 5/23/2018 1:56:41 AM
**Subject:** Re: DCM Firms

Thank you Dave! What is the B5

B5

I am heading on leave until Friday, but let's regroup then and come up with a plan! Thanks again!

Martine

---

**From:** Rotstein, David <David.Rotstein@fda.hhs.gov>
**Date:** May 22, 2018 at 4:11:39 PM EDT
**To:** Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>, Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>, Queen, Jackie L <Jackie.Queen@fda.hhs.gov>
**Cc:** Rotstein, David <David.Rotstein@fda.hhs.gov>
**Subject:** DCM Firms

B4, B5

# B4, B5

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
**240-506-6763 (BB)**


**From:** Rotstein, David </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=0A3B17EBFCF14A6CB8E94F322906BADD-DROTSTEI>
**To:** Hartogensis, Martine; Palmer, Lee Anne; Jones, Jennifer L; Queen, Jackie L
**Sent:** 5/23/2018 9:00:48 AM
**Subject:** Re: DCM Firms

Martine,

Thank you.

B5

Dave

---

**From:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
**Date:** May 22, 2018 at 9:56:42 PM EDT
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>, Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>, Queen, Jackie L <Jackie.Queen@fda.hhs.gov>
**Cc:** Rotstein, David <David.Rotstein@fda.hhs.gov>
**Subject:** Re: DCM Firms

Thank you Dave! What is the B5

B5

I am heading on leave until Friday, but let's regroup then and come up with a plan! Thanks again!

Martine

---

**From:** Rotstein, David <David.Rotstein@fda.hhs.gov>
**Date:** May 22, 2018 at 4:11:39 PM EDT
**To:** Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>, Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>, Queen, Jackie L <Jackie.Queen@fda.hhs.gov>
**Cc:** Rotstein, David <David.Rotstein@fda.hhs.gov>
**Subject:** DCM Firms

B4, B5

B4, B5

B4, B5

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
**240-506-6763 (BB)**


## Report Details - EON-365610

| | | |
|---|---|---|
| **ICSR:** | 2054966 | |
| **Type Of Submission:** | Initial | |
| **Report Version:** | FPSR.FDA.PETF.V.V1 | |
| **Type Of Report:** | Adverse Event (a symptom, reaction or disease associated with the product) | |
| **Reporting Type:** | Voluntary | |
| **Report Submission Date:** | 2018-09-16 11:38:42 EDT | |
| **Reported Problem:** | **Problem Description:** | DCM and CHF - had been having respiratory signs for ~1 month prior to diagnosis at Tufts Littermate is B6 (already reported by owner after being diagnosed with DCM and CHF in July 2018) Owner has another Great Dane at home (~1 year of age) eating the same diet that will be screened soon Owner approved submission of this report and talking to FDA Will send rest of medical records by email (sorry - too many to upload) |
| | **Date Problem Started:** | 09/12/2018 |
| | **Concurrent Medical Problem:** | Yes |
| | **Pre Existing Conditions:** | B6 |
| | **Outcome to Date:** | Stable |
| **Product Information:** | **Product Name:** | 4Health beef stew canned |
| | **Product Type:** | Pet Food |
| | **Lot Number:** | |
| | **Package Type:** | CAN |
| | **Product Use Information:** | **Description:** 2 tablespoons 3x/day since Jan, 2017 |
| | **Manufacturer /Distributor Information:** | |
| | **Purchase Location Information:** | |
| | **Product Name:** | Taste of the Wild Prey Beef dry (will provide full diet history) |
| | **Product Type:** | Pet Food |
| | **Lot Number:** | |
| | **Package Type:** | BAG |
| | **Product Use Information:** | **Description:** 9 cups/day<br>**First Exposure Date:** 06/01/2018 |
| | **Manufacturer /Distributor Information:** | |
| | **Purchase Location Information:** | |
| **Animal Information:** | **Name:** | B6 |
| | **Type Of Species:** | Dog |
| | **Type Of Breed:** | Great Dane |
| | **Gender:** | Male |
| | **Reproductive Status:** | Neutered |
| | **Weight:** | 69.5 Kilogram |
| | **Age:** | B6 Years |
| | **Assessment of Prior Health:** | Excellent |
| | **Number of Animals Given the Product:** | 2 |
| | **Number of Animals Reacted:** | 1 |
| | **Owner Information:** | **Owner** Yes |

| | | | |
|---|---|---|---|
| | | **Information provided:** | |
| | | **Contact:** | **Name:** B6 |
| | | | **Phone:** B6 |
| | | | **Email:** |
| | | **Address:** | B6<br>United States |
| | **Healthcare Professional Information:** | **Practice Name:** | Tufts Cummings School of Veterinary Medicine |
| | | **Contact:** | **Name:** Lisa Freeman |
| | | | **Phone:** (508) 887-4523 |
| | | | **Email:** lisa.freeman@tufts.edu |
| | | **Address:** | 200 Westboro Rd<br>North Grafton<br>Massachusetts<br>01536<br>United States |
| **Sender Information:** | **Name:** | Lisa Freeman | |
| | **Address:** | 200 Westboro Rd<br>North Grafton<br>Massachusetts<br>01536<br>United States | |
| | **Contact:** | **Phone:** | 5088874523 |
| | | **Email:** | lisa.freeman@tufts.edu |
| | **Permission To Contact Sender:** | Yes | |
| | **Preferred Method Of Contact:** | Email | |
| **Additional Documents:** | | | |
| | **Attachment:** | taurine.pdf | |
| | **Description:** | Taurine results | |
| | **Type:** | Laboratory Report | |

3 S 20658

**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/aal/aal.html

**Vet/Tech Contact:** B6
**Company Name:** B6
**Address:** B6
B6

**Email:** B6
**Tel:** B6 **Fax:** B6

**Billing Contact** B6 **TAX ID:**
**Email** B6 **Tel:** B6

**Patient Name** B6
**Species:** Great Dane
**Owner's Name:** B6

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

**Taurine Results (nmol/ml)**

Plasma:_____ Whole Blood B6 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 |

| | |
|---|---|
| **From:** | PFR Event <pfreventcreation@fda.hhs.gov> |
| **To:** | Cleary, Michael *; HQ Pet Food Report Notification; B6 |
| **Sent:** | 9/22/2018 10:40:31 PM |
| **Subject:** | Taste of the Wild-Last 1-2 bags (for 2 dogs) before diagnosis were Southwest Canyon flavor-Before that: Lisa Freeman - EON-366516 |
| **Attachments:** | 2055229-report.pdf; 2055229-attachments.zip |

A PFR Report has been received and PFR Event [EON-366516] has been created in the EON System.

A "PDF" report by name "2055229-report.pdf" is attached to this email notification for your reference. Please note that all documents received in the report are compressed into a zip file by name "2055229-attachments.zip" and is attached to this email notification.

Below is the summary of the report:

**EON Key:** EON-366516
**ICSR #:** 2055229
**EON Title:** PFR Event created for Taste of the Wild Last 1-2 bags (for 2 dogs) before diagnosis were Southwest Canyon flavor Before that fed 3-4 bags of Pine Forest Before that had been feeding Pacific Stream for several years; 2055229

| | | | |
|---|---|---|---|
| **AE Date** | 09/08/2018 | **Number Fed/Exposed** | 2 |
| **Best By Date** | | **Number Reacted** | 1 |
| **Animal Species** | Dog | **Outcome to Date** | Stable |
| **Breed** | Doberman Pinscher | | |
| **Age** | B6 Years | | |
| **District Involved** | PFR-New England DO | | |

**Product information**
**Individual Case Safety Report Number:** 2055229
**Product Group:** Pet Food
**Product Name:** Taste of the Wild Last 1-2 bags (for 2 dogs) before diagnosis were Southwest Canyon flavor Before that, fed 3-4 bags of Pine Forest Before that, had been feeding Pacific Stream for several years
**Description:** DCM and CHF Probably primary DCM in predisposed breed but given diet history, some

possibility of diet-associated DCM Taurine WNL
**Submission Type:** Initial
**Report Type:** Adverse Event (a symptom, reaction or disease associated with the product)
**Outcome of reaction/event at the time of last observation:** Stable
**Number of Animals Treated With Product:** 2
**Number of Animals Reacted With Product:** 1

| Product Name | Lot Number or ID | Best By Date |
|---|---|---|
| Taste of the Wild Last 1-2 bags (for 2 dogs) before diagnosis were Southwest Canyon flavor Before that, fed 3-4 bags of Pine Forest Before that, had been feeding Pacific Stream for several years | | |

**Sender information**
Lisa Freeman
200 Westboro Rd
North Grafton, MA 01536
USA

**Owner information**
B6
USA

To view this PFR Event, please click the link below:
https://eon.fda.gov/eon//browse/EON-366516

To view the PFR Event Report, please click the link below:
https://eon.fda.gov/eon//EventCustomDetailsAction!viewReport.jspa?decorator=none&e=0&issueType=12&issueId=383430

==========

from further disclosure. The information in the report is intended for situational awareness and should not be shared or acted upon independently. Any and all actions regarding this information should be coordinated through your local district FDA office.

Failure to adhere to the above provisions could result in removal from the approved distribution list. If you think you received this email in error, please send an email to FDAReportableFoods@fda.hhs.gov immediately.

## Report Details - EON-366516

| | | |
|---|---|---|
| **ICSR:** | 2055229 | |
| **Type Of Submission:** | Initial | |
| **Report Version:** | FPSR.FDA.PETF.V.V1 | |
| **Type Of Report:** | Adverse Event (a symptom, reaction or disease associated with the product) | |
| **Reporting Type:** | Voluntary | |
| **Report Submission Date:** | 2018-09-22 18:33:37 EDT | |
| **Reported Problem:** | **Problem Description:** | DCM and CHF Probably primary DCM in predisposed breed but given diet history, some possibility of diet-associated DCM Taurine WNL |
| | **Date Problem Started:** | 09/08/2018 |
| | **Concurrent Medical Problem:** | No |
| | **Outcome to Date:** | Stable |
| **Product Information:** | **Product Name:** | Taste of the Wild Last 1-2 bags (for 2 dogs) before diagnosis were Southwest Canyon flavor Before that, fed 3-4 bags of Pine Forest Before that, had been feeding Pacific Stream for several years |
| | **Product Type:** | Pet Food |
| | **Lot Number:** | |
| | **Package Type:** | BAG |
| | **Product Use Information:** | **Description:** Owner has given consent to have FDA contact her for any additional questions |
| | **Manufacturer /Distributor Information:** | |
| | **Purchase Location Information:** | |
| **Animal Information:** | **Name:** | B6 |
| | **Type Of Species:** | Dog |
| | **Type Of Breed:** | Doberman Pinscher |
| | **Gender:** | Male |
| | **Reproductive Status:** | Neutered |
| | **Weight:** | 34.2 Kilogram |
| | **Age:** | B6 Years |
| | **Assessment of Prior Health:** | Excellent |
| | **Number of Animals Given the Product:** | 2 |
| | **Number of Animals Reacted:** | 1 |
| | **Owner Information:** | **Owner Information provided:** Yes |
| | | **Contact:** **Name:** B6 |
| | | **Phone:** |
| | | **Email:** |
| | | **Address:** B6 United States |
| | **Healthcare Professional Information:** | **Practice Name:** Tufts Cummings School of Veterinary Medicine |
| | | **Contact:** **Name:** Lisa Freeman |
| | | **Phone:** (508) 887-4523 |

| | | | |
|---|---|---|---|
| | | Email: | lisa.freeman@tufts.edu |
| | | Address: | 200 Westboro Rd<br>North Grafton<br>Massachusetts<br>01536<br>United States |

| | | | |
|---|---|---|---|
| **Sender Information:** | **Name:** | Lisa Freeman | |
| | **Address:** | 200 Westboro Rd<br>North Grafton<br>Massachusetts<br>01536<br>United States | |
| | **Contact:** | **Phone:** | 5088874523 |
| | | **Email:** | lisa.freeman@tufts.edu |
| | **Permission To Contact Sender:** | Yes | |
| | **Preferred Method Of Contact:** | Email | |

| | | |
|---|---|---|
| **Additional Documents:** | | |
| | **Attachment:** | discharge 9-20-18.pdf |
| | **Description:** | Discharge 9-20-18 |
| | **Type:** | Medical Records |
| | **Attachment:** | bnp.pdf |
| | **Description:** | BNP |
| | **Type:** | Laboratory Report |
| | **Attachment:** | cardio appointment 9-20-18.pdf |
| | **Description:** | Cardio appt 9-20-18 |
| | **Type:** | Echocardiogram |
| | **Attachment:** | cardio consult 9-8-18.pdf |
| | **Description:** | cardio consult 9-8-18 |
| | **Type:** | Echocardiogram |
| | **Attachment:** | discharge 9-9-18.pdf |
| | **Description:** | Discharge 9-9-18 |
| | **Type:** | Medical Records |

**Lab Results IDEXX CARDIOPET proBNP 9/10/18**

IDEXX Reference Laboratories

Client: B6 Patient: B6

IDEXX VetConnect 1-888-433-9987

Client: B6
Patient: B6
Species: CANINE
Breed: DOBERMAN_PINSCH
Gender: MALE NEUTERED
Age: 8Y

Date: 09/10/2018
Requisition #: B6
Accession #: B6
Ordered by:

TUFTS UNIVERSITY
200 WESTBORO RD
NORTH GRAFTON, Massachusetts 01536-1828
508-839-5395

Account #80735

**CARDIOPET proBNP - CANINE**

| Test | Result | Reference Range | Low | Normal | High |
|---|---|---|---|---|---|
| CARDIOPET proBNP - CANINE | B6 | 0 - 900 pmol/L | HIGH | B6 | |

**Comments:**

1 Cardiopet proBNP >1800pmol/L

B6

Please note: Complete interpretive comments for all concentrations of Cardiopet proBNP are available in the online directory of services. Serum specimens received at room temperature may have decreased NT-proBNP concentrations.

Cummings
Veterinary Medical Center
AT TUFTS UNIVERSITY
Cardiology Liaison: 508-887-4696

B6

Patient ID: B6
B6 Canine
B6 Years Old Male (Neutered) Doberman Pinscher
Black/Tan

## Cardiology Appointment Report

**Date:** 9/20/2018

**Attending Cardiologist:**
John E. Rush DVM, MS, DACVIM (Cardiology), DACVECC

B6

**Cardiology Resident:**
B6 B6

**Cardiology Technician:**
B6

**Student:** B6

**Presenting Complaint:**
Recheck of DCM with active CHF

**General Medical History:**
Has been his normal self, went on one 20 min walk since he was last here and had no trouble at all.
PU/PD (due to B6

**Diet and Supplements:**
Fromm Mature: 4 cups a day

**Cardiovascular History:**
Prior CHF diagnosis? Yes
Prior heart murmur? Yes, grade III/VI
Prior ATE?, N
Prior arrhythmia? Y, VPCs
Monitoring respiratory rate and effort at home? N, but havent noticed any labored breathing
Cough? N
Shortness of breath or difficulty breathing? N
Syncope or collapse? N
Sudden onset lameness? N
Exercise intolerance? N

**Current Medications Pertinent to CV System:**

B6

**Cardiac Physical Examination:**

B6

**Muscle condition:**

- [x] Normal
- [ ] Mild muscle loss
- [ ] Moderate cachexia
- [ ] Marked cachexia

**Cardiovascular Physical Exam:**

**Murmur Grade:**

- [ ] None
- [ ] I/VI
- [ ] II/VI
- [ ] III/VI
- [x] IV/VI
- [ ] V/VI
- [ ] VI/VI

**Murmur location/description:**

**Jugular vein:**

- [x] Bottom 1/3 of the neck
- [ ] Middle 1/3 of the neck
- [ ] 1/2 way up the neck
- [ ] Top 2/3 of the neck

**Arterial pulses:**

- [ ] Weak
- [ ] Fair
- [x] Good
- [ ] Strong
- [ ] Bounding
- [ ] Pulse deficits
- [ ] Pulsus paradoxus
- [ ] Other:

**Arrhythmia:**

- [x] None
- [ ] Sinus arrhythmia
- [ ] Premature beats
- [ ] Bradycardia
- [ ] Tachycardia

Gallop:

- [ ] Yes
- [x] No
- [ ] Intermittent
- [ ] Pronounced
- [ ] Other:

Pulmonary assessments:

- [x] Eupneic
- [ ] Mild dyspnea
- [ ] Marked dyspnea
- [ ] Normal BV sounds
- [x] Pulmonary crackles
- [ ] Wheezes
- [ ] Upper airway stridor

Abdominal exam:

- [x] Normal
- [ ] Hepatomegaly
- [ ] Abdominal distension
- [ ] Mild ascites
- [ ] Marked ascites

**Problems:**

**Differential Diagnoses:**

**Diagnostic plan:**

- [ ] Echocardiogram
- [ ] Chemistry profile
- [ ] ECG
- [ ] Renal profile
- [ ] Blood pressure
- [ ] Dialysis profile
- [ ] Thoracic radiographs
- [ ] NT-proBNP
- [ ] Troponin I
- [ ] Other tests:

**Assessment and recommendations:**

**Final Diagnosis:**

**Heart Failure Classification Score:**

ISACHC Classification:

- [ ] Ia
- [ ] Ib
- [ ] II
- [ ] IIIa
- [ ] IIIb

ACVIM Classification:

- [ ] A
- [ ] B1
- [ ] B2
- [ ] C
- [ ] D

# TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 7/28/2017 | UC Davis | B6 | | 8/17/2017 | Normal | No Prior Clearance | | 4health | Large Breed Adult Chicken & Vegetables | More than 1 Year | |
| | Golden/Border Collie/Misc | | 4/26/2018 | UC Davis | | | n/a | n/a | | | 4health | Special Care Weight MAnagement | 6 mo - 1 yr | various canned food, homecooked venison, chicken, turkey, beef with U-Stew |
| | Golden Retriever | | 01/02/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | | Acana | Rotated - pork, lamb, etc. | More than 1 yr | no |
| | Golden Retriever | | 3/22/2017 | UC Davis | | | | Mild/Moderate DCM | Clear Auscultation | 16 mo. | Acana | Pork & Squash | More than 1 Year | |
| | Golden Retriever | | 4/18/2017 | UC Davis | | | | Severe DCM & CHF | Clear Auscultation | 2 yrs | Acana | Pork & Squash | More than 1 Year | |
| | Golden Retriever | | 3/1/2017 | UC Davis | | | | Severe DCM & CHF | Clear Auscultation | 16 mo. | Acana | Pork & Squash | More than 1 Year | |
| | Golden Retriever | | 5/2/2017 | Wisconsin Veterinary Diagnostic Lab | | | 05/02/2017 Mod | Moderate DCM (see Additional Info column) | | | Acana | Pork & Squash | 6 Months to 1 Year | Stella and Chewy Freeze Dried Patties(Pheasant, Venison) |
| | Golden Retriever | | 4/21/2017 | Wisconsin Veterinary Diagnostic Lab | | | 05/02/2017 | Moderate DCM (see Additional Info column) | Auscultation | 01/21/2014 | Acana | Pork & Squash | More than 1 Year | Stella and Chewy Paddies (Pheasant, Venison) |
| | Golden Retriever | | 7/21/2017 | UC Davis | | | July 2017 | Normal | | | Acana | Lamb & Apple | More than 1 Year | |
| | Golden Retriever | | 7/21/2017 | UC Davis | | | July 2017 | Normal | | 2009 | Acana | Lamb & Apple | More than 1 Year | |
| | Golden Retriever | | 07/21/2017 | UC Davis | | | N/A | | No Prior Clearance | | Acana | Duck & Bartlett Pear Singles | More than 1 yr | |
| | Golden Retriever | | 07/21/2017 | UC Davis | | | N/A | | Auscultation | 05/14/2016 | Acana | Duck & Bartlett Pear Singles | More than 1 yr | |
| | Golden Retriever | | 04/25/2018 | UC Davis | | | 05/15/2018 | No DCM - minor issues | No Prior Clearance | | Acana | Lamb & Apple | 6 mo - 1 yr | None |
| | Golden Retriever | | 04/2017 | Antech | | | 6/2017 | Mild to moderate enlargement of heart | Auscultation | Age 2 | Acana | Pork & Squash | More than 1 yr | Occasional chicken, ground beef, ground turkey, chicken eggs, |
| | Golden Retriever | | 04/2017 | Antech | | | 6/17 | Leaking valve | Auscultation | 01/2009 age 25 months | Acana | Pork & Squash | More than 1 yr | Ground turkey, chicken, beef, eggs on occasion |
| | Golden Retriever | | April 17/18 | UC Davis | | | 6/25/18 | Minor Issues | No Prior Clearance | | Acana | Lamb & Apple | Less than 6 mo | |
| | Golden Retriever | | 04/17/2018 | UC Davis | | | 06/25/18 | Minor Issues | No Prior Clearance | | Acana | Lamb & Apple | Less than 6 mo | |
| | Golden Retriever | | 04/2017 | UC Davis | | | 6/2017 | mild DCM | | | Acana | Pork & Squash | More than 1 yr | |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Standard Poodle | B6 | 6/26/2018 | UC Davis | B6 | | 6/25/18 | DCM | Echo cardiogram | 17 months | Acana | Lamb & Apple Singles | More than 1 yr | |
| | Golden Retriever | | 6/18/2018 | Wisconsin Veterinary Diagnostic Lab | | | 7/13/18 | No DCM, slight murmur | No Prior Clearance | | Acana | Mackarel and Greens Singles | More than 1 yr | Cooked chicken, cooked beef, cooked eggs |
| | Goldendoodle | | 7/30/2018 (Results to be posted) | | | | 06/28/18 | Moderate DCM - treatment started immediately by cardiologist. | | | Acana | Venison 2 yrs then Wild Atlantic fish | More than 1 yr | salmon, ground lamb, bison added occasionally |
| | Golden Retriever | | 5/2/2017 | Wisconsin Veterinary Diagnostic Lab | | | 05/09/2017 | Mild systolic dysfunction (details in comments) | | | Acana | Pork & Squash | Less than 6 Months | Stella and Chewy Freeze-dried Patties(Pheasant, Venison) |
| | Golden Retriever | | 07/2017 | UC Davis | | | n/a | n/a | Auscultation | 21 months | Annamaet | Grain Free Salcha | More than 1 Year | |
| | Golden Retriever | | 5/16/2018 | UC Davis | | | N/a | | No Prior Clearance | | Blue Buffalo | Turkey and potato | 6 mo - 1 yr | |
| | Golden Retriever | | 7/17/2018 | UC Davis | | | Echo scheduled for 8/3/18 | N/A | No Prior Clearance | | Blue Buffalo | Blue Wilderness Chicken and Healthy Weight | More than 1 yr | |
| | Golden Retriever | | 7/1/2017 | UC Davis | | | 10/10/2017 | Normal | No Prior Clearance | | Canidae | Chicken Meal & Rice Formula | More than 1 Year | Red Barn Canned Beef |
| | Golden Retriever | | 06/13/2018 | UC Davis | | | n/a | | Auscultation | 10/22/2015 | Canidae | Chicken All life stages | 6 mo - 1 yr | none |
| | Golden Retriever | | 7/17/2017 | UC Davis | | | 07/25/17 | Normal | Auscultation | 02/07/2013 | Canidae | Pure Sky Grain Free | More than 1 yr | |
| | Border Collie | | 7/17/2017 | UC Davis | | | 07/27/2017 | No DCM | No Prior Clearance | | Canidae | Pure Sky Grain Free | More than 1 yr | |
| | Golden Retriever | | 7/17/2017 | UC Davis | | | at this time, this # was considered normal so no echo done | N/A | Auscultation | 10/06/2016 | Canidae | Pure Sky Grain Free | Less than 6 mo | |
| | Golden Retriever | | 05/02/2018 | UC Davis | | | n/a | N/a | No Prior Clearance | | Darwin's Raw | Beef, chicken, duck | More than 1 yr | |
| | Golden Retriever | | 08/30/2017 | IDEXX | | | n/a | n/a | No Prior Clearance | | Earthborn | Holistic Coastal Catch | More than 1 yr | |
| | Golden Retriever | | 3/6/2018 | UC Davis | | | n/a | n/a | n/a | n/a | Earthborn | Holistic Coastal Catch | More than 1 Year | |
| | Golden Retriever | | 3/6/2018 | UC Davis | | | | | | | Earthborn | Holistic Coastal Catch | More than 1 Year | |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 02/27/2018 | Wisconsin Veterinary Diagnostic Lab | B6 | | 2/27/2019 | CHF DCM | No Prior Clearance | | Earthborn | Holistic Great Plains Feast | More than 1 yr | |
| | Golden Retriever | | 3/27/2018 | UC Davis | | | | | No Prior Clearance | | Earthborn | Coastal Catch | More than 1 yr | |
| | Golden Retriever | | 7/3/2017 | UC Davis | | | | Normal | Auscultation | Nov 14, 2014 | Eukanuba | Premium Sport 28/18 Condition | More than 1 Year | |
| | Golden Retriever | | 7/3/2017 | UC Davis | | | | Normal | Auscultation | 2/21/2011 | Eukanuba | Premium Sport 28/18 Condition | More than 1 Year | |
| | Golden Retriever | | 4/20/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | | Eukanuba | Large Breed Puppy/chicken | More than 1 yr | Cooked egg yolks, chicken white/dark, zucchini, potatoes, yams, sweet potatoes, broccoli. Sardines in water. |
| | Golden Retriever | | 6//11/18 | UC Davis | | | | n/a | | | Farmina | Ancient Grains Chicken and Pomegranate | Less than 6 mo. | |
| | Golden Retriever | | 03/2018 | UC Davis | | | n/a | n/a | | | Farmina | Pumpkin formula Chicken and Pomegranate Maxi | More than 1 Year | |
| | Golden Retriever | | 8/25/2017 | UC Davis | | | n/a | n/a | Auscultation | 4/17/2015 | Farmina | Cod and Ancestral Low-Grain | 6 mo - 1 yr | Small Batch Freeze Dried Turkey nuggets; raw chicken eggs |
| | Golden Retriever | | ***6/19/2018 RETEST*** | UC Davis | | | n/a | n/a | No Prior Clearance | n/a | Farmina | Ancestral Grains, Chicken and Pomegranate | 6 mo - 1 yr | Greek yogurt, eggs (raw with shell), raw hamburger, raw bison, chicken hearts/livers, calf liver (see additional info for details) |
| | Golden Retriever | | ***12/01/2017 2nd TEST*** | UC Davis | | | July 2017 | Normal | No Prior Clearance | | Farmina | Ancestral Grain Chicken/Pomegrante | Less than 6 mo | None |
| | Golden Retriever | | ***6/29/018 3rd RETEST*** | UC Davis | | | July 2017 | Normal | No Prior Clearance | | Farmina | Ancestral Grain Chicken/Pomegrante | More than 1 yr | None |
| | Golden Retriever | | 7/16/2018 | UC Davis | | | n/a | | Auscultation | 04/11/2009 | Farmina, Natural Balance | Farmina Farmina N&D Grain-Free Wild Boar & Natural Balance LID Potato & Duck | 6 mo - 1 yr | yogurt and powdered goat's milk daily |
| | Golden Retriever | | 10/12/0017 | UC Davis | | | 08/09/2017 | Normal | No Prior Clearance | 09/26/2017 | Farmina, Ziwipeak | N&D Quinoa Functional Canine Quinoa & Herring, Duck or Quail; Mackerel & Lamb | Less than 6 mo | 1/4 c raw chicken hearts |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 10/12/2017 | UC Davis | B6 | | 08/09/2017 | no DCM | No Prior Clearance | | Farmina, Ziwipeak | N&D Quinoa Functional Canine Quinoa & Herring, Duck or Quail; Mackerel & Lamb | Less than 6 mo | 1/4 cup raw chicken hearts |
| | Golden Retriever | | 8/2017 | Wisconsin Veterinary Diagnostic Lab | | | 9/12/2017 | Mild DCM | No Prior Clearance | | Fromm | Lamb and Lentil | More than 1 Year | |
| | Golden Retriever | | 6/18/2018 | Antech | | | n/a | | Auscultation | 1 yr | Fromm | Fromm Pork and Applesauce; Chicken Recipe (raw patties) | More than 1 yr | |
| | Golden Retriever | | 5/15/2018 | Tufts - North Grafton, MA | | | n/a | n/a | No Prior Clearance | n/a | Fromm | Gold Large Breed Adult | More than 1 Year | |
| | Golden Retriever | | 10/10/2017 | UC Davis | | | n/a | n/a | n/a | | Fromm | Grain Free Tunalini | More than 1 Year | Canned pumpkin, greek yogurt |
| | Golden Retriever | | 10/17/2017 | UC Davis | | | n/a | n/a | Clear Auscultation | 10/15/2016 3 yrs | Fromm | Grain Free Tunalini | More than 1 Year | Canned pumpkin, greek yogurt |
| | Golden Retriever | | 10/10/2017 | UC Davis | | | 10/16/2017 | Clear | n/a | | Fromm | Grain Free Tunalini | More than 1 Year | Canned pumpkin, greek yogurt |
| | Golden Retriever | | 10/02/2017 | UC Davis | | | n/a | | No Prior Clearance | | Fromm | 50% Adult Gold +50% Salmon A La Veg or Salmon Tunalini | More than 1 yr | |
| | Golden Retriever | | ***7/9/2018 Retest*** | UC Davis | | | n/a | n/a | No Prior Clearance | | Fromm | Duck and Sweet Potato | 6 mo - 1 yr | lean ground beef, lean ground turkey, chicken breast, freeze dried raw chicken, freeze dried raw beef, liver treats (beef & chicken liver), eggs, pure pumpkin |
| | Golden Retriever | | ***7/9/2018 Retest*** | UC Davis | | | n/a | n/a | No Prior Clearance | | Fromm | Duck and Sweet Potato | 6 mo - 1 yr | lean ground beef, lean ground turkey, chicken breast, freeze dried raw chicken, freeze dried raw beef, liver treats (beef & chicken liver), eggs, pure pumpkin |
| | Golden Retriever | | 3/28/2018 ***RETEST*** | UC Davis | | | 04/11/2018 | Progression of DCM from 9/17 echo | Echo cardiogram | 7 years 4 months | Fromm | Salmon a la veg | Less than 6 mo | Gizzards and hearts and pro bloom in goats milk |
| | Golden Retriever | | 5/15/2018 ***RESTEST-1*** | UC Davis | | | N/A | | | | Fromm | Salmon A La Veg | Less than 6 mo | Raw egg 3 times a week |
| | Golden Retriever | | 6/26/2018 ***RETEST-2*** | UC Davis | | | | | | | Fromm | Salmon A La Veg | Less than 6 mo | Pumpkin, Goats milk yogurt, Egg |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 7/23/2018 | UC Davis | B6 | | NA | | | | Fromm | Four Star Nutrionals Game Bird Recipe | 6 mo - 1 yr | |
| | Golden Retriever | | 3/9/2018 | UC Davis | | | | | | | Fromm | Grain Fee Salmon Tunalini | 6 mo - 1 yr | |
| | Golden Retriever | | 07/21/2017 | UC Davis | | B6 | n/a | | No Prior Clearance | | Fromm | Gold Black Bag | 6 mo - 1 yr | |
| | Golden Retriever | | 5/9/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | n/a | GO! | Salmon Limited Ingredient Grain Free | More than 1 Year | |
| | Golden Retriever | | 7/23/2018 | UC Davis | | | NA | | | | Holistic Select | Grain Free Salmon Anchovy and Sardine Meal Recipe | More than 1 yr | |
| | Golden Retriever | | 6/27/2017 | UC Davis | | | n/a | n/a | | | Home Cooked | Home cooked | 6 mo - 1 yr | yogurt, cottage cheese |
| | Golden Retriever | | 04/09/18 | UC Davis | | | n/a | | No Prior Clearance | | Home Cooked | Home Cooked | Less than 6 mo | |
| | Golden Retriever | | 11/2017 | UC Davis | | | | | Auscultation | | Honest Kitchen | Limited ingredient grain free white fish | More than 1 Year | Hard boiled eggs, goat milk, sardines, chopped clams (3x wk) |
| | Aussiedoodle | | 4/3/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | | Honest Kitchen | Preference (50%), Zeal (25%), Verve (12.5%) Hope (12.5%) | More than 1 yr | Chicken, fish, pork, turkey with base; bison heart, liver, kidneys |
| | Golden Retriever | | 7/28/0017 | UC Davis | | | 08/09/17 | no DCM | No Prior Clearance | | Honest Kitchen | Embark & Love | More than 1 yr | |
| | Golden Retriever | | 7/28/2017 | UC Davis | | | 08/09/2017 | Normal | No Prior Clearance | 09/26/2017 | Honest Kitchen, Other | Love & Embark | More than 1 yr | |
| | Golden Retriever | | 6/10/2018 | UC Davis | | | n/a | | No Prior Clearance | | K-9 Kraving | Chicken & Vegetables | 6 mo - 1 yr | Low Fat Organic Plain Yogurt, Zukes PB&Oats, String Cheese |
| | Golden Retriever | | 3/26/18 | UC Davis | | | n/a | | No Prior Clearance | | Kasiks | Grain Free Fish | Less than 6 mo | |
| | Golden Retriever | | 03/2018 | UC Davis | | | n/a | n/a | | | Natural Balance | LTD Venison and Sweet Potato | More than 1 Year | |
| | English Setter | | 07/02/2015 | UC Davis | | B6 | 07/02/2015 | Normal | No Prior Clearance | | Nature's Domain | Beef | More than 1 Year | |
| | English Setter | | 06/16/2015 | UC Davis | | | 06/16/2015 | Severe Dilated Cardiomyopathy | No Prior Clearance | | Nature's Domain | Beef | More than 1 Year | |
| | Golden Retriever | | 5/1/2018 | UC Davis | B6 | | n/a | n/a | No Prior Clearance | | Nature's Logic | Beef or Pork | 6 mo - 1 yr | |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 1/5/2018 | Idexx | B6 | B6 | 01/05/2018 | Enlarged heart, fluid, DCM | Echo cardiogram | 2 years old | Nature's Variety | Instinct Limited Ingredient Grain Free Lamb and Rice | More than 1 yr | |
| | Golden Retriever | | 3/28/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | n/a | Nulo | Salmon & Peas | More than 1 Year | |
| | Golden Retriever | | 04/03/2017 | UC Davis | | | n/a | n/a | Auscultation | 12/04/2012 | Nutrisca | Lamb | More than 1 yr | Eggs, chicken, turkey, lamb |
| | Golden Retriever | | 04/03/2017 | UC Davis | | | n/a | n/a | No Prior Clearance | | Nutrisca | Lamb | More than 1 yr | Eggs, chicken, beef, lamb, cheese. About 4oz/day |
| | Golden Retriever | | a6/15/2017 | Wisconsin Veterinary Diagnostic Lab | | | 6/15/17 | Severe DCM | | 7/24/2012 | NutriSource | Chicken with peas | More than 1 yr | |
| | Golden Retriever | | 6/5/17 | Wisconsin Veterinary Diagnostic Lab | | | 6/20/2017 | Normal | No Prior Clearance | | NutriSource | Chicken with peas | Less than 6 mo | |
| | Golden Retriever | | 5/2/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | | Orijen | Original Formula | More than 1 Year | |
| | Golden Retriever | | 5/2/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | | Orijen | Original Formula | More than 1 Year | |
| | Golden Retriever | | 3/29/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | | Orijen | Original Formula | More than 1 Year | |
| | Golden Retriever | | 7/14/2017 | Wisconsin Veterinary Diagnostic Lab | | | n/a | | Auscultation | 03/08/15 | Orijen | Original Formula | More than 1 yr | |
| | Golden Retriever | | 7/14/2017 | Wisconsin Veterinary Diagnostic Lab | | | n/a | | Auscultation | 03/08/2009 | Orijen | Original Formula | More than 1 yr | |
| | Golden Retriever | | 7/23/2018 | UC Davis | | B6 | N/A | | | | Orijen | Regional Red (1 cup - 2 times daily) | More than 1 yr | 1/2 boiled chicken breast 2X daily , 1/4 cup cottage cheese, 1X daily |
| | Golden Retriever | | 7/23/2018 | IDEXX but testing done by UC Davis lab per my vet | | | | | No Prior Clearance | | Performatrin | Ultra Grain Free Large Breed Adult and prior LB Puppy | More than 1 yr | Small amt canned Performatrin with Tylan daily; various treats esp. Natural Balance LID treats |
| | Golden Retriever | | 5/13/2017 | IDEXX UCD | | B6 | 4/20/2017 | Clear | Clear | 10/16/2016 | Pure-Vita | Venison and Lentil | More than 1 Year | |
| | Golden Retriever | | 5/31/2017 | Antech | | | 6/20/2017 | Mild enlargement & decreased contractility | No Prior Clearance | | Pure-Vita | Turkey & Sweet Potato GF | More than 1 yr | |
| | Golden Retriever | | 4/1/2018 | UC Davis | | | 05/08/2018 | normal | Echo cardiogram | 05/11/2011 | Purina | Pro Plan Active Sport All Life stages 26/16 | More than 1 yr | none |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | | UC Davis | B6 | | n/a | | Echo cardiogram | 11/08/2016 | Purina | Pro Plan Sport Active All Life Stages 26/16 | More than 1 yr | none |
| | Golden Retriever | | 05/19/2017 | UC Davis | | | 06/07/2016 | Normal | Echo cardiogram | 06/07/2016 | Purina | ProPlan Sensitive Stomach Salmon and Rice | More than 1 yr | Digestive enzymes, Show Stopper |
| | Golden Retriever | | 05/19/2017 | UC Davis | | | 06/07/2016 | Normal | Echo cardiogram | 06/07/2016 | Purina | ProPlan Sensitive Skin and Stomach (pre formula change) | More than 1 yr | Extensive coat conditioners, vitamins, enzymes |
| | Golden Retriever | | July 2017 | UC Davis | | | n/a | n/a | Auscultation | 1 year | Purina | Pro Plan Sport 30/20 Chicken | More than 1 yr | Whole raw eggs |
| | Golden Retriever | | 7/9/2018 | UC Davis | | B6 | 09/28/2017 | clear | Echo cardiogram | 09/28/2017 | Rachael Ray | Zero Grain Salmon & Sweet Potato | More than 1 yr | |
| | Golden Retriever | | 7/17/2018 | UC Davis | | | n/a | n/a | Auscultation | 07/17/2018 | Rawz | Meal Free Dry Food, Dehydrated Chicken, Turkey, & Chicken recipe | 6 mo - 1 yr | Stella and Chewy's Freeze Dried Raw Meal Mixers, Chewy's Chicken recipe |
| | Golden Retriever | | 10/15/2017 | Antech | | | n/a | | No Prior Clearance | | Royal Canin | Golden Retriever Puppy | More than 1 yr | Three 1000Mg Salmon Oil caplets daily |
| | Golden Retriever | | 10/15/2017 | Antech | | | 03/23/2012 | Normal | OFA Clearance | 4/2/2012 | Royal Canin | Golden Retriever Adult | More than 1 yr | Three 1000 mg Salmon oil caplets daily |
| | Golden Retriever | | 2/14/2018 | UC Davis | | | 3/2/2018 | Normal | No Prior Clearance | | Royal Canin | Golden Retriever Adult | More than 1 Year | |
| | Golden Retriever | | 4/10/2018 | UC Davis | | | n/a | n/a | Auscultation | 05 years | Royal Canin | Renal Support | More than 1 yr | |
| | Golden Retriever | | 4/10/2018 | UC Davis | | | n/a | n/a | Auscultation | 05 years | Royal Canin | Renal Support | More than 1 yr | |
| | Golden Retriever | | 7/19/2018 | UC Davis | | | n/a | | No Prior Clearance | | Royal Canin | Golden Retriever Adult canned | 6 mo - 1 yr | Eggs, chicken liver, hamburger, lamb, chicken thighs |
| | Golden Retriever | | 4/20/2018 | UC Davis | | | 2011 | Clear | Auscultation | 2011 age 2 years | Royal Canin | PR Rabbit Veterinary Diet | More than 1 yr | none |
| | Golden Retriever | | 4/20/2018 | UC Davis | | | 04/20/2018 | Normal | No Prior Clearance | 04/20/2018 | Royal Canin & Northwest Naturals Raw | Golden Retriever Adult | Less than 6 Months | Raw Chicken formula and hearts, freeze-dried chicken hearts, fish oil |
| | Golden Retriever | | 4/20/2018 | UC Davis | | | 2010 | Clear | Auscultation | done at 2 years old | Royal Canin/ Nutro Combined | RC: PR Rabbit Veterinary Diet Nutro: Limited Ingredient Venison Meal & Sweet Potato | More than 1 yr | Cooked egg yolks, chicken white/dark, zucchini, potatoes, yams, sweet potatoes, broccoli. Sardines in water. |
| | Golden Retriever | | 4/20/2018 | UC Davis | | | 2015 | Minor issue no DCM | Echo cardiogram | 2015 | Royal Canin/ Nutro Combined | RC: PR Rabbit Veterinary Diet Nutro: Limited Ingredient Venison Meal & Sweet Potato | More than 1 yr | Cooked egg yolks, chicken white/dark, zucchini, potatoes, yams, sweet potatoes, broccoli. Sardines in water. |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 06/23/2018 | UC Davis | B6 | | n/a | | No Prior Clearance | | Science Diet (Hills) | Adult Advanced Fitness - dry | Less than 6 mo | pork, hamburger, steak or cheese (training treats) |
| | Golden Retriever | | ***9/25/2017 RETEST*** | UC Davis | | | 10/10/2017 | Normal | | | Sojo's | Turkey | Less than 6 mo | Raw goats milk, chicken gizzards and hearts, chicken thighs, Stella & Chewy Turkey Mixers |
| | Golden Retriever | | 7/6/2017 | UC Davis | | | n/a | n/a | No Prior Clearance | n/a | Sojo's Honest Kitchen | Sojo's Turkey Honest Kitchen Dried Fish | 6 mo - 1 yr | Fresh meat (raw & cooked), sardines, salmon, eggs, raw goat milk |
| | Golden Retriever | | 7/6/2017 | UC Davis | | | n/a | n/a | No Prior Clearance | n/a | Sojo's Honest Kitchen | Turkey Fish | 6 mo - 1 yr | Meat, (some raw, some cooked), Sardines, Salmon, eggs, and raw goat milk; Lots of beef lung treats. |
| | Golden Retriever | | 4/16/2018 | UC Davis | | | NA | NA | Auscultation | 08/27/2017 | SportDog Elite | Elite Whitefish | 6 Months to 1 Year | Goat milk & Veggies |
| | Golden Retriever | | 5/15/2018 | UC Davis | | B6 | 6/3/2018 | Clear | Auscultation | 2 yrs | Taste of the Wild | Salmon | More than 1 Year | |
| | Golden Retriever | | 5/2/2018 | UC Davis | | | n/a | n/a | Auscultation | 11/2/2013 | Taste of the Wild | Roasted Lamb | More than 1 Year | |
| | Golden Retriever | | 8/13/2017 | UC Davis | | | n/a | n/a | Auscultation | 5/10/2008 | Taste of the Wild | Roasted Lamb | More than 1 Year | |
| | Golden Retriever | | 08/04/2017 | UC Davis | | | n/a | n/a | | | Taste of the Wild | Pacific Stream | More than 1 Year | Hill's canned W/D |
| | Golden Retriever | | 4/27/2018 | UC Davis | | | 5/1/2017 | Moderate to severe DCM and ventricular ectopy | No Prior Clearance | | Taste of the Wild | Lamb, Venison, Bison | More than 1 yr | |
| | Golden Retriever | | 6/13/18 | UC Davis | | | n/a | | No Prior Clearance | | Taste of the Wild | Sierra Mountain (lamb) | More than 1 yr | n/a |
| | Golden Retriever | | 5/8/2017 | IDEXX UCD | | B6 | 7/7/2017 | Mild DCM | MVD and equivocal SAS | July 2016 | Taste of the Wild | Pine Forrest, Venison, Legumes | More than 1 Year | |
| | Golden Retriever | | 5/13/2017 | IDEXX UCD | | | | | Auscultation | 1 yr | Taste of the Wild | Pine Forrest, Venison, Legumes | More than 1 Year | |
| | Golden Retriever | | 4/2/2018 | UC Davis | | | N/A | N/A | No Prior Clearance | | Taste of the Wild | Chicken | 6 Months to 1 Year | |
| | Golden Retriever | | 5/15/2018 | UC Davis | | | 6/3/2018 | Normal | Auscultation | 2 yrs | Taste of the Wild | Salmon | More than 1 Year | |
| | Golden Retriever | | 06/2018 | UC Davis | | | 06/26/2018 | Normal | No Prior Clearance | | Taste of the Wild | Pacific Stream | More than 1 yr | Eggs |
| | Golden Retriever | | 08/18/2017 | UC Davis | | | n/a | n/a | B6 was a rescue - no prior clearance | | Taste of the Wild | Pacific Stream (Salmon) alternated with Sierra Mountain (roasted lamb) | More than 1 yr | spoonful of canned food/sardines/chicken eggs/yogurt |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 08/18/2017 | UC Davis | B6 | | 09/28/2017 | no DCM/mild aortic stenosis | Auscultation | 11/18/2010 | Taste of the Wild | Pacific Stream (salmon) alternated with Sierra Mountain (roasted lamb) | More than 1 yr | spoonful of canned food/sardines/chicken eggs/yogurt |
| | Golden Retriever | | 7/9/2018 | UC Davis | | B6 | n/a | n/a | No Prior Clearance | n/a | Taste of the Wild | Fish | More than 1 yr | n/a |
| | Golden Retriever | | 7/9/2018 | UC Davis | | | n/a | n/a | No Prior Clearance | n/a | Taste of the Wild | Trout and Salmon | More than 1 yr | |
| | Golden Retriever | | 7/17/2018 | UC Davis | B6 | | N/a | n/a | Auscultation | | Taste of the Wild | High Prairie Roasted Bison Roasted Venison | More than 1 yr | Chicken eggs, occasional cooked chicken |
| | Golden Retriever | | 08/04/2017 | UC Davis | | | 02/14/2017 | Normal | Echo cardiogram | 02/14/2017 | Taste of The Wild | Pacific Stream | More than 1 Year | |
| | Golden Retriever | | ***5/1/18 RETEST*** | UC Davis | | | n/a | | Auscultation | 5/10/2008 | Victor | Professional | Less than 6 mo | cooked turkey |
| | Golden Retriever | | 4/26/2018 | UC Davis | | | 05/22/18 | "Normal" with concern for one area that was borderline... (See Additional Information column) | Auscultation | 11/19/13 | Wellness | Wellness: Core Ocean | More than 1 yr | 1/2 C cooked chicken breast or 93% ground beef, 1/4C sweet potato, 1/4C various vegetables (broccoli, cauliflower, Kale, squash), 15 blueberries....2oz of yogurt in am |
| | Golden Retriever | | 5/31/2018 | UC Davis | | | 06/27/2018 | | | | Wellness | Complete Health Whitefish and Sweet Potato Recipe | More than 1 Year | |
| | Golden Retriever | | 5/31/2018 | UC Davis | | | 06/27/2018 | | | | Wellness | Complete Health Whitefish and Sweet Potato Recipe | More than 1 Year | |
| | Golden Retriever | | 6/27/2018 | UC Davis | | | n/a | n/a | | none | Wellness | CORE Ocean | More than 1 yr | |
| | Golden Retriever | | 6/27/2018 | UC Davis | | | n/a | n/a | | none | Wellness | CORE Ocean | More than 1 yr | |
| | Golden Retriever | | 6/5/2018 | UC Davis | | | 07/11/2018 | No sign of DCM. Minor valve issue, but normal for dog's age | | | Wellness | 1/2 Whitefish and sweet potato, 1/2 Healthy Weight chicken and peas | More than 1 yr | |
| | Golden Retriever | | 05/2018 | UC Davis | | | N/A | N/A | | | Wellness Core Grain Free | Reduced Fat Turkey and Chicken | More than 1 yr | Eggs/ Omega 3 oils/ meats |
| | Golden Retriever | | 05/2018 | UC Davis | | | N/A | N/A | | | Wellness Core Grain Free | Reduced Fat Turkey and Chicken | More than 1 yr | Eggs/ meats/ omega 3 oils |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Golden Retriever | B6 | 7/16/2018 | UC Davis | B6 | | n/a | | Echo cardiogram | 06/18/2010 | West Coast Canine Life | No Calcium Kidney Recipe | Less than 6 mo | yogurt and powdered goat's milk |
| | Golden Retriever | | 5/7/2018 | UC Davis | | | Scheduled | Pending | No Prior Clearance | NA | Whole Earth | Chicken and Turkey GF | More than 1 yr | Eggs or left over meats 2-3 times a week |
| | Golden Retriever | | 5/7/2018 | UC Davis | | | Scheduled | Pending | Auscultation | 05/01/2017 | Whole Earth Farms | Grain Free Recipe with Chicken & Turkey | More than 1 yr | Eggs or left over meats 2-3 times a week |
| | Golden Retriever | | 4/23/2018 | UC Davis | | | n/a | n/a | None | n/a | Zignature | Turkey | 6 mo - 1 yr | |
| | German Shepherd | | 11/7/2017 | UC Davis | | | 10/31/2017 | CHF due to DCM, left and right sided CHF | | | Zignature | Kangaroo | More than 1 Year | |
| | Golden Retriever | | 03/22/2018 | UC Davis | | | 05/03/2018 | Normal | No Prior Clearance | | Zignature | Zssentials grain free | More than 1 yr | Eggs, meat home cooked, pure pumpkin, vegetables, yogurt |
| | Golden Retriever | | 2/12/2018 | B6 | | | | DCM | No Prior Clearance | | Zignature | Lamb & Rice | More than 1 Year | |
| | Dutch Shepherd | | 5/21/2018 | UC Davis | | | 12/21/2016 | Normal heart size and function. Heart murmur from increase aortic outflow velocity | Auscultation | | Zignature | Kangaroo, Turkey, Duck | More than 1 Year | |
| | Belgian Malinois | | 5/21/2018 | UC Davis | | | n/a | n/a | Auscultation | 3/2018 | Zignature | Duck | More than 1 Year | |
| | Boxer | | 06/2018 | UC Davis | | | 06/17/2018 | DCM Stage C CHF | No Prior Clearance | | Zignature | Lamb Formula | More than 1 yr | |
| | Golden Retriever | | 8/13/2017 | UC Davis | | | n/a | n/a | No Prior Clearance | n/a | Zignature | Kangaroo | More than 1 yr | lean ground beef, lean ground turkey, chicken breast, freeze dried raw chicken, freeze dried raw beef, liver treats (beef & chicken), eggs, pure pumpkin |
| | Golden Retriever | | 8/13/2017 | UC Davis | | | n/a | n/a | No Prior Clearance | | Zignature | Kangaroo | More than 1 yr | lean ground beef, lean ground turkey, chicken breast, freeze dried raw chicken, freeze dried raw beef, liver treats (beef & chicken liver), eggs, pure pumpkin |
| | Golden Retriever | | 3/22/2018 | Antech | | B6 | April 17, 2018 | Normal | No Prior Clearance | | Zignature | Zignature Kangaroo | More than 1 yr | |

## TAURINE TEST RESULTS - Sorted by Food Brand

Golden Normal = Whole Blood is >250
Other Breed Normal = Whole Blood is >200
Plasma Normal = >60

Yellow = LOW TAURINE RESULT

| Owner's Name | Breed | Dog DOB | Date of Taurine Test | LAB | Whole Blood | Plasma | Date of Echo | Echo Results | Heart Clearance Prior to Taurine Test | Date of Heart Clearance | Brand | Formula | Duration | Protein Toppers/Treats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | English Mastiff | B6 | 5/1/2018 | B6 | B6 | | 05/2018 | DCM | | | Zignature | Kangaroo | More than 1 Year | Beef liver |
| | Golden Retriever | | 4/27/2018 | Antech | | | | I-II mesosystolic murmur; normal echo except mildly decreased systolic function | Normal Echocardiogram w/no murmur | January 2017 | Zignature and Earthborn | ZEssentials, Lamb Holistic Meadowfeast | More than 1 Year | |
| | Golden Retriever | | 6/27/2017 | UC Davis | | | 08/17/2015 | Benign physiologic flow murmur | Echo cardiogram | 08/17/2015 | Ziwi Peak | Ziwipeak Venison | More than 1 yr | yogurt |

# Withheld in Full as B5

# Withheld in Full as B5, B6

# DOCUMENT PRODUCED IN NATIVE

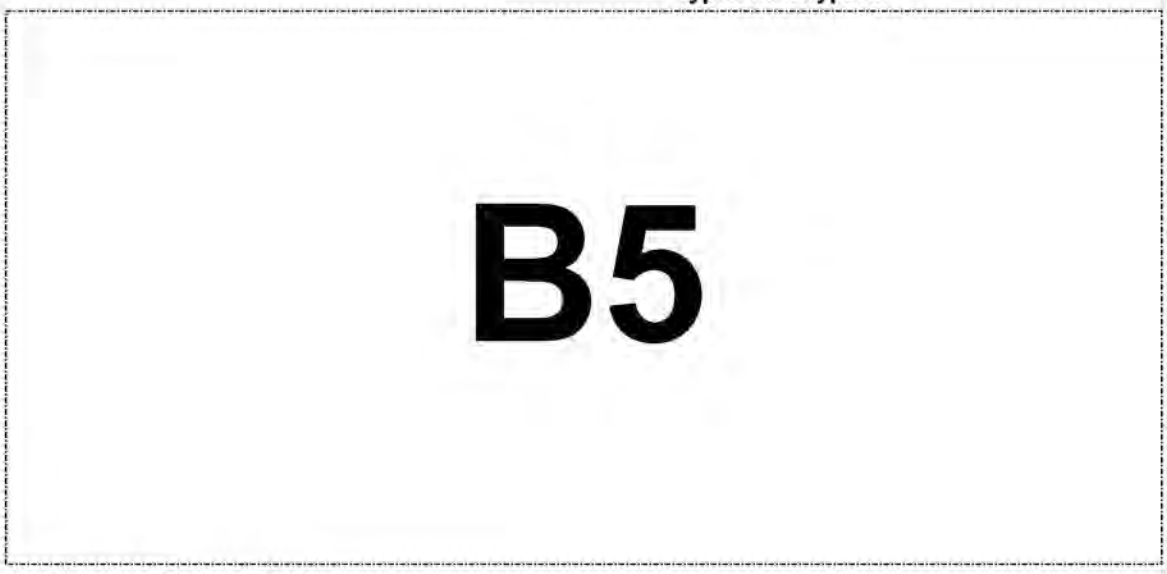
# B5

# B5

# B5

# B5

**Typical vs Atypical**




1 row has been excluded.

---
Document properties
---

Author: Palmer, Lee Anne
Template: Normal.dotm
Page count: 2
Paragraph count: 1525
Line count: 1529
Word count: 1739
Character count (spaces excluded): 6739
Character count (spaces included): 6956

# B5

# B5

# B5

# B5

B5

---
Document properties
---

Author: Palmer, Lee Anne
Template: Normal.dotm
Page count: 5
Paragraph count: 655
Line count: 656
Word count: 851
Character count (spaces excluded): 3992
Character count (spaces included): 4189

# Clinical Nutrition Service

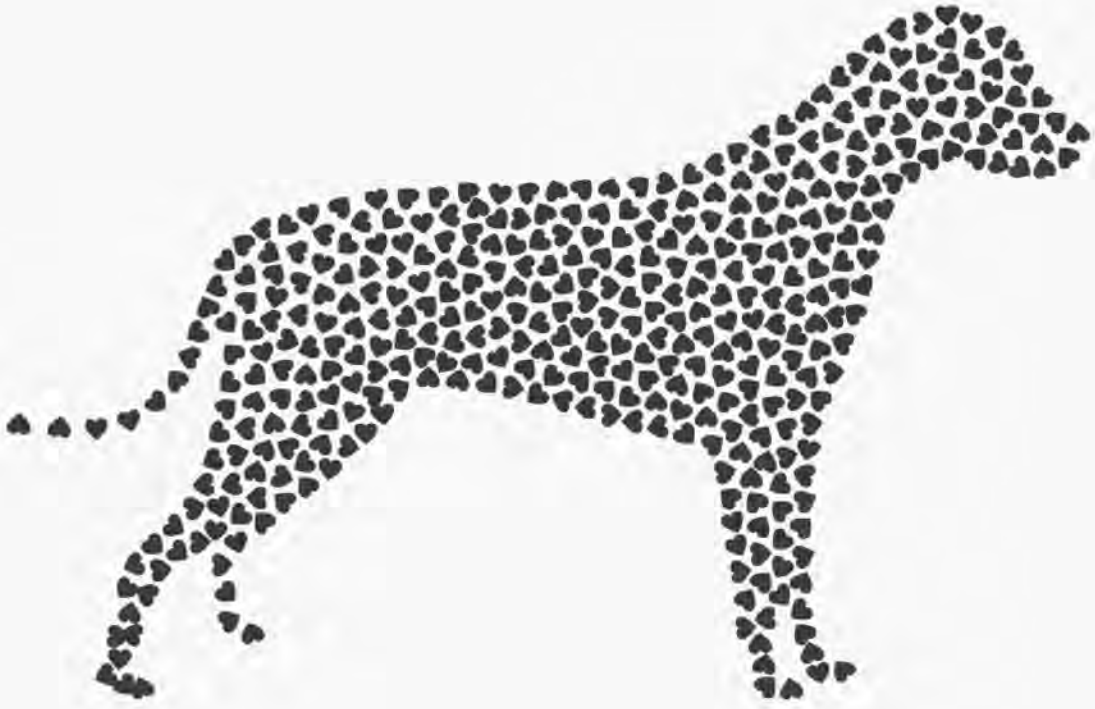
Cummings Veterinary Medical Center AT TUFTS UNIVERSITY



Petfoodology

**the scientific study of pet nutrition by veterinary nutrition specialists and experts.**

# A broken heart: Risk of heart disease in boutique or grain-free diets and exotic ingredients



by Lisa M. Freeman, DVM, PhD, DACVN

JUNE 04, 2018 IN FINDING THE BEST FOOD FOR YOUR PET, PETFOODOLOGY BLOG

Earlier this year, Peanut, a 4-year-old male Beagle/Lab mix was diagnosed with a life-threatening heart disease at our hospital. Peanut had been lethargic, not eating well, and occasionally coughing. The veterinary cardiologist seeing him asked what he was eating and found that his owner, in a desire to do the best thing for Peanut, was feeding a boutique, grain-free diet containing kangaroo and chickpeas. Peanut required several medications to treat his heart failure but the owner also changed his diet. And today, now 5 months later, Peanut's heart is nearly normal!

Heart disease is common in our companion animals, affecting *10-15% of all dogs and cats*, with even higher rates in Cavalier King Charles Spaniels, Doberman Pinschers, and Boxer dogs. Most nutritional recommendations focus on treating dogs and cats with heart disease and there is much less information on the role of diet in causing heart disease. However, a recent increase in heart disease in dogs eating certain types of diets may shed light on the role of diet in causing heart disease. It appears that diet may be increasing dogs' risk for heart disease because owners have fallen victim to the many myths and misperceptions about pet food. If diet proves to be the cause, this truly is heart-breaking to me.

In my 20 years as a veterinary nutritionist, I've seen vast improvements in our knowledge about pet nutrition, in the quality of commercial pet foods, and in our pets' nutritional health (other than the unfortunate rise in obesity). However, in the last few years I've seen more cases of nutritional deficiencies due to people feeding unconventional diets, such as unbalanced home-prepared diets, raw diets, vegetarian diets, and boutique commercial pet foods. The pet food industry is a competitive one, with more and more companies joining the market every year. Marketing is a powerful tool for selling pet foods and has initiated and expanded fads, that are unsupported by nutritional science, including grain-free and exotic ingredient diets. All this makes it difficult for pet owners to know what is truly the best food for their pet (as opposed to the one with the loudest or most attractive marketing). Because of the thousands of diet choices, the creative and persuasive advertising, and the vocal opinions on the internet, pet owners aren't able to know if the diets they're feeding have nutritional deficiencies or toxicities – or could potentially even cause heart disease.

**Dilated cardiomyopathy**

Dilated cardiomyopathy or DCM occurs in cats where it is associated with a nutritional deficiency (see below). DCM is a serious disease of the heart muscle which causes the heart to beat more weakly and to enlarge. DCM can result in abnormal heart rhythms, congestive heart failure (a build-up of fluid in the lungs or abdomen), or sudden death. In dogs, it typically occurs in large- and giant-breeds, such as Doberman pinschers, Boxers, Irish Wolfhounds, and Great Danes, where it is thought to have a gene[tic] component. Recently, some veterinary cardiologists have been reporting increased rates of DCM in dogs – in both the typical breeds and in breeds not usually associated with DCM, such as Miniature

Schnauzers or French Bulldogs. There is suspicion that the disease is associated with eating boutique or grain-free diets, with some of the dogs improving when their diets are changed. The US Food and Drug Administration (FDA) Center for Veterinary Medicine and veterinary cardiologists are currently investigating this issue.

**Is diet the cause?**

It's not yet clear if diet is causing this issue. The first thought was a deficiency of an amino acid called taurine. DCM used to be one of the most common heart diseases in cats but in 1987, it was discovered that feline DCM was caused by insufficient taurine in the diet. It was shown that DCM in cats could be reversed with taurine supplementation, and now all reputable commercial cat foods contain enough taurine to prevent the development of this lethal disease. We still occasionally see taurine deficiency-induced DCM in cats but it is usually when owners are feeding a vegetarian or home-prepared diet, supplemental diets, or a diet made by a manufacturer with inadequate nutritional expertise or quality control.

In dogs, Golden Retrievers and Cocker Spaniels were found to be at risk for DCM caused by taurine deficiency, and one study showed that Cocker Spaniels with DCM improved when given taurine supplementation. Since then, additional studies have shown associations between dietary factors and taurine deficiency in dogs, such as lamb, rice bran, high fiber diets, and very low protein diets. And certain other breeds were found to be at increased risk for taurine deficiency and DCM, including Newfoundlands, St. Bernards, English Setters, Irish Wolfhounds, and Portuguese Water Dogs. The reasons for taurine deficiency in dogs are not completely understood but could be reduced production of taurine due to dietary deficiency or reduced bioavailability of taurine or its building blocks, increased losses of taurine in the feces, or altered metabolism of taurine in the body.

No matter what the reason, the number of dogs with taurine deficiency and DCM subjectively appeared to decrease since the early 2000's. However, recently, some astute cardiologists noticed higher rates of DCM including Golden retrievers and in some atypical dog breeds. They also noticed that both the typical and atypical breeds were more likely to be eating boutique or grain-free diets, and diets with exotic ingredients – kangaroo, lentils, duck, pea, fava bean, buffalo, tapioca, salmon, lamb, barley, bison, venison, and chickpeas. Even some vegan diets have been associated. It has even been seen in dogs eating raw or home-prepared diets.

So, is this latest rash of DCM caused by taurine deficiency? Most of these affected dogs were eating boutique, grain-free, or exotic ingredient diets. Some of the dogs had low taurine levels and improved with taurine supplementation. But even some of those dogs that were not taurine deficient improved with

taurine supplementation and diet change. Fortunately, cardiologists reported the issue to the FDA which is currently investigating this issue. [Note: Dr. Joshua Stern from the University of California Davis is conducting research on taurine deficiency and DCM in Golden Retrievers.

**It's not so simple**

Currently, it seems that there may be two separate problems occurring – one related to taurine deficiency and a separate and yet unknown problem (with a third group of dogs likely having DCM completely unrelated to diet). Identifying the potential dietary factors contributing to DCM in the non-taurine deficient dogs is more difficult, but the FDA and cardiologists are hard at work trying to solve it. What seems to be consistent is that it does appear to be more likely to occur in dogs eating boutique, grain-free, or exotic ingredient diets.

**Exotic ingredients are on the rise**

Why are pet owners feeding these exotic ingredients? I think is it primarily because pet owners are falling victim to marketing which portrays exotic ingredients as more natural or healthier than typical ingredients. There is no truth to this marketing – and there is no evidence that these ingredients are any more natural or healthier than more typical ingredients. This is just good marketing that preys on our desire to do the best for our pets.

**There is no proof that grain-free is better!**

Many pet owners have, unfortunately, also bought into the grain-free myth. The fact is that food allergies are very uncommon, so there's no benefit of feeding pet foods containing exotic ingredients. And while grains have been accused on the internet of causing nearly every disease known to dogs, grains do not contribute to any health problems and are used in pet food as a nutritious source of protein, vitamins, and minerals.

**Exotic ingredients are more difficult to use**

Not only are the more exotic ingredients unnecessary, they also require the manufacturer to have much more nutritional expertise to be nutritious and healthy. Exotic ingredients have different nutritional profiles and different digestibility than typical ingredients, and also have the potential to affect the metabolism of other nutrients. For example, the bioavailability and metabolism of taurine is different in a lamb-based diet compared to a chicken-based diet or can be affected by the amount and types of fiber in the diet.

**Small pet food manufacturers might be better at marketing than at nutrition and quality control**

Making high quality, nutritious pet food is not easy! It's more than using a bunch of tasty-sounding ingredients. The right nutrients in the right proportions have to be in the diet, the effects of processing (or not processing) the food need to be considered, and the effects of all the other ingredients in the food need to be addressed, in addition to ensuring rigorous quality control and extensive testing. Not every manufacturer can do this.

**How could diet be increasing the risk for DCM?**

What is the consistent factor between the diets being implicated in diet-related DCM? It may be related to companies' inadequate nutritional expertise or rigorous quality control. We published a study several years ago in which we measured a single nutrient in 90 canned cat foods that all claimed to be nutritionally complete and balanced. We found that 15% of the diets were deficient in that nutrient (all of those diets were made by small companies). If companies don't have the quality control to ensure all nutrients are at the minimum levels, deficiencies could occur and could contribute to DCM. However, these problems could also be related to problems with bioavailability or interaction with other ingredients in the diet (especially the more exotic ingredients, which are not as well studied or understood). And DCM could even be the result of an ingredient in the diet that is toxic to the heart. The FDA is investigating this potential association between diet and DCM but, in the meantime, there are some things you can do.

**What should you do?**

- Reconsider your dog's diet. If you're feeding a boutique, grain-free, or exotic ingredient diets, I would reassess whether you could change to a diet with more typical ingredients made by a company with a long track record of producing good quality diets. And do yourself a favor – stop reading the ingredient list! Although this is the most common way owners select their pets' food, it is the least reliable way to do so. And be careful about currently available pet food rating websites that rank pet foods either on opinion or on based on myths and subjective information. It's important to use more objective criteria (e.g., research, nutritional expertise, quality control in judging a pet food). The best way to select what is really the best food for your pet is to ensure the manufacturer has excellent nutritional expertise and rigorous quality control standards (see our "Questions you should be asking about your pet's food" post).
- If you're feeding your dog a boutique, grain-free, or exotic ingredient diet, watch for early signs of heart disease – weakness, slowing down, less able to exercise, short of breath, coughing, or fainting. You veterinarian will listen for a heart murmur or abnormal heart rhythm and may do additional tests (or send

you to see a veterinary cardiologist), such as x-rays, blood tests, electrocardiogram, or ultrasound of the heart (echocardiogram).

- If your dog is diagnosed with DCM and eating one of these diets, I'd recommend the following steps:
  - Ask your veterinarian to test whole blood and plasma taurine levels (I recommend the University of California Davis Amino Acid Laboratory
  - Report it to the FDA. This can be done either online or by telephone. The FDA may be able to help with testing costs for your dog. Reporting it will also help us to identify and solve this current problem.
  - Change your dog's diet to one made by a well-known reputable company and containing standard ingredients (e.g., chicken, beef, rice, corn, wheat). Changing to a raw or homecooked diet will not protect your dog from this issue (and may increase the risk for other nutritional deficiencies). If your dog requires a homecooked diet or has other medical conditions that require special considerations, be sure to talk to a veterinarian or a veterinary nutritionist (acvn.org) before making a dietary change. You can contact the Cummings Nutrition Service to schedule an appointment (vetnutrition@tufts.edu)
  - Start taurine supplementation. Your veterinarian or veterinary cardiologist can recommend an appropriate dose for your dog. Be sure to use a brand of taurine with good quality control.
  - Any improvements in your dog's DCM can take 3-6 months. Your dog will need regular monitoring and may require heart medications during this time. There's no guarantee she'll improve but is certainly worth a try.
  - Make sure your dog is getting the best combination of medications to treat his heart disease, as this can make a difference in his outcome. You can find a board-certified veterinary cardiologist near you on this website: http://find.vetspecialists.com/

Sometimes, the changes we make in pet nutrition advance our knowledge and the health of our pets. In other cases, we can take a step in the wrong direction when the marketing outpaces the science. Hopefully, identifying this current issue will allow us to set a new, more science-based approach to the optimal nutrition of our pets.

For more information about heart disease in dogs, please see our HeartSmart website.

Share

GRAIN-FREE HEART DISEASE


## Lisa M. Freeman, DVM, PhD, DACVN

Dr. Freeman is a veterinary nutritionist and a professor at Cummings School of Veterinary Medicine at Tufts University. She is on the cutting-edge of science, with hundreds of articles in prestigious journals, speaking engagements at national and international conferences, and awards for her scientific achievements. However, she also is passionate about providing objective and accurate information on pet nutrition to veterinarians, pet owners, and other animal enthusiasts.




## Recommended Posts

## Research Review: A Simple Step for Extending Your Dog's Life

*April 01, 2019*

## Puzzle Feeders for Cats: Dogs Shouldn't Have All the Fun!

*March 26, 2019*

## Stop reading your pet food ingredient list!

*March 01, 2019*

## SUBSCRIBE

Want to read more information on feeding your pet? Subscribe to always know when we add new material!

your email here..

SUBSCRIBE

## SEARCH

Search

## PETFOODOLOGY ON TWITTER

FOLLOW US ON twitter

Follow us on Twitter @petfoodology

Popular

Who we are and why you can trust us

*Jul 28, 2016*

A broken heart: Risk of heart disease in boutique or grain-free diets and exotic ingredients

*Jun 04, 2018*

What's your Pet Food IQ?

*Nov 30, 2016*

It's Not Just Grain-Free: An Update on Diet-Associated Dilated Cardiomyopathy

*Nov 29, 2018*

My pet has kidney disease – what kind of diet should I feed?

*May 03, 2016*

Browse All Pet Nutrition Articles

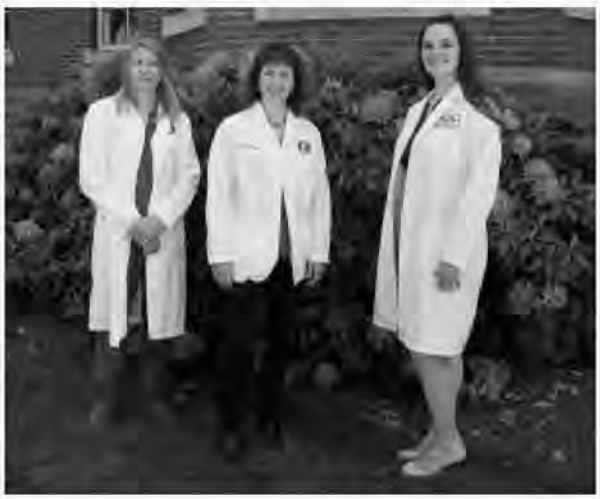
## TAG CLOUD

AAFCO allergies allergy appetite by-product calories carbohydrate ca

cat nutrition cats dog dog nutrition dogs environment fat

grain-free grains heart disease Home-cooked diet home-prepared diet human grade ingredient list ingredients kidney disease kitten labels large breed media multiple pets muscle condition score nutrition nutritional adequacy obesity overweight pet food Petfoodology protein puppy speaking engagements supplements therapeutic diets treats vegetarian vitamin weight loss

# Why Trust Us?



As you're on this website right now, we can assume that you love pets and likely have a special dog or cat (or many) in your life. We love them, too! And not only do we love the pets, we also love their people, and you are our reason for making this site.

Learn more about the Clinical Nutrition Team at Tufts

## PETFOODOLOGY TOPICS

## CLINICAL NUTRITION SERVICE AT TUFTS

The Clinical Nutrition Service at Foster Hospital for Small Animals offers in-person and telephone appointments to pet owners and case consultations to veterinarians within the Foster Hospital and throughout the country.

MAKE AN APPOINTMENT

## TUFTS OBESITY CLINIC FOR ANIMALS

The Tufts Obesity Clinic for Animals specializes in customized weight management plans that allow for safe weight loss with expert guidance from a board-certified veterinary nutritionist within the Clinical Nutrition Service.

MAKE AN APPOINTMENT

## SUBSCRIBE VIA EMAIL FOR UPDATES

your email here..

SUBSCRIBE

Cummings Veterinary Medical Center
at Tufts University
Foster Hospital for Small Animals
55 Willard St.
North Grafton, MA 01536





American Journal of Medical Genetics 95:57–66 (2000)

# Inherited Infantile Dilated Cardiomyopathy in Dogs: Genetic, Clinical, Biochemical, and Morphologic Findings

**Joseph Alroy,[1,2]* John E. Rush,[3] Lisa Freeman,[3] M. S. Amarendhra Kumar,[2] Ashok Karuri,[2] Kevin Chase,[4] and Satyapriya Sarkar[2,5]**

[1]*Department of Pathology, Tufts University Schools of Medicine and Veterinary Medicine, and New England Medical Center, Boston, Massachusetts*
[2]*Department of Biomedical Sciences, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts*
[3]*Department of Clinical Sciences, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts*
[4]*Department of Biology, University of Utah, Salt Lake City, Utah*
[5]*Department of Anatomy and Cellular Biology, Tufts University Health Science Schools, Boston, Massachusetts*

**Dilated cardiomyopathy, a lethal disease characterized by left ventricular dilation and systolic dysfunction, is relatively common in humans and other mammals. Idiopathic dilated cardiomyopathy (IDCM) is a primary myocardial disease of unknown cause and can be a familial disorder. This report describes autosomal recessive IDCM in dogs. It occurs in Portuguese Water Dog (PWD) pups and is manifested by acute, vague clinical signs and sudden death. Affected pups have progressive reduction of fractional shortening that can be demonstrated by echocardiography prior to the development of clinical signs. Furthermore, these pups have low plasma taurine levels when consuming certain diets. Affected pups had dilation of the left ventricle and alterations in the sarcomere appearance, while immunohistochemical and biochemical studies demonstrate an increase in desmin, a cytoskeleton protein. The clinical and morphologic findings of IDCM in PWDs are distinct from those reported in adult IDCM. Finally, the clinical and echocardiographic manifestations were reversible in some pups following oral taurine supplementation for 2 months. These results suggest that IDCM in PWDs is correlated with low plasma taurine levels. Am. J. Med. Genet. 95:57–66, 2000.** © 2000 Wiley-Liss, Inc.

**KEY WORDS: biochemistry; canine infantile dilated cardiomyopathy; pathology; population genetics**

B4

*Correspondence to: Joseph Alroy, D.V.M., Department of Pathology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111.
E-mail: Joseph Alroy@FacStaff@VET

Received 23 February 2000; Accepted 5 June 2000

# B4

# B4

# B4

# B4

# B4

# B4

# B4

# B4

B4

Veterinary Teaching Hospital
College of Veterinary Medicine
Washington State University

# Cardiology Service Nutritional Statement 2019

## What's all the hype about grain-free diets and heart disease?

Much information has been circulated on the internet and other in sources regarding grain-free diets and associated heart disease in dogs. The recent announcement from the US FDA alerting pet owners and veterinarians about reports of Dilated Cardiomyopathy (DCM) in dogs eating pet foods containing peas, lentils, other legume seeds, or other exotic ingredients has raised questions among the public. We hope to address the most current understanding of the problem and the most commonly asked questions here. While we do not yet have all the answers, we do have some information and recommendations to share with veterinarians, clients, and breeders.

## What we know:

Over the past 8-10 years there have been increased reports of DCM in dogs that are eating grain-free, exotic-ingredient, vegetarian/vegan, or home-prepared diets. This increase has appeared to have correlated with the rapid expansion in unique and trendy pet foods (boutique diets). While there has historically been some evidence of diet-responsive DCM in some breeds (Golden Retrievers, Cocker Spaniels, Newfoundlands, Irish wolfhounds, Saint Bernards), the incidence in these breeds has appeared to increase when eating grain-free, vegetarian/vegan, or exotic-ingredient foods. In other cases, the breeds of dogs developing DCM appear unusual, meaning that the dog does not have a breed history of an inherited type of DCM and/or the dog may be very young.

**KEY POINTS:**

- Taurine deficiency may be a factor in the unique foods, but it is unclear whether taurine deficiency is a cause or merely an association with yet unknown other dietary components.
- Some breeds may be more sensitive to changes in nutritional factors (such as taurine). This may suggest breed-related differences in metabolism.
- Dogs with DCM that have been eating the diets described above may reverse the condition (if caught early) or respond favorably to a change in diet and taurine supplementation, regardless of normal taurine blood levels.

WSU College of Veterinary Medicine Veterinary Teaching Hospital
Phone (509) 335-0711 • Fax (509) 335-3330

## What we recommend:

- If your dog does not have a medical condition requiring alterations in specific dietary ingredients, we recommend the owner feed a diet made by a well-established manufacturer that contains standard ingredients (e.g. chicken, beef, rice, corn, and wheat).

- If your dog has been diagnosed with DCM and is eating a diet with non-standard ingredients, we recommend changing the diet as above and measuring whole blood and plasma taurine levels.
    - o If taurine levels are low or low end of normal range, dietary supplementation of taurine should be added.
    - o Follow-up echocardiography should be performed in 3, 6, and 12 months to assess for improvement in heart function.
    - o Screening echocardiography for DCM should be performed in all dogs of the same household eating the non-standard diet.

- If your dog does have a medical condition that requires a non-standard diet, we suggest a diet made by a well-established manufacturer that has undergone extensive Association of American Feed Control Officials (AAFCO) feeding trials. Your veterinarian can help you choose an appropriate diet for your dog's medical condition.

- Owners of dogs with possible diet-associated DCM should save samples (and product labels) of all dietary components they are currently feeding, including not only the main food itself but also all treats, chews, and supplements.

- With complete diet information in hand, the veterinarian or owner should report the case to the FDA, which can be done either online or by telephone as this will help the agency identify possible underlying causes as quickly as possible.

  Web site: https://www.fda.gov/AnimalVeterinary/SafetyHealth/ReportaProblem/

**WSU Clinical Cardiology:**
Pamela Lee, DVM, ACVIM-Cardiol & O. Lynne Nelson, DVM, ACVIM- Cardiol, IM

---

**Related Publications:**

Kaplan JL. Stern JA, Fascetti AJ, et al. Taurine deficiency and dilated cardiomyopathy in golden retrievers fed commercial diets. PLoS One. 2018 Dec 13;13(12):e0209112. doi: 10.1371/journal.pone.0209112.

Mansilla WD, Marinangeli CPF, Ekenstedt KJ, et al. The association between pulse ingredients and canine dilated cardiomyopathy: addressing the knowledge gaps before establishing causation. J Anim Sci. 2019 Jan 7. doi: 10.1093/jas/sky488.

Freeman LM, Stern JA, Fries R, et al. Diet-associated dilated cardiomyopathy in dogs: what do we know? J Am Vet Med Assoc. 2018 Dec 1;253(11):1390-1394. doi: 10.2460/javma.253.11.1390.

# (C29) Amino Acid Concentrations and Echocardiographic Findings in Dogs Fed a Commercial Plant-Based Diet

*Sarah M. Cavanaugh, DVM, MS, DACVIM (Cardiology) - Ross University School of Veterinary Medicine*

Ryan Cavanaugh, DVM, DACVS-SA, ACVS Founding Fellow, Surgical Oncology – Ross University School of Veterinary Medicine; Gregory Gilbert, EdD, MSPH, PStat – Adtalem Global Education; Elena Leavitt, BS – Ross University School of Veterinary Medicine; Jennifer Ketzis, PhD – Ross University School of Veterinary Medicine; Aline Vieira, DVM, MSc, PhD – Ross University School of Veterinary Medicine

Diet and nutrition studies in people have shown that plant-based diets are capable of preventing, arresting, and reversing heart disease and other chronic debilitating diseases. To date, there are no studies evaluating the effects of a plant-based diet on any naturally-occurring chronic disease in dogs. Moreover, studies evaluating the nutritional adequacy and safety of plant-based diets formulated for pets are sparse. This prospective study investigated whether differences in amino acid concentrations and left ventricular echocardiographic findings occur in dogs who are transitioned from a traditional diet to a plant-based diet.

Thirty-eight client-owned healthy adult dogs were enrolled; 34 dogs to receive a commercial plant-based diet (PB diet) and 4 dogs to remain on their regular diet. Amino acid analysis (including plasma and whole blood taurine levels) was performed in all dogs, and echocardiography was performed in 37 dogs at baseline. Thirty-four dogs were transitioned from their traditional diet (≥1 animal ingredient, commercially-available, non-prescription, non-grain-free) to the PB diet. Thirty-four of 38 dogs completed the study. Amino acid analysis was repeated in 34 dogs (30 PB diet, 4 comparison group) after 30 days, and echocardiography was repeated in 33 dogs (29 PB diet, 4 comparison group) after 90 days. Pre- and post PB diet data were analyzed using a paired t test or Wilcoxon's signed rank test.

Nineteen of 28 amino acids showed statistically significant differences in pre- and post-diet values. There was a significant increase in arginine ($P = .0031$), asparagine ($P = .0022$), aspartic acid ($P = .0170$), cystathionine ($P = .0027$), glutamic acid ($P < .0001$), histidine ($P < .0001$), isoleucine ($P = .0405$), phenylalanine ($P = .0185$), taurine ($P < .0001$), threonine ($P = .0005$), tryptophan ($P = .0114$), tyrosine ($P = .0002$), valine ($P = .0185$), and whole blood taurine ($P < .0001$). There was a significant decrease in glutamine ($P < .0001$), glycine ($P = .0017$), 3-methylhistidine ($P < .0001$), methionine ($P = .0209$), and hydroxyproline ($P = .0026$). All post-diet amino acid values were within or above the established reference intervals except glutamine. There was no significant difference in normalized left ventricular internal systolic diameter ($P = .2068$) or fractional shortening ($P = .4889$) between pre- and post-diet. The normalized left ventricular internal diastolic diameter was higher post-diet (Mdn: 1.46) compared to pre-diet (Mdn: 1.41, $P = .0006$), but still within

the reference interval (1.27-1.85). No significant changes were seen in the comparison group.

The results of this study suggest dogs transitioned from a traditional diet to a plant-based diet undergo changes to their amino acid profile. Further, three-fourths of the amino acids (including taurine) significantly increased after 30 days on a plant-based diet suggesting that meat/animal ingredients are not essential for amino acid homeostasis in dogs. Additional studies are needed to determine whether the significant changes in amino acid concentrations observed in this study are due to normal day-to-day variation or due to differences in type, quality, and/or quantity of nutrients in plant-based diets compared to traditional diets. After 90 days on a plant-based diet, no dogs had echocardiographic evidence of left ventricular systolic dysfunction or dilated cardiomyopathy.

| | |
|---|---|
| **From:** | Palmer, Lee Anne </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=CF7C8BD53B6C45A39318A596ACEA7C53-LPALMER> |
| **To:** | Hartogensis, Martine; Jones, Jennifer L; Rotstein, David |
| **CC:** | Burkholder, William; Carey, Lauren; Norris, Anne; DeLancey, Siobhan; Lovell, Randall A; Reimschuessel, Renate; Ceric, Olgica; Nemser, Sarah |
| **Sent:** | 5/17/2018 6:05:58 PM |
| **Subject:** | RE: 800.267-DCM and meetign with Cardiac Care for Pets |
| **Attachments:** | Dilated Cardiomyopathy Cases.docx; E-266814 Cat case.pdf; photos and ingred product info.docx |

B5

**From:** Hartogensis, Martine
**Sent:** Thursday, May 17, 2018 11:59 AM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Lovell, Randall A <Randall.Lovell@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Nemser, Sarah <Sarah.Nemser@fda.hhs.gov>
**Subject:** RE: 800.267-DCM and meetign with Cardiac Care for Pets

Excellent work Jen!!

**B5**

Martine

**From:** Jones, Jennifer L
**Sent:** Thursday, May 17, 2018 11:29 AM
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Lovell, Randall A <Randall.Lovell@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Nemser, Sarah <Sarah.Nemser@fda.hhs.gov>
**Subject:** RE: 800.267-DCM and meetign with Cardiac Care for Pets

**B5**

Please see the PPT for the rationale/summary

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Rotstein, David
**Sent:** Monday, May 14, 2018 10:22 AM
**To:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Lovell, Randall A <Randall.Lovell@fda.hhs.gov>

**Subject:** RE: DCM and meetign with Cardiac Care for Pets

B5 Once we get a better handle on a specific cause, we can work on that.

---

**From:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
**Date:** May 14, 2018 at 9:09:17 AM EDT
**To:** Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>, Rotstein, David <David.Rotstein@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>, Carey, Lauren <Lauren.Carey@fda.hhs.gov>, Norris, Anne <Anne.Norris@fda.hhs.gov>, DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>, Lovell, Randall A <Randall.Lovell@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

This is very interesting. B5

B5

Martine

**From:** Palmer, Lee Anne
**Sent:** Friday, May 11, 2018 4:30 PM
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Lovell, Randall A <Randall.Lovell@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

B5

**From:** Rotstein, David
**Sent:** Friday, May 11, 2018 4:14 PM
**To:** Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Lovell, Randall A <Randall.Lovell@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

Lee Anne,

This is fantastic. B5

B5

B5

**From:** Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Date:** May 11, 2018 at 4:06:05 PM EDT
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>, Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>, Carey, Lauren <Lauren.Carey@fda.hhs.gov>, Norris, Anne <Anne.Norris@fda.hhs.gov>, DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>, Lovell, Randall A <Randall.Lovell@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

B5

**From:** Rotstein, David

**Sent:** Wednesday, May 9, 2018 4:13 PM
**To:** Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

Sounds very intriguing!!!

---

**From:** Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Date:** May 9, 2018 at 4:09:18 PM EDT
**To:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>, Rotstein, David <David.Rotstein@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>, Carey, Lauren <Lauren.Carey@fda.hhs.gov>, Norris, Anne <Anne.Norris@fda.hhs.gov>, DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

**B5**

**From:** Hartogensis, Martine
**Sent:** Wednesday, May 9, 2018 2:17 PM
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

Awesome, thank you Dave!

Martine

**From:** Rotstein, David
**Sent:** Wednesday, May 09, 2018 2:06 PM
**To:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Cc:** Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
**Subject:** RE: DCM and meetign with Cardiac Care for Pets

Good Afternoon,

I spoke with Dr **B6** @ Cardiac Care for Pets. He is going to look into times/dates with the cardiologists there and we can set the meeting up from that point.

Just some basic information:

**B5**

As a side note, there is a facebook page dedicated to this issue:

https://www.facebook.com/groups/1952593284998859/about/

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
**240-506-6763 (BB)**




**From:** Hartogensis, Martine
**Sent:** Tuesday, May 08, 2018 10:58 AM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>
**Subject:** RE: DCM

Thank you Jen and Dave! Very interesting and sounds like you all are on it! **B6**

**B6**

Keep us posted!

Thanks again!

Martine

Hi Martine,

**B5**

B5

I'm happy to share more info as needed.
Jen

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421

**From:** Rotstein, David
**Sent:** Tuesday, May 08, 2018 9:45 AM
**To:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Subject:** RE: DCM

Martine,

Jen and I spoke with cardiologists from NCSU and a few other institutions. That's how we were able to get the information.

B5

Looping in Jen.

Thanks for the update!

dave

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
**240-506-6763 (BB)**



**From:** Hartogensis, Martine
**Sent:** Tuesday, May 08, 2018 9:00 AM
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>

**Subject:** RE: DCM

Thank you Dave! We did not go around yesterday because the Vet-LIRN folks came in for their routine presentation.

B5

Might be a good opportunity for us to partner with some cardiology folks….

Martine

**From:** Rotstein, David
**Sent:** Monday, May 07, 2018 1:13 PM
**To:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
**Subject:** Re: DCM

Martine

B5

There's a way to go on this moving forward.

Dave

---

**From:** Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
**Date:** May 7, 2018 at 1:03:13 PM EDT
**To:** Rotstein, David <David.Rotstein@fda.hhs.gov>
**Subject:** DCM

Hi Dave!

Do you have any more details on the DCM and grain free diet issue?

Martine

**From:** Reimschuessel, Renate </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=4C00C47AE2794134B2906D6B9252FCF6-RREIMSCH>
**To:** Jones, Jennifer L
**Sent:** 10/22/2018 2:39:54 PM
**Subject:** Fwd: DCM question

You might want to share w Martine and communications to formulate a response

---

**From:** Joshua A Stern <jstern@ucdavis.edu>
**Date:** October 19, 2018 at 12:12:22 PM CDT
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>, Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** DCM question

HI Jennifer & Renate -
I'm giving a presentation at the ACVIM Cardiology conference (all board certified vet cardiologists will be the audience) and I'm giving an update on our work in golden retrievers. I will however be highlighting that goldens are a bit different and may not represent the problem so accurately.

I was hoping I could get a tiny bit of general info from you to update my slides. I'm happy to chat by phone if that is easier.

Here a few questions - that I would love to know if you are able to answer:

1.) how many confirmed cases are now part of your investigation (dogs, vs. cats)
2.) How many of your confirmed cases have measured low taurine levels
3.) Can you give me any idea of breed distribution (how many goldens, what are the top breeds, etc)
4.) Have all of the companies that manufacture diets fed to the cases been notified of the case being reported? If possible roughly how many diets are found in your case investigation (more than 20, more than 50, more than 100, etc)

I really appreciate your help if possible. The cardiologists would love to have a tiny bit more info and I'm in a position to pass this on to a captive audience tomorrow afternoon.

Also I'll be reviewing the recommendations for reporting and necropsy info too - so this will hopefully give the investigation a bit of a boos.

Best

Josh

Joshua Stern, DVM, PhD, DACVIM (Cardiology)
Associate Professor of Cardiology
Department of Medicine & Epidemiology
University of California Davis; CCAH Room 258
(614) 390.1516 cell (530) 752.2475 office

jstern@ucdavis.edu

Associate Editor - Journal of Veterinary Cardiology
www.journals.elsevier.com/journal-of-veterinary-cardiology

**From:** Jones, Jennifer L </O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=0F6CA12EAA9348959A4CBB1E829AF244-JENNIFER.JO>
**To:** DeLancey, Siobhan; Norris, Anne
**CC:** Reimschuessel, Renate; Hartogensis, Martine
**Sent:** 10/22/2018 3:47:38 PM
**Subject:** RE: Please advise-Fwd: DCM question

Thank you both! I'll use your format, Anne, and incorporate Siobhan's suggestion.

---

**From:** DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
**Date:** October 22, 2018 at 10:45:45 AM CDT
**To:** Norris, Anne <Anne.Norris@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>
**Cc:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>, Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
**Subject:** RE: Please advise-Fwd: DCM question

B5

**From:** Norris, Anne
**Sent:** Monday, October 22, 2018 11:42 AM
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
**Cc:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
**Subject:** RE: Please advise-Fwd: DCM question

Hi Jen,

Here's a suggestion below. B5

B5

B5

B5

At the time of FDA's public notification on 7/12/18, FDA had received sporadic reports involving 30 dogs and seven cats. Since that update, we have received approximately 110 additional reports of dilated cardiomyopathy involving approximately 120 dogs and one cat. Those numbers are current as of August 23, 2018.]

Hope that helps. Glad to help modify as needed.

Thanks,
Anne

**From:** Jones, Jennifer L

**Sent:** Monday, October 22, 2018 11:30 AM
**To:** DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>
**Cc:** Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
**Subject:** Please advise-Fwd: DCM question

Hi Comms Team,

**B5**

**B5**

Thank you as always, Jen

---

**From:** Joshua A Stern <jstern@ucdavis.edu>
**Date:** October 19, 2018 at 12:12:22 PM CDT
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>, Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** DCM question

HI Jennifer & Renate -
I'm giving a presentation at the ACVIM Cardiology conference (all board certified vet cardiologists will be the audience) and I'm giving an update on our work in golden retrievers. I will however be highlighting that goldens are a bit different and may not represent the problem so accurately.

I was hoping I could get a tiny bit of general info from you to update my slides. I'm happy to chat by phone if that is easier.

Here a few questions - that I would love to know if you are able to answer:

1.) how many confirmed cases are now part of your investigation (dogs, vs. cats)
2.) How many of your confirmed cases have measured low taurine levels
3.) Can you give me any idea of breed distribution (how many goldens, what are the top breeds, etc)
4.) Have all of the companies that manufacture diets fed to the cases been notified of the case being reported? If possible roughly how many diets are found in your case investigation (more than 20, more than 50, more than 100, etc)

I really appreciate your help if possible. The cardiologists would love to have a tiny bit more info and I'm in a position to pass this on to a captive audience tomorrow afternoon.

Also I'll be reviewing the recommendations for reporting and necropsy info too - so this will hopefully give the investigation a bit of a boos.

Best

Josh

---

Joshua Stern, DVM, PhD, DACVIM (Cardiology)
Associate Professor of Cardiology
Department of Medicine & Epidemiology
University of California Davis; CCAH Room 258
(614) 390.1516 cell (530) 752.2475 office
jstern@ucdavis.edu

Associate Editor - Journal of Veterinary Cardiology
www.journals.elsevier.com/journal-of-veterinary-cardiology

**From:** Jones, Jennifer L </o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0f6ca12eaa9348959a4cbb1e829af244-Jennifer.Jo>
**To:** Joshua A Stern
**CC:** 'Reimschuessel, Renate (Renate.Reimschuessel@fda.hhs.gov)'
**Sent:** 11/2/2018 3:09:39 PM
**Subject:** RE: DCM question

Good morning Josh,
Thank you for your patience. At this time, we are limited in the information we can provide, as this is an ongoing investigation. We are eager to cooperate and as soon as we are able to share more, we'll be sure to let you know. We are planning to publicly share updates in the investigation as it progresses and will definitely reach out as that goes forward to make sure that you are briefed on the latest.

At the time of FDA's public notification on 7/12/18, FDA had received sporadic reports involving 30 dogs and seven cats. Since that update, we have received approximately 110 additional reports of dilated cardiomyopathy involving approximately 120 dogs and one cat. Those numbers are current as of August 23, 2018.

Andrea Fascetti mentioned you'd be interested in looking at the urine Taurine data we've collected. We're interested in sharing that data but must first establish a Material Transfer Agreement. I'm working with our team here to get it written and will send it to you for approval/signature. Did you want to add any other collaborators other than B6? Please let me know.

Thank you and take care,
Jen

Jennifer Jones, DVM
Veterinary Medical Officer
Tel: 240-402-5421
U.S. FOOD & DRUG ADMINISTRATION

**From:** Jones, Jennifer L
**Sent:** Monday, October 22, 2018 9:26 AM
**To:** Joshua A Stern <jstern@ucdavis.edu>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** Re: DCM question

Hi Josh,
I've been at a conference the last week and will get back with you. I'm sorry for the belated response.
Jen

---

**From:** Joshua A Stern <jstern@ucdavis.edu>
**Date:** October 19, 2018 at 12:12:22 PM CDT
**To:** Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>, Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>
**Subject:** DCM question

HI Jennifer & Renate -
I'm giving a presentation at the ACVIM Cardiology conference (all board certified vet cardiologists will be the audience) and I'm giving an update on our work in golden retrievers. I will however be highlighting that goldens are a bit different and may not represent the problem so accurately.

I was hoping I could get a tiny bit of general info from you to update my slides. I'm happy to chat by phone if that is easier.

Here a few questions - that I would love to know if you are able to answer:

1.) how many confirmed cases are now part of your investigation (dogs, vs. cats)
2.) How many of your confirmed cases have measured low taurine levels
3.) Can you give me any idea of breed distribution (how many goldens, what are the top breeds, etc)
4.) Have all of the companies that manufacture diets fed to the cases been notified of the case being reported? If possible roughly how many diets are found in your case investigation (more than 20, more than 50, more than 100, etc)

I really appreciate your help if possible. The cardiologists would love to have a tiny bit more info and I'm in a position to pass this on to a captive audience tomorrow afternoon.

Also I'll be reviewing the recommendations for reporting and necropsy info too - so this will hopefully give the investigation a bit of a boos.

Best

Josh

Joshua Stern, DVM, PhD, DACVIM (Cardiology)
Associate Professor of Cardiology
Department of Medicine & Epidemiology
University of California Davis; CCAH Room 258
(614) 390.1516 cell (530) 752.2475 office
jstern@ucdavis.edu

Associate Editor - Journal of Veterinary Cardiology
www.journals.elsevier.com/journal-of-veterinary-cardiology

# DOCUMENT PRODUCED IN NATIVE

*Toxins* **2010**, *2*, 2289-2332; doi:10.3390/toxins2092289

OPEN ACCESS

*toxins*

**ISSN 2072-6651**

www.mdpi.com/journal/toxins

*Review*

# Naturally Occurring Food Toxins

**Laurie C. Dolan *, Ray A. Matulka and George A. Burdock**

Burdock Group, 801 N. Orange Ave., Suite 710, Orlando FL 32801, USA;
E-Mails: rmatulka@burdockgroup.com (R.A.M.); gburdock@burdockgroup.com (G.A.B.)

* Author to whom correspondence should be addressed; E-Mail: ldolan@burdockgroup.com; Tel.: +1-407-802-1400; Fax: +1-407-802-1405.

*Received: 12 August 2010; in revised form: 2 September 2010 / Accepted: 13 September 2010 / Published: 20 September 2010*

**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 |
| UGT and GST | Brussels sprouts, cabbage, watercress, broccoli | Acetaminophen, oxazepam, morphine, ibuprofen |
| P-glycopeptide and OATP | Vegetables, fruit juice, St. Johns wort | Digoxin, cyclosporine, parvastatin |

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

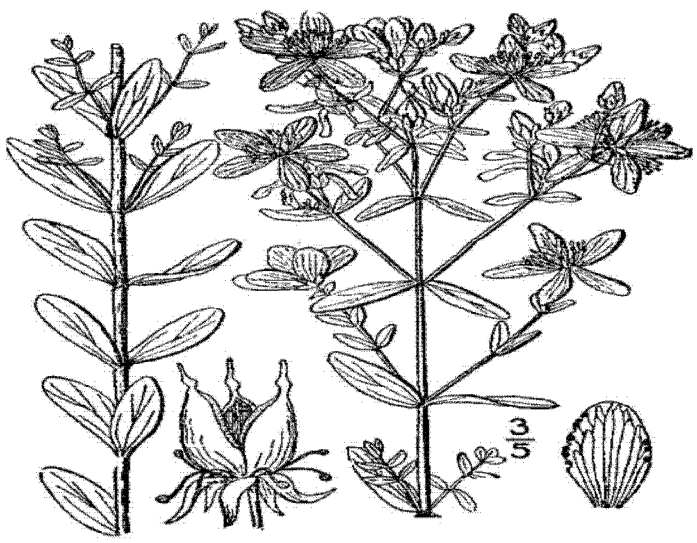
negligible levels are present in the fleshy parts of the fruit [35]. In the west African tropics, cassava is consumed as a dietary staple and inappropriate handling of the cassava prior to processing and consumption can result in a chronic form of cyanide poisoning termed "tropical ataxic neuropathy",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

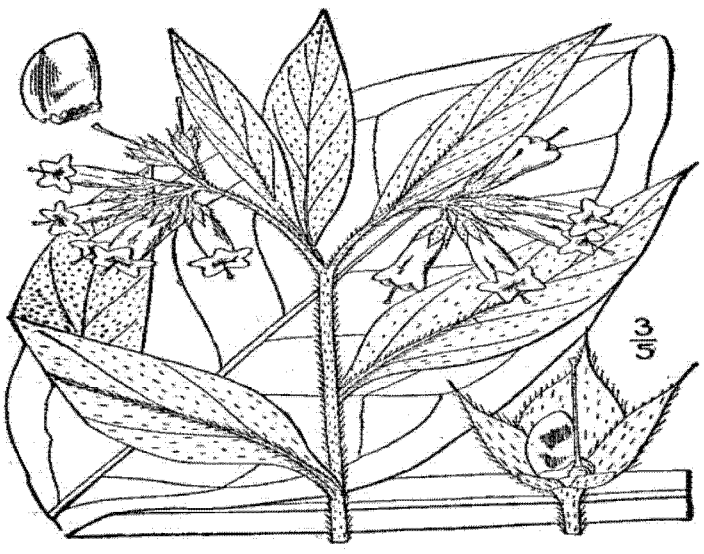
Thiamine is an essential vitamin involved in energy production. Thiamine deficiency is associated with impaired pyruvate utilization, resulting in a shortage of cellular ATP. In humans, thiamine deficiency may lead to weakness and weight loss. Severe thiamine deficiency produces “beri-beri”, a disease 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).

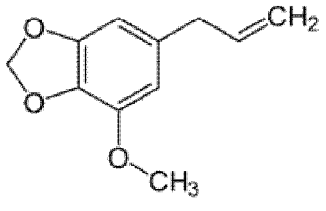
At a concentration of 1% in the diet, safrole produces weight loss, testicular atrophy, bone marrow depletion and malignant liver tumors in rats [106]. Based on sufficient evidence of carcinogenicity in experimental animals, safrole is reasonably anticipated to be a human carcinogen [107]. The mechanism 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

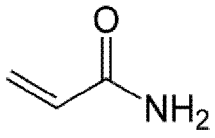
Polycyclic aromatic hydrocarbons (PAHs) are known carcinogens that are formed from the incomplete combustion of fossil fuels such as wood, coal and oil. PAHs can enter the food chain from environmental contamination or from food processing. Foods containing the highest concentrations of PAHs include 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 °C (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].

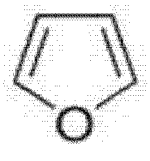
In rats and mice, 3-MCPD is toxic to the kidney, producing renal tubule hyperplasia. It is also carcinogenic in rats when given in high doses over prolonged periods. Although 3-MCPD is genotoxic *in vitro*, it is not *in vivo*. The UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products 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,

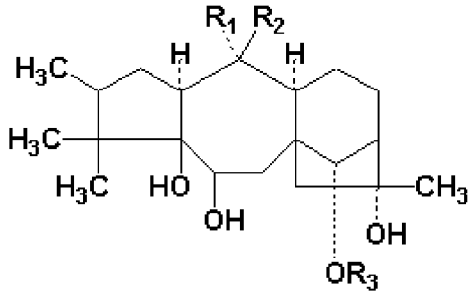
producing ataxia in both man and dogs. However, the flesh may be consumed if boiled several times with changes of water, or as the Inuit prepares it, by burying it in the ground and allowing the meat to go through several freezing and thawing cycles [170–172].

### *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.

## References

1. Institute of Medicine (IOM). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*; National Academies Press: Washington, DC, USA, 2001.
2. Burton, G.W.; Ingold, K.U. beta-Carotene: An unusual type of lipid antioxidant. *Science* **1984**, *224*, 569–573.
3. Bannister, B.; Gibsburg, G.; Shneerson, T. Cardiac arrest due to liquoriceinduced hypokalaemia. *Br. Med. J.* **1977**, *2*, 738–739.
4. Isbrucker, R.A.; Burdock, G.A. Risk and safety assessment on the consumption of licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regul. Toxicol. Pharmacol.* **2006**, *46*, 167–192.

5. United States Government Accountability Office (GAO). Food safety. FDA should strengthen its oversight of food ingredients determined to be generally recognized as safe (GRAS). GAO-10-246, February, 2010. Available online: http://www.gao.gov/new.items/d10246.pdf (accessed on 21 July 2010).
6. Food and Drug Law Institute. Sec. 201. [321] Definitions. In *FDCA Statutory Supplement Including FDA Amendments Act of 2007 and Related Sections of Additional Statutes*; Food and Drug Law Institute: Washington, DC, USA, 2008; pp. 1–2.
7. Food and Drug Law Institute. Sec. 402. [342] Adulterated Food. In *FDCA Statutory Supplement Including FDA Amendments Act of 2007 and Related Sections of Additional Statutes*; Food and Drug Law Institute: Washington, DC, USA, 2008; p. 31.
8. Food and Drug Law Institute. Sec. 406. [346] Tolerances for Poisonous Ingredients in Food. In *FDCA Statutory Supplement Including FDA Amendments Act of 2007 and Related Sections of Additional Statutes*; Food and Drug Law Institute: Washington, DC, USA, 2008; p. 31.
9. Kracov, D.A. The regulation of foods and food additives. In *A Practical Guide to Food and Drug Law Regulation*, 2nd ed.; Piña, K.R., Pines, W.L., Eds.; Food and Drug Law Institute: Washington, DC, USA, 2002; pp. 159–214.
10. Tullo, A. Newscripts: Vile weed or essential ingredient? *Chem. Eng. News* **2010**, *88*, 72.
11. Fischer, R.; Griffin, F.; Kaplan, A.R. Taste thresholds, cigarette smoking, and food dislikes. *Med. Exp. Int. J. Exp. Med.* **1963**, *9*, 151–167.
12. Goff, S.A.; Klee, H.J. Plant volatile compounds: Sensory clues for health and nutritional value? *Science* **2006**, *311*, 815–819.
13. National Organization for Rare Disorders (NORD), 2010. Available online: http://www.rarediseases.org (accessed on 21 July 2010).
14. Carabin, I.G.; Magnuson, B.A. New Labeling Requirements for Food Allergens, April, 2006. Nutritional Outlook. Available online: http://www.nutritionaloutlook.com/article.php?ArticleID=2096 (accessed on 21 July 2010).
15. Kotsonis, F.N.; Burdock, G.A. Food Toxicology. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 7th ed.; Klaassen, C.D., Ed.; McGraw-Hill: New York, NY, USA, 2008; pp. 1191–1236.
16. Sors, T.G.; Ellis, D.R.; Salt, D.E. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynth. Res.* **2005**, *86*, 373–389.
17. Yang, G.; Wang, S.; Zhou, R.; Sun, S. Endemic selenium intoxication of humans in China. *Am. J. Clin. Nutr.* **1983**, *37*, 872–881.
18. Reilly, C. Selenium: Physiology, dietary sources and requirements. In *Encyclopaedia of Human Nutrition*; Sadler, M.J., Ed.; Academic: San Diego, CA, USA, 1998; pp. 1752–1758.
19. United States Environmental Protection Agency (EPA). Selenium and compounds (CASRN 7782-49-2), March 1, 1991. Available online: http://www.epa.gov/iris/subst/0472.htm (accessed on 21 July 2010).
20. Waldron, H.A. Did the Mad Hatter have mercury poisoning? *Br. Med. J.* **1983**, *287*, 1961.

21. Carrington, C.; Bolger, M. An Exposure Assessment for Methylmercury from Seafood for Consumers in the United States. Available online: http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/Seafood/FoodbornePathogensContaminants/Methylmercury/UCM114740.pdf (accessed on 21 July 2010).
22. United States Food and Drug Administration (FDA). Chapter 10: Methyl Mercury. In *Fish and Fisheries Products Hazards and Controls Guidance*, 3rd ed, June, 2001. Available online: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/ucm092041.htm (accessed on 21 July 2010).
23. Hutt, P.B.; Merrill, R.A.; Grossman, L.W. *Food and Drug Law*, 3rd ed.; Foundation Press: New York, NY, USA, 2007; p. 369.
24. European Commission. Scientific Committee on Food. Opinion of the scientific committee on food on thujone, February 6, 2003. Available online: http://ec.europa.eu/food/fs/sc/scf/out162_en.pdf (accessed on 21 July 2010).
25. United States Food and Drug Administration. *Code of Federal Regulations (CFR) 21 § 172.510*; U.S. Government Printing Office: Washington, DC, USA, 2006; pp. 55–57.
26. Galli, C.L.; Galli, G.; Tragni, E.; Caruso, D.; Fiecchi A. Quantitative analysis of alpha, beta-thujone, pulegone, safrole, coumarin and beta-asarone in alcoholic beverages by selected-ion monitoring. *J. Appl. Toxicol.* **1984**, *4*, 273–276.
27. Lawrence, B.M. Progress in essential oils. Sage oil. In *Essential Oils: 2001–2004*; Allured Publishing: Carol Stream, IL, USA, 2006; pp. 25–30.
28. Ben Farhat, M.; Jordán, M.J.; Chaouech-Hamada, R.; Landoulsi, A.; Sotomayor, J.A. Variations in essential oil, phenolic compounds, and antioxidant activity of tunisian cultivated *Salvia officinalis* L. *J. Agric. Food Chem.* **2009**, *57*, 10349–10356.
29. Patocka, J.; Plucar, B. Pharmacology and toxicology of absinthe. *J. Appl. Biomed.* **2003**, *1*, 199–205.
30. Millet, Y.; Jouglard, J.; Steinmetz, M.D.; Tognetti, P.; Joanny, P.; Arditti, J. Toxicity of some essential plant oils. Clinical and experimental study. *Clin. Toxicol.* **1981**, *18*, 1485–1498.
31. Bonkovsky, H.L.; Cable, E.E.; Cable, J.W.; Donohue, S.E.; White, E.C.; Greene, Y.J.; Lambrecht, R.W.; Srivastava, K.K.; Arnold, W.N. Porphyrogenic properties of the terpenes camphor, pinene, and thujone. *Biochem. Pharmacol.* **1992**, *43*, 2359–2368.
32. United States National Toxicology Program (NTP). Alpha-Thujone, December 10, 1997. Available online: http://ntp.niehs.nih.gov/index.cfm?objectid=03DB8C36-E7A1-9889-3BDF8436F2A8C51F (accessed on 21 July 2010).
33. Hold, K.M.; Sirisoma, N.S.; Casida, J.E. Detoxification of alpha- and beta-thujones (the active ingredients of absinthe): Site specificity and species differences in cytochrome P450 oxidation *in vivo* and *in vivo*. *Chem. Res. Toxicol.* **2001**, *14*, 589–595.
34. Perdue University, Cooperative Extension Service (Perdue). Indiana plants poisonous to livestock and pets. Available online: http://www.vet.purdue.edu/toxic/plant46.htm (accessed on 21 July 2010).
35. Merck. Cyanide Poisoning: Introduction. In *The Merck Veterinary Manual*; 2008. Available online: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/210800.htm&word=prussic%2cacid (accessed on 21 July 2010).

36. Panter, K.E. Natural toxins of plant origin. In *Toxins in Food*; Dabrowski, W.M., Sikorski, Z.E., Eds.; CRC Press: Boca Raton, FL, USA, 2004; pp. 11–63.
37. Wentworth, J.M.; Agostini, M.; Love, J.; Schwabe, J.W.; Chatterjee, V.K. St John's wort, a herbal antidepressant, activates the steroid X receptor. *J. Endocrinol.* **2000**, *166*, R11–R16.
38. Karioti, A.; Bilia, A.R. Hypericins as potential leads for new therapeutics. *Int. J. Mol. Sci.* **2010**, *11*, 562–594.
39. Hammerness, P.; Basch, E.; Ulbricht, C.; Barrette, E.P.; Foppa, I.; Basch, S.; Bent, S.; Boon, H.; Ernst, E. St. John's Wort: A systematic review of adverse effects and drug interactions for the consultation psychiatrist. *Psychosomatics* **2003**, *44*, 271–282.
40. Britton, N.L.; Brown, A. *Hypericum perforatum* L. In *An illustrated Flora of the Northern United States, Canada and the British Possessions*; Charles Scribner's Sons: New York, NY, USA, 1913; Volume 2, p. 533. USDA-NRCS PLANTS Database. Available online: http://plants.usda.gov/java/profile?symbol=HYPE&photoID=hype_001_avd.tif (accessed on 31 August 2010).
41. State of Victoria Department of Primary Industries (Victoria). Landcare notes. St. John's wort, 2007. Available online: http://www.dpi.vic.gov.au/dpi/nreninf.nsf/93a98744f6ec41bd4a256c8e00013aa9/9f65b9c41bbc7aa5ca25737500119160/$FILE/LC0177_Sep07.pdf (accessed on 21 July 2010).
42. Greer, M.A. Goitrogenic substances in food. *Am. J. Clin. Nutr.* **1957**, *5*, 440–444.
43. Conn, E.E. Cyanogenetic Glycosides. In *Toxicants Occurring Naturally in Foods*, 2nd ed.; Committee on Food Protection, Food and Nutrition Board, National Research Council, National Academy of Sciences: Washington, DC, USA, 1973; pp. 299–308.
44. VenEtten, C.H.; Wolff, I.A. Natural sulfur compounds. In *Toxicants Occurring Naturally in Foods*, 2nd ed.; Committee on Food Protection, Food and Nutrition Board, National Research Council, National Academy of Sciences: Washington, DC, USA, 1973; pp. 210–234.
45. United States Department of Agriculture (USDA). Plants Profile: *Brassica napus* L. Available online: http://plants.usda.gov/java/profile?symbol=BRNA (accessed on 21 July 2010).
46. Carroll, K.K. Erucic acid as the factor in rape oil affecting adrenal cholesterol in the rat. *J. Biol. Chem.* **1953**, *200*, 287–292.
47. Chien, K.R.; Bellary, A.; Nicar, M.; Mukherjee, A.; Buja, L.M. Induction of a reversible cardiac lipidosis by a dietary long-chain fatty acid (erucic acid). *Am. J. Pathol.* **1983**, *112*, 68–77.
48. Ratanasethkul, C.; Riddell, C.; Salmon, R.E.; O'Neil, J.B. Pathological changes in chickens, ducks and turkeys fed high levels of rapeseed oil. *Can. J. Comp. Med.* **1976**, *40*, 360–369.
49. Mattson, F.H. Potential toxicity of food lipids. In *Toxicants Occurring Naturally in Foods*, 2nd ed.; Committee on Food Protection, Food and Nutrition Board, National Research Council, National Academy of Sciences: Washington, DC, USA, 1973; pp. 189–209.
50. Mori, H.; Tanaka, T.; Hirono, I. Toxicants in Food: Naturally Occurring. In *Nutrition and Chemical Toxicity*; Ioannides, C., Ed.; John Wiley & Sons: West Sussex, England, UK, 1998; pp. 1–27.

51. Biotechnology Australia (Australian Government). "What is canola?" A problem with weeds—the canola story. Available online: http://www.biotechnologyonline.gov.au/foodag/weeds.html (accessed on 21 July 2010).
52. Health Canada. "Low Erucic Acid Rapeseed (Lear) Oil Derived From Canola-quality *Brassica juncea* (L.) CZERN. Lines PC 97-03, PC98-44 AND PC98-45", March 27, 2003. Available online: http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/low_erucic-faible_erucique-eng.php (accessed on 21 July 2010).
53. Wagstaff, D. Dietary exposure to furocoumarins. *Regul. Toxicol. Pharmacol.* **1991**, *14*, 261–272.
54. Ashwood-Smith, M.J.; Ceska, O.; Chaudhary, S.K.; Warrington, P.J.; Woodcock, P. Detection of furocoumarins in plants and plant products with an ultrasensitive biological photoassay employing a DNA-repair-deficient bacterium. *J. Chem. Ecol.* **1986**, *12*, 915–932.
55. Zobel, A.M.; Brown, S.A. Dermatitis-inducing psoralens on the surfaces of seven medicinal plant species. *J. Toxicol. Cutaneous Ocul. Toxicol.* **1991**, *10*, 223–231.
56. Dunnick, J.K. NTP Technical Report on the Toxicology and Carcinogenesis Studies of 8-Methoxypsoralen (CAS No. 298-81-7) in F344/N Rats. NIH Publication No. 89-2814. National Toxicology Program: Research Triangle Park, NC, USA, 1989.
57. International Agency for Research on Cancer (IARC). Summaries & Evaluations, 8-Methoxypsoralen (Methoxsalen) plus ultraviolet radiation. *IARC* **1987**, *7* (Suppl.), 261. Available online: http://www.inchem.org/documents/iarc/suppl7/methoxypsoralen-8.html (accessed on 21 July 2010).
58. Stern, R.S.; Nichols, K.T.; Vakeva, L.H. Malignant melanoma in patients treated for psoriasis with methoxsalen (psoralen) and ultraviolet A radiation (PUVA). The PUVA follow-up study. *N. Engl. J. Med.* **1997**, *336*, 1041–1045.
59. International Agency for Research on Cancer (IARC) Summaries & Evaluations, 5-Methoxypsoralen. *IARC* **1986**, *40*, 327. Available online: http://www.inchem.org/documents/iarc/vol40/5-methoxypsoralen.html (accessed on 21 July 2010).
60. Girennavar, B.; Poulose, S.M.; Jayaprakasha, G.K.; Bhat, N.G.; Patil, B.S. Furocoumarins from grapefruit juice and their effect on human CYP3A4 and CYP1B1 isoenzymes. *Bioorg. Med. Chem.* **2006**, *14*, 2606–2612.
61. Bailey, D.G.; Malcom, J.; Arnold, O.; Spence, J.D. Grapefruit juice-drug interactions. *Br. J. Clin. Pharmacol.* **1998**, *46*, 101–110.
62. Duke, J.A. *Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants*; CRC Press: Boca Raton, FL, USA, 1992; pp. 171, 174, 180, 183.
63. Placzek, M.; Fromel, W.; Eberlein, B.; Gilbertz, K.P.; Przybilla, B. Evaluation of phototoxic properties of fragrances. *Acta Derm. Venereol.* **2007**, *87*, 312–316.
64. Marzulli, F.N.; Maibach, H.I. Perfume phototoxicity. *J. Soc. Cosmet. Chem.* **1970**, *21*, 695–715.
65. Coulumbe, R.A., Jr. Natural toxins and chemopreventives in plants. In *Food Toxicology*; Helferich, W., Winter, C.K., Eds.; CRC Press: Boca Raton, FL, USA, 2001; p. 152.
66. Schlatter, J.; Zimmerli, B.; Dick, R.; Panizzon, R.; Schlatter, C. Dietary intake and risk assessment of phototoxic furocoumarins in humans. *Food Chem. Toxicol.* **1991**, *29*, 523–530.

67. Deshpande, S.S. Food Additives. In *Handbook of Food Toxicology*; Marcel Dekker: New York, NY, USA, 2002a; pp. 219–284.
68. Nutrilab, Inc. v. S. Schweiker, 713 F.2d 335 (7th Cir. 1983). Available online: http://openjurist.org/713/f2d/335 (accessed on 21 July 2010).
69. Franken, J.; Stephan, U.; Meyer, H.E.; Konig, W. Identification of alpha-amylase inhibitor as a major allergen of wheat flour. *Int. Arch. Allergy Appl. Immunol.* **1994**, *104*, 171–174.
70. Moreno-Ancillo, A.; Dominguez-Noche, C.; Gil-Arados, A.C.; Cosmes, P.M. Bread eating induced oral angiodema due to a-amylase allergy. *J. Investig. Allergol. Clin. Immunol.* **2004**, *14*, 346–347.
71. Granum, P.E. Studies on α-amylase in foods. *Food Chem.* **1979**, *4*, 173–178.
72. Phadia, A.B. http://www.immunocapinvitrosight.com/ImmunoCAPDefault____23027.aspx (accessed on 14 September 2010).
73. Jones, J.M.J. *Food Safety*; Eagan Press: St. Paul, MN, USA, 1995; pp. 71, 77, 84, 87.
74. Shibamoto, T.; Bjeldanes, L.F. Natural toxins in plant foodstuffs. In *Introduction to Food Toxicology*; Academic Press: San Diego, CA, USA, 1993; pp. 78–79, 82–84.
75. Omaye, S.T. Toxicity of Nutrients. In *Food and Nutritional Toxicology*; CRC Press: Boca Raton, FL, USA, 2004; pp. 205–213.
76. Banwell, J.G.; Boldt, D.H.; Meyers, J.; Weber, F.L., Jr. Phytohemagglutinin derived from red kidney bean (Phaseolus vulgaris): A cause for intestinal malabsorption associated with bacterial overgrowth in the rat. *Gastroenterology* **1983**, *84*, 506–515.
77. Dobbins, J.W.; Laurenson, J.P.; Gorelick, F.S.; Banwell, J.G. Phytohemagglutinin from red kidney bean (Phaseolus vulgaris) inhibits sodium and chloride absorption in the rabbit ileum. *Gastroenterology* **1986**, *90*, 1907–1913.
78. United States Food and Drug Administration (FDA). Bad Bug Book. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. Phytohaemagglutinin, May 14, 2009. Available online: http://www.fda.gov/food/foodsafety/foodborneillness/foodborneillnessfoodbornepathogensnaturaltoxins/badbugbook/ucm071092.htm (accessed on 21 July 2010).
79. Buhler, R. Eating raw, undercooked beans can be unpleasant. High Plains/Midwest AG Journal. Available online: http://www.hpj.com/archives/2004/nov04/nov15/Eatingrawundercookeddrybean.cfm (accessed on 21 July 2010).
80. Cornell University. Plants poisonous to livestock. Thiaminases. Available online: http://www.ansci.cornell.edu/plants/toxicagents/thiaminase.html (accessed on 21 July 2010).
81. Deshpande, S.S. Toxicants and antinutrients in plant foods. In *Handbook of Food Toxicology*; Marcel Dekker: New York, NY, USA, 2002b; pp. 331–372.
82. Prakash, A.S.; Pereira, T.N.; Reilly, P.E.B.; Seawright, A.A. Pyrrolizidine alkaloids in human diet. *Mutat. Res.* **1999**, *443*, 53–67.
83. Britton, N.L.; Brown, A. *Symphytum officinale* L. In *An Illustrated Flora of the Northern United States, Canada and the British Possessions*; Charles Scribner's Sons: New York, NY, USA, 1913; Volume 2, p. 92. USDA-NRCS PLANTS Database. Available online: http://plants.usda.gov/java/profile?symbol=SYOF&photoID=syof_001_avd.tif (accessed on 31 August 2010).

84. Dharmananda, S. Safety issues affecting herbs: Pyrollizidine alkaloids, November, 2001. Available online: http://www.itmonline.org/arts/pas.htm (accessed on 21 July 2010).
85. Lowry, N. Rhubarb and Oxalic Acid. Available online: http://helios.hampshire.edu/~nlNS/mompdfs/oxalicacid.pdf (accessed on 21 July 2010).
86. Finkelstein, V.A.; Goldfarb, D.S. Strategies for preventing calcium oxalate stones. *Can. Med. Assoc. J.* **2006**, *174* (10), 1407–1409, DOI:10.1503/cmaj.051517.
87. Subbiah, V. Method of isolating cucurbitacin, July 20, 1999. Available online: http://www.freepatentsonline.com/5925356.html (accessed on 21 July 2010).
88. Martin, P.A.W.; Blackburn, M.; Schroder, R.F.W.; Matsuo, K.; Li, B.W. Stabilization of cucurbitacin E-glycocide, a feeding stimulant for diabroticite beetles, extracted from bitter Hawkesbury watermelon. *J. Insect Sci.* **2002**, *2*, 1–6.
89. Feather, S. Growing zucchini. Why your garden zucchinis might taste bitter. Available online: http://www.donnan.com/Zucchini.htm (accessed on 21 July 2010).
90. Browning, S.; Hodges, L. Bitterness in Zucchini Squash and Cucumber, February 19, 2010. Available online: http://cuke.hort.ncsu.edu/cucurbit/cuke/cukehndbk/cukebitterness.html (accessed on 21 July 2010).
91. Burfield, T. Coumarin: The real story, January, 2008. Available online: http://www.leffingwell.com/Coumarin%20-%20the%20real%20story%20update2.pdf (accessed on 21 July 2010).
92. Cornell University. Plants poisonous to livestock. Coumarin Glycosides. Available online: http://www.ansci.cornell.edu/plants/toxicagents/coumarin.html (accessed on 21 July 2010).
93. Lake, B.G. Coumarin metabolism, toxicity and carcinogenicity: Relevance for human risk assessment. *Food Chem. Toxicol.* **1999**, *37*, 423–453.
94. Fallon, S.; Enig, M.G. Cinderella's dark side. Available online: http://www.mercola.com/article/soy/avoid_soy.htm (accessed on 21 July 2010).
95. Kumar, V.; Sinha, A.K.; Makkar, H.P.S.; Becker, K. Dietary roles of phytate and phytase in human nutrition: A review. *Food Chem.* **2010**, *120*, 945–959.
96. Baruah, K.; Sahu, N.P.; Pal, A.K.; Debnath, D. Dietary phytase: An ideal approach for a cost effective and low-polluting aquafeed. *NAGA, WorldFish Center Quarterly* **2004**, *27* (3 & 4), 15–19.
97. Schecter, J.C.; Wiener, S.W. Plant Poisoning, Hypoglycemics, December 16, 2009. Available online: http://emedicine.medscape.com/article/817325-overview (accessed on 21 July 2010).
98. Lancashire, R.J. Jamaican Ackee, November 21, 2008. Available online: http://wwwchem.uwimona.edu.jm/lectures/ackee.html (accessed on 21 July 2010).
99. Sherratt, H.S.A. Hypoglycin, the famous toxin of the unripe Jamaican ackee fruit. *Trends Pharmacol. Sci.* **1986**, *7*, 186–191.
100. United States Food and Drug Administration (FDA). Haitian ackee fruit, January, 2010. Available online: http://www.fda.gov/Food/NewsEvents/WhatsNewinFood/ucm197850.htm (accessed on 21 July 2010).
101. Henry, S.H.; Page, S.W.; Bolger, P.M. Hazard assessment of ackee fruit (Blighia sapida). *Hum. Ecol. Risk Assess.* **1998**, *4*, 1175–1187.

102. Blake, O.A.; Jackson, J.C.; Jackson, M.A.; Gordon, C.L.A. Assessment of dietary exposure to the natural toxin hypoglycin in ackee (Blighia sapida) by Jamaican consumers. *Food Res. Int.* **2004**, *37*, 833–838.
103. Blake, O.A.; Bennink, M.R.; Jackson, J.C. Ackee (*Blighia sapida*) hypoglycin A toxicity: Dose response assessment in laboratory rats. *Food Chem. Toxicol.* **2006**, *44*, 207–213.
104. United States Food and Drug Administration (FDA). Detention without Physical Examination of Ackees. Import Alert 21–11, June 3, 2010. Available online: http://www.accessdata.fda.gov/cms_ia/importalert_64.html (accessed on 21 July 2010).
105. McGuffin, M. *American Herbal Product Association's Botanical Safety Handbook*; CRC Press: Boca Raton, FL, USA, 1997; pp. 149–152.
106. Homburger, F.; Boger, E. The carcinogenicity of essential oils, flavors and spices: A review. *Cancer Res.* **1968**, *28*, 2372–2374.
107. United States National Institute of Environmental Health Sciences (NIEHS). Substance Profiles: Safrole (CAS No. 94-59-7). Report on Carcinogens, Eleventh Edition; January 31, 2005. Available online: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s159safa.pdf (accessed on 21 July 2010).
108. Wislocki, P.G.; Miller, E.C.; Miller, J.A.; McCoy, E.C.; Rosenkranz, H.S. Carcinogenic and mutagenic activities of safrole, 1'-hydroxysafrole, and some known or possible metabolites. *Cancer Res.* **1977**, *37*, 1883–1891.
109. Burfield, T. Safrole: Human carcinogenicity risk over-stated? September, 2009. Available online: http://www.cropwatch.org/Safrole%20human%20carcinogenicity.pdf (accessed on 21 July 2010).
110. Hallstrom, H.; Thuvander, A. Toxicological evaluation of myristicin. *Nat. Toxins* **1997**, *5*, 186–192.
111. Arneson, P.A.; Drubin, R.D. Studies on the mode of action of tomatine as a fungitoxic agent. *Plant Physiol.* **1968**, *43*, 683–686.
112. Rick, C.M.; Uhlig, J.W.; Jones, A.D. High alpha-tomatine content in ripe fruit of Andean Lycopersicon esculentum var. cerasiforme: developmental and genetic aspects. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 12877–12881.
113. Friedman, M.; Levin, C.E. α-Tomatine content in tomato and tomato products determined HPLC with pulsed amperometric detection. *J. Agric. Food Chem.* **1995**, *43*, 1507–1511.
114. Friedman, M.; Fitch, T.E.; Yokayama, W.E. Lowering of plasma LDL cholesterol in hamsters by the tomato glycoalkaloid tomatine. *Food Chem. Toxicol.* **2000**, *38*, 549–553.
115. Ize-Ludlow, D.; Ragone, S.; Bruck, I.S.; Bernstein, J.N.; Duchowny, M.; Pena, M.G. Neurotoxicities in infants seen with consumption of star anise tea. *Pediatrics* **2004**, *114*, e653–e656.
116. United States Food and Drug Administration (FDA). Inspections, Compliance, Enforcement and Criminal Investigations. Available online: http://www.fda.gov/ICECI/EnforcementActions/EnforcementStory/EnforcementStoryArchive/ucm095929.htm (accessed on 21 July 2010).
117. Tice, R. α-Chaconine [20562-03-2] and α-Solanine [20562-02-1]. Review of toxicological literature. Prepared for Errol Zeiger, National Institute of Environmental Health Sciences, February, 1998. Available online: http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/ChaconineSolanine.pdf (accessed on 21 July 2010).

118. Surak, J.G. Phytoalexins and human health—a review. *FSHS Proc.* **1978**, *91*, 256–258.
119. United States Food and Drug Administration (FDA). FDA Poisonous Database, January 1, 2008. Available online: http://www.accessdata.fda.gov/scripts/Plantox/Detail.CFM?ID=6537 (accessed on 21 July 2010).
120. Dinkins, C.L.P.; Peterson, R.K.D. A human dietary risk assessment associated with glycoalkaloid response of potato to Colorado potato beetle defoliation. *Food Chem. Toxicol.* **2008**, *46*, 2837–2840.
121. Ceska, O.; Chaudhary, S.K.; Warrington, P.J.; Ashwood-Smith, M.J. Naturally-occurring crystals of photocarcinogenic furocoumarins on the surface of parsnip roots sold as food. *Experentia* **1986**, *42*, 1302–1304.
122. Ostertag, E.; Becker, T.; Ammon, J.; Bauer-Aymanns, H.; Schrenk, D. Effects of storage conditions on furocoumarin levels in intact, chopped, or homogenized parsnips. *J. Agric. Food Chem.* **2002**, *50*, 2565–2570.
123. Turesky, R.J. Heterocyclic Aromatic Amines (Part 2.3). In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; pp. 75–115.
124. United States National Institute of Environmental Health Sciences (NIEHS). Selected Heterocylclic Amines. Report on Carcinogens, Eleventh Edition; January 31, 2005. Available online: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s092vhca.pdf (accessed on 21 July 2010).
125. Park, J.-H.; Penning, T.M. Polyaromatic Hydrocarbons (Part 2.8). In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; pp. 243–282.
126. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Sixty-fourth meeting (64/SC). Section 2.6. Available online: http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf (accessed on 21 July 2010).
127. Mills, C.; Mottram, D.S.; Wedzicha, B.L. Acrylamide (Part 2.1). In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; pp. 23–50.
128. Exon, J.H. A review of the toxicology of acrylamide. *J. Toxicol. Environ. Health* **2006**, *9*, 397–412.
129. Hamlet, C.G.; Sadd, P.A. Chloropropanols and Chloroesters (Part 2.6). In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; pp. 175–214.
130. Watkins, C. Chloroesters in foods: An emerging issue; April, 2009. Available online: http://www.aocs.org/Membership/FreeCover.cfm?itemnumber=1084 (accessed on 21 July 2010).
131. Directorate-General Health and Consumer Protection. Reports on tasks for scientific cooperation. Collection and collation of data on levels of 3-monochloropropanediol (3-MCPD) and related substances in foodstuffs; June, 2004. Available online: http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-9_final_report_chloropropanols_en.pdf (accessed on 21 July 2010).

132. Food Standards Australia New Zealand (FSANZ). *Chloropropanols in Food—an Analysis of Public Health Risk*; Technical Report Series No. 15; Food Standards Australia New Zealand: Canberra, Australia, 2003.
133. United States Food and Drug Administration (FDA). Sec. 500.500 Guidance levels for 3-MCPD (3-chloro-1,2-propanediol) in acid-hydrolyzed protein and asian-style sauces; March 2008. Available online: http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidance Manual/ucm074419.htm (accessed on 31 August 2010).
134. Carthew, P.; DiNovi, M.; Setzer, R.W. Application of the margin of exposure (MoE) approach to substances in food that are genotoxic and carcinogenic. Example: Furan (CAS No. 110-00-9). *Food Chem. Toxicol.* **2010**, *48*, S69–S74.
135. Bolger, P.M.; Tao, S.; Dinovi, M. Hazards of Dietary Furan. In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; pp. 117–133.
136. Moser, G.J.; Foley, J.; Burnett, M.; Goldsworthy, T.L.; Maronpot, R. Furan-induced dose-response relationships for liver cytotoxicity, cell proliferation, and tumorigenicity (furan-induced liver tumorigenicity). *Exp. Toxicol. Pathol.* **2009**, *61*, 101–111.
137. Cordelli, E.; Leopardi, P.; Villani, P.; Marcon, F.; Macri, C.; Caiola, S.; Siniscalchi, E.; Conti, L.; Eleuteri, P.; Malchiodi-Albedi, F.; Crebelli, R. Toxic and genotoxic effects of oral administration of furan in mouse liver. *Mutagenesis* **2010**, *25*, 305–314.
138. Leopardi, P.; Cordelli, E.; Villani, P.; Cremona, T.P.; Conti, L.; DeLuca, G.; Crebelli, R. Assessment of *in vivo* genotoxicity of the rodent carcinogen furan: Evaluation of DNA damage and induction of micronuclei in mouse splenocytes. *Mutagenesis* **2010**, *25*, 57–62.
139. Sadler, M.J. Health effects of trans fatty acids. In *Encyclopedia of Human Nutrition*; Sadler, M.J., Strain, J.J., Caballero, B., Eds.; Academic: San Diego, CA, USA, 1999; Volume 2, pp. 769–776.
140. Ascherio, A.; Katan, M.B.; Zock, P.L.; Stampfer, M.J.; Willett, W.C. Trans fatty acids and coronary heart disease. *N. Eng. J. Med.* **1999**, *340*, 1994–1998.
141. Baxter, S.D. Nutrition for Healthy Children and Adolescents Aged 2 to 18 Years. In *Handbook of Nutrition and Food*, 2nd ed.; Berdanier, C.D., Dwyer, J., Feldman, E.B., Eds.; CRC Press: Boca Raton, FL, USA, 2008; p. 295.
142. United States Food and Drug Administration (FDA). Federal Register—68 FR 41433 July 11, 2003: Food Labeling; Trans Fatty Acids in Nutrition Labeling; Consumer Research to Consider Nutrient Content and Health Claims and Possible Footnote or Disclosure Statements; Final Rule and Proposed Rule. Available online: http://www.fda.gov/food/labelingnutrition/labelclaims/nutrientcontentclaims/ucm110179.htm (accessed on 21 July 2010).
143. United States Department of Agriculture (USDA). Dietary Guidelines for Americans 2005. Available online: http://www.cnpp.usda.gov/publications/dietaryguidelines/2005/2005DGpolicydocument.pdf (accessed on 21 July 2010).
144. Motarjemi, Y.; Stadler, R.H.; Studer, A.; Damiano, V. Application of the HAACP Approach for the Management of Processing Contaminants. In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; p. 573.

145. United States National Institute of Environmental Health Sciences (NIEHS). *N*-Nitrosodimethylamine. Report on Carcinogens, Eleventh Edition; January 31, 2005. Available online: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s128nitr.pdf (accessed on 21 July 2010).
146. Habermeyer, M.; Eisenbrand, G. *N*-Nitrosmaines, including *N*-Nitrosoaminoacids and potential further nonvolatiles (Part 4.1). In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; pp. 365–386.
147. United States National Institute of Environmental Health Sciences (NIEHS). *N*-Nitrosopyrrolidine. Report on Carcinogens, Eleventh Edition; January 31, 2005. Available online: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s137nsop.pdf (accessed on 21 July 2010).
148. United States National Institute of Environmental Health Sciences (NIEHS). *N*-Nitrosopiperidine. Report on Carcinogens, Eleventh Edition; January 31, 2005. Available online: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s136nsop.pdf (accessed on 21 July 2010).
149. Jakszyn, P.; Gonzalez, C.A. Nitrosamine and related food intake and gastric and oesophageal cancer risk: A systematic review of the epidemiogical evidence. *World J. Gastroenterol.* **2006**, *12*, 4296–4303.
150. Sarkadi, L.S. Biogenic Amines (Part 3.2). In *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R.H., Lineback, D.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2009; pp. 321–361.
151. United States Food and Drug Administration (FDA). Fish and Fisheries Products Hazards and Controls Guidance, Third Edition. Chapter 7: Scombrotoxin (Histamine) Formation (A Chemical Hazard), June 2001. Available online: http://www.fda.gov/Food/GuidanceComplianceRegulatory Information/GuidanceDocuments/Seafood/FishandFisheriesProductsHazardsandControlsGuide/ ucm091910.htm (accessed on 31 August 2010).
152. Van Dolah, F.M. Marine algal toxins: Origins, health effects, and their increased occurrence. *Environ. Health Perspect.* **2000**, *108*, 133–141.
153. Woods Hole Oceanographic Institution (WHOI). Human illness associated with harmful algae. Available online: http://www.whoi.edu/science/B/redtide/illness/illness.html (accessed on 21 July 2010).
154. United States Food and Drug Administration (FDA). Fish and Fisheries Products Hazards and Controls Guidance, Third Edition. Chapter 6: Natural Toxins (A Chemical Hazard). Available online: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/Guidance Documents/Seafood/ucm091782.htm (accessed on 29 August 2010).
155. Beauchamp, R.A.; Wiles, K.; Hendricks, K. Red Tide Information; May 20, 2008. Available online: http://www.dshs.state.tx.us/seafood/redtide.shtm (accessed on 21 July 2010).
156. University System of Maryland (USM). Harmful algal blooms. Available online: http://aquaticpath.umd.edu/toxalg/nsp.html (accessed on 21 July 2010).
157. United States Department of Commerce National Oceanic and Atmospheric Administration (NOAA). Microscopic image of Pseudo-nitzschia. Available online: http://www.noaanews. noaa.gov/stories2009/20091116_razor.html (accessed on 31 August 2010).

158. United States Department of Commerce National Oceanic and Atmospheric Administration (NOAA). Scientists report first remote, underwater detection of harmful algae, toxins; June 14, 2009. Available online: http://www.physorg.com/news166807443.html (accessed on 21 July 2010).
159. Kleivdal, H.; Kristiansen, S.; Nilsen, M.V. Single-laboratory validation of the Biosense Direct Competitive Enzyme-Linked Immunosorbent Assay (ELISA) for determination of domoic acid toxins in shellfish. *J. AOAC Int.* **2007**, *90*, 1000–1010.
160. Dickey, R.W.; Plakas, S.M. Ciguatera: A public health perspective. *Toxicon* **2009**, DOI:10.1016/j.toxicon.2009.09.008.
161. United States Food and Drug Administration (FDA). Bad Bug Book. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. BBB-Gemphylotoxin; May 20, 2010. Available online: http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodborne PathogensNaturalToxins/BadBugBook/ucm071191.htm (accessed on 21 July 2010).
162. Ukishima, Y.; Masui, T.; Masubara, S.; Goto, R.; Okada, S.; Tsuji, K.; Kosuge, T. Wax components of escolar (Lepidocybium flavobrunneum) and its application to base of medicine and cosmetics. *Yakugaku Zasshi* **1987**, *107*, 883–890.
163. Nicholas, P.D.; Mooney, B.D.; Elliott, N.G. Unusually high levels of non-saponifiable lipids in the fishes escolar and rudderfish identification by gas and thin-layer chromatography. *J. Chromatogr. A* **2001**, *936*, 183–191.
164. Berman, P.; Harley, E.H.; Spark, A.A. Keriorrhoea—the passage of oil per rectum—after ingestion of marine wax esters. *S. Afr. Med. J.* **1981**, *59*, 791–792.
165. SEFSC Pascagoula Laboratory; Collection of Brandi Noble. Photograph of Juvenile Oilfish (Ruvettus pretiosus), NOAA/NMFS/SEFSC. NOAA Photo Library. Available online: http://www.photolib.noaa.gov/htmls/fish4425.htm (accessed on 15 September 2010).
166. Reid, T.M.S.; Gould, I.M.; Mackie, I.M.; Ritchie, A.H.; Hobbs, G. Food poisoning due to the consumption of red whelks (*Neptunea antiqua*). *Epidemiol. Infect.* **1988**, *101*, 419–424.
167. Kim, J.H.; Lee, K.J.; Suzuki, T.; Kim, C.M.; Lee, J.Y.; Mok, J.S.; Lee, T.S. Identification of tetramine, a toxin in whelks, as the cause of a poisoning incident in Korea and the distribution of tetramine in fresh and boiled whelk (*Neptunea intersculpta*). *J. Food Prot.* **2009**, *72*, 1935–1940.
168. Power, A.J.; Keegan, B.G.; Nolan, K. The seasonality and role of the neurotoxin tetramine in the salivary glands of the red whelk *Neptunea antiqua* (L.). *Toxicon* **2002**, *40*, 419–425.
169. Anthoni, U.; Bohlin, L.; Larsen, C.; Nielsen, P.; Nielsen, N.H. The toxin tetramine from "edible" whelk *Neptunea antiqua. Toxicon* **1989**, *27*, 717–723.
170. Anthoni, U.; Christophersen, C.; Gram, L.; Nielsen, N.H.; Nielsen, P. Poisonings from flesh of the Greenland shark *Somniosus microcephalus* may be due to trimethylamine. *Toxicon* **1991**, *29*, 1205–1212.
171. Benz, G.W.; Hocking, R.; Kowunna, Sr.A.; Bullard, S.A.; George, J.C. A second species of Arctic shark: Pacific sleeper shark *Somniosus pacificus* from Point hope Alaska. *Polar Biol.* **2004**, *27*, 250–252.
172. Idboro, C.J. The pangnirtung inuit and the greenland shark. Masters Thesis. University of Manitoba, Canada, November, 2008. Available online: http://www.umanitoba.ca/institutes/

natural_resources/canadaresearchchair/thesis/Idrobo.Masters%20Thesis.Feb%2009.pdf (accessed on 21 July 2010).

173. United States Food and Drug Administration (FDA). Bad Bug Book. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. BBB-Grayanotoxin. Available online: http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/ucm071128.htm (accessed on 29August 2010).
174. Laborde, A. *Nerium oleader* L. Poisons Information Monograph 366. International Programme on Chemical Safety (INCHEM); November, 1989. Available online: http://www.inchem.org/documents/pims/plant/pim366.htm (accessed on 21 July 2010).
175. Panter, K.E.; James, L.F. Natural plant toxicants in milk: A review. *J. Anim. Sci.* **1990**, *68*, 892–904.
176. Lee, S.T.; Davis, T.Z.; Gardner, D.R.; Stegelmeier, B.L.; Evans, T.J. Quantitative method for the measurement of three benzofuran ketones in rayless goldenrod (*Isocoma pluriflora*) and white snakeroot (*Ageratina altissima*) by high-performance liquid chromatography (HPLC). *J. Agric. Food Chem.* **2009**, *57*, 5639–5643.
177. National Park Service (NPS). Lincoln Boyhood National Memorial. The plant that killed Nancy Hanks Lincoln. Available online: http://www.nps.gov/archive/libo/white_snakeroot3.htm (accessed on 21 July 2010).

IVYSPRING INTERNATIONAL PUBLISHER

*International Journal of Medical Sciences*

2012; 9(6):506-512. doi: 10.7150/ijms.4787

Research Paper

# Mannose Binding Lectin and Macrophage Migration Inhibitory Factor Gene Polymorphisms in Turkish Children with Cardiomyopathy: No Association with MBL2 Codon 54 A/B Genotype, but an Association between MIF -173 CC Genotype

**Nilgun Col-Araz[1], Sibel Oguzkan-Balci[2]✉, Osman Baspinar[3], Tugce Sever[2], Ayse Balat[4], Sacide Pehlivan[2],**

1. University of Gaziantep, Faculty of Medicine, Department of Pediatrics, Gaziantep, Turkey.
2. University of Gaziantep, Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey.
3. University of Gaziantep, Faculty of Medicine, Department of Pediatric Cardiology, Gaziantep, Turkey.
4. University of Gaziantep, Faculty of Medicine, Department of Pediatric Nephrology, Gaziantep, Turkey.

✉ Corresponding author: Assos. Prof. Dr. Sibel Oguzkan Balci, University of Gaziantep, Faculty of Medicine, Department of Medical Biology and Genetics, 27310 Gaziantep, Turkey. E-mail: oguzkan@gantep.edu.tr Tel: +90 342 3606060, Fax: +90 342 3601617.



Received: 2012.06.27; Accepted: 2012.08.19; Published: 2012.08.22

## Abstract

Myocardial inflammation is one of the commonest mechanisms in cardiomyopathy (CMP). Mannose binding lectin (MBL) is a key molecule in innate immunity, while macrophage migration inhibitory factor (MIF) is a constitutive element of the host defenses. We investigated the possible association between polymorphisms of MBL2 and MIF genes and CMP in Turkish children. Twenty-children with CMP and 30 healthy controls were analyzed for codon 54 A/B polymorphism in MBL, and -173 G/C polymorphism in MIF genes by using PCR-RFLP methods. No significant difference was found between genotypes and alleles of MBL2 gene codon 54 A/B polymorphism in patients and controls ($p>0.05$). However, serum uric acid levels was found higher in dilated CMP patients with AA genotype. Frequency of MIF -173 CC genotype was significantly higher in patients ($p<0.05$), and sodium levels were higher in patients with MIF -173 CC genotype. This study is the first to investigate the MBL and MIF gene polymorphisms in Turkish children with CMP. We conclude that CC genotype of MIF (-173) polymorphism may be a risk factor for CMP patients. However, further studies with larger samples are needed to address the exact role of this polymorphism in CMP.

Key words: Cardiomyopathy, Children, Macrophage migration inhibitory factor, Mannose binding lectin, polymorphism.

## Introduction

Cardiomyopathy (CMP) is defined as "diseases of the myocardium associated with cardiac disfunction" by World Health Organization (WHO), and it is an important cause of chronic congestive cardiac failure in children. The reported incidence for cardiomyopathies is 1,13-1,24 per 100,000 children [1, 2]. Although the pathogenesis of disease is not fully understood, disturbances of the cellular and humoral immune system are frequently observed in CMPs, and myocardial inflammation is one of the commonest mechanisms in cardiomyopathy [3].

Mannose binding lectin (MBL) and Macrophage

migration inhibitory factor (MIF) play substantial roles in the pathogenesis of several inflammatory and autoimmune disorders [4, 5]. Mannose binding lectin is a key molecule in innate immunity with the capacity to bind to microorganisms and kill them by initiating the lectin pathway of complement activation [5]. Furthermore, MBL has a major role in the modulation of inflammation but the mechanisms responsible for MBL interactions with inflammatory pathways is remain unclear [6]. Several studies suggest that, there is a modulatory role of MBL in autoimmune disease such as rheumatoid arthritis and systemic lupus erythematosus [6-8]. Previous studies show that the absence of MBL may affect occurrence of cardiovascular complications and myocardial ischemia/reperfussion injury, and CMP in MBL null animal models (9-11). Mannose Binding Lectin deficiency has been reported by three single nucleotide polymorphisms (SNPs) in codon 52, 54 and 57 of exon 1 in the MBL2 gene [6]. These SNPs are frequently referred to as variants B, C, and D (B, C, and D, denoting the substitution of aspartic acid for glysine codon 54, the substitution of gutamic acid for glycine codon 57, and the substitution of cysteine for arginine codon 52, respectively). Each of these variant alleles affect the stability of the final protein product, resulting in decreased serum levels and a dysfunctional MBL variant with a lower molecular weight than the normal MBL [6, 12].

Macrophage migration inhibitory factor is a constitutive element of the host antimicrobial defences and stress response that promotes proinflammatory function of the innate and acquired immune system. Macrophage migration inhibitory factor plays a regulator role in the immune response system and promotes proinflammatory biological activities. MIF is constitutively expressed in variety types of tissue and cells, including innate immune cells such as monocytes and macrophages [4]. Recently, it has been shown that MIF gene expression is higher in the heart with impaired glucose tolerance with cardiac dysfunction in rats, and elevated levels of MIF were associated with cardiac dysfunction in diabetic patients [13]. Mutations of the human MIF gene would predispose affected hosts to altered to sensibility or severity of inflammatory diseases such as juvenile idiopathic rheumatoid arthritis, and glomerulonephritis [4, 14, 15].

To our knowledge, no studies have investigated the possible roles of MBL2 and MIF gene polymorphisms in children with CMP. The aim of the present study is to investigate any possible association between polymorphisms of MBL2 and MIF genes and CMP in a group of Turkish children, and to investigate the association between the identified genotypes and their clinical features.

## Materials and Methods

**Patients and controls:** Twenty unrelated Turkish children with CMP, followed up in the Paediatric Cardiology Clinic of the Gaziantep University, Medical Faculty, were compared with 30 age-and sex-matched healthy controls. Relatives of CMP patients did not included as healthy controls. The diagnosis of CMP were made by signs and symptoms (irritability, feeding difficulties, weakness, fatigue, dizziness, syncope, tachypnea, tachycardia, hepatomegaly, and evidence of fluid retension), chest X ray (cardiomegaly, pulmonary venous congestion, pulmonary oedema), electrocardiography (hypertrophy of left ventricle with strain, low voltage complexes) and echocardiographic signs. Cardiomyopathies were classified according to their structural and functional abnormalities such as dilated, in the setting of reduced left ventricular systolic function; hypertrophic, in the presence of unexplained septal hypertrophy of the left ventricule; restrictive, when impaired diastolic filling with preserved systolic function and normal ventricular wall thickness [1].The study was approved by the Local Ethics Committee of the Faculty of Medicine, and informed consents were obtained from the parents of children. The medical records of all children with CMP were reviewed for information about age, sex, and to document clinical presentation including symptoms, family history, laboratory and echocardiographic findings.

**Genotyping**: All patients and controls were analyzed for codon 54 A/B (gly54asp) variation in exon 1 of MBL2 gene and -173 G/C polymorphism in MIF gene. Genomic DNA was extracted from peripheral blood samples using the salting out procedure [16].

**Genotyping of MBL2 gene codon 54 A/B:** Polymerase Chain reaction (PCR) was performed using a forward (5′-TAGGACAGAGGGCATGCTC-3′) and a reverse (5′-CAGGCAGTTTCCTCTGGAAGG-3′) primers in a 25 µl volume containing 50 ng DNA, 2 mM dNTPs, 2 nmol of each primer, 1.5 mM $MgCl_2$ and 3U Taq polymerase. The product 349 bp was digested with restriction enzyme *Ban*I (Fermentas) identify codon 54 polymorphism, respectively. BanI digestion was performed at 50 °C for 60 minutes with 5 U enzyme. After enzyme digestion, products were visualized by electrophoresis on 3% agarose gel. The BanI restriction site is present on wild type allele A and absent on variant allele B [17].

**Genotyping of MIF gene -173 G/C:** PCR was performed using a forward (5′-ACTAAGAAAGACCCGAGGC-3′) and reverse (5′-GGGGCACGTTGGTGTTTAC-3′) primers. For

MIF (-173), a 330 bp fragment was amplified, which was then digested with AluI restriction enzyme (Fermentas), overnight at 37 °C. The products were then separated on 3% agarose gel. The PCR product contains two restriction site for allele C and one of these sites is destroyed when the presence of allele G [18].

**Statistical Analysis:** All statistical analyses were performed with the Statistical Package for the Social Science for Windows (version 18.0; SPSS Inc, Chicago, IL, U.S.A.).Results are given as mean±SD, while allele frequencies and the distribution of genotype are given as %. Clinical features and MBL/MIF gene polymorphisms were compared using the chi-square and the Fisher's exact tests. Differences between groups were compared by Kruskal-Wallis variant analysis and the Mann-Whitney U-test. Statistical significance was considered at $p<0.05$. Hardy-Weinberg equilibrium (HWE) was calculated using De-finetti program [19]. Differences in allele and genotype distributions were assessed using odds ratios (ORs) and 95% confidence intervals. Sample size was estimated using a power calculation based on other studies [20]. The minimum sample size was determined as 44 person in each group at the 80% power level with an α error of 5%.

## Results

**Clinical features:** The age ranged from 3 months to 13 years (mean: 3.47 ± 3.38 years; median: 2.00-IR:3.00) in patients with CMP (n=20, 10 females/10 males). According to the echocardiographic evaluation, 80% (n=16) of the patients had dilated cardiomyopathy, 15% (n=3) hypertrophic cardiomyopathy and 5% (n=1) restrictive cardiomyopathy. All patients had clinical findings of CMP. Echocardiographic findings of CMP patients were shown in Table 1.

### Genotype frequencies of MBL2 and MIF genes

The distribution of AA, AB, and BB genotypes for MBL codon 54 were 65 % (13), 25 % (5) and 10 % (2) in CMP compared with 76.7 % (23), 23.3 % (7) and 0 % (0) in the controls. The allele frequency of A/B in MBL was 77.5 % (31), and 22.5 % (9) in CMP compared with 88.3 % (53), and 11.7 % (7) in the controls. No significant difference was found between genotypes and alleles of MBL2 gene in patients and controls ($p>0.05$).

The distribution of GG, GC, and CC genotypes for MIF (-173) were 50 %, 30 %, and 20 % in CMP compared with 56.7 %, 43.3 % and 0 % in the controls (Table 2). CC genotype was significantly higher in patient group ($p=0.0210$, Table 2). The allele frequency of G/C in MIF was 65 %, and 35 % in CMP compared with 78.3 %, and 21.7 % in the controls. The observed genotype counts were not deviated significantly from those expected according to the Hardy-Weinberg Equilibrium for MBL and MIF gene polymorphisms ($p>0.05$).

### Genotype frequencies of MBL2 and MIF genes

The distribution of AA, AB, and BB genotypes for MBL codon 54 were 65 % (13), 25 % (5) and 10 % (2) in CMP compared with 76.7 % (23), 23.3 % (7) and 0 % (0) in the controls. The allele frequency of A/B in MBL was 77.5 % (31), and 22.5 % (9) in CMP compared with 88.3 % (53), and 11.7 % (7) in the controls. No significant difference was found between genotypes and alleles of MBL2 gene in patients and controls ($p>0.05$).

The distribution of GG, GC, and CC genotypes for MIF (-173) were 50 %, 30 %, and 20 % in CMP compared with 56.7 %, 43.3 % and 0 % in the controls (Table 2). CC genotype was significantly higher in patient group ($p=0.0210$, Table 2). The allele frequency of G/C in MIF was 65 %, and 35 % in CMP compared with 78.3 %, and 21.7 % in the controls. The observed genotype counts were not deviated significantly from those expected according to the Hardy-Weinberg Equilibrium for MBL and MIF gene polymorphisms ($p>0.05$).

**Association between the identified genotypes and patients clinical/laboratory characteristics:** We investigated correlations of MBL/MIF genotypes with clinical and laboratory findings of patients such as duration of symptoms, ejection fraction (EF), fractional shortening (FS), left ventricle end diastolic diameter (LVEDD), left ventricle end systolic diameter (LVESD), left ventricle end diastolic volume (LVEDV), and left ventricle end systolic volume (LVESV). No relationship was found between MBL/MIF genotypes and these parameters (data not shown).

In children with dilated CMP (n=16), serum uric acid levels were higher in patients with MBL AA genotype ($p=0.033$, Table 3), while plasma sodium (Na) levels were higher in patients with MIF CC genotype ($p=0.042$, Table 4).

**Table 1.** Echocardiographic signs of children with cardiomyopathy (CMP).

| | Dilated CMP Mean ± SD (min-max) (n=16) | Hypertrophic CMP Mean ± SD (min-max) (n=3) | Restrictive CMP (n=1) |
|---|---|---|---|
| Ejection fraction (EF) (%) | 33.75±11.13 (19-58) | 77.00±3.60 (74-81) | 58.00 |
| Fractional shortening (FS) (%) | 15.07±5.89 (8-29) | 35.67±6.02 (30-42) | 29.00 |
| Left ventricle end diastolic diameter (LVEDD) (cm) | 4.75±1.04 (2.40-5.90) | 2.83±0.72 (2.00-3.30) | 2.40 |
| Left ventricle end systolic diameter (LVESD) (cm) | 3.98±1.07 (1.70-5.40) | 1.63±0.40 (1.20-2.00) | 1.70 |
| Left ventricle end diastolic volume (LVEDV) (ml) | 119.94±42.08 (29.60-173.00) | 32.600±17.30 (12.70-44.10) | 45.00 |
| Left ventricle end systolic volume (LVESV) (ml) | 82.15±37.53 (18.10-141.00) | 8.15±4.67 (3.36-12.70) | 22.00 |

**Table 2.** Genotype and allele frequencies of Macrophage Migration Inhibitory Factor (MIF) gene -173 G/C polymorphism in children with cardiomyopathy (CMP).

| MIF Genotype | Control n(%) | CMP Patients n(%) | Odds Ratio (95%C.I.) | p |
|---|---|---|---|---|
| GG | 17 (56.7) | 10 (50) | 0.823 (0.266 - 2.541) | 0.4793[a] |
| GC | 13 (43.3) | 6 (30) | 0.560 (0.169 - 1.858) | 0.2578[a] |
| CC | 0 (0) | 4 (20) | 16.636 (0.842 - 328.60) | 0.0210[a] |
| MIF Allele | | | | |
| G | 47 (78.3) | 26 (65) | 0.513 (0.210 - 1.256) | 0.1072 |
| C | 13 (21.7) | 14 (35) | 1.947 (0.796 - 4.761) | 0.1072 |
| HWE (p) | 0.129 | 0.127 | | |

[a]Fisher exact test, HWE: Hardy-Weinberg Equilibrium.

**Table 3.** Association with serum uric acid levels and Mannose Binding Lectin (MBL2) gene codon 54 A/B polymorphism in children with dilated cardiomyopathy.

| MBL genotypes | Serum uric acid level (mg/dL) Mean ± SD (min-max) | 95% CI | p |
|---|---|---|---|
| AA | 6,139±1,508 (4,5-8,4) | 4,979- 7,299 | 0.033 |
| AB | 3,000±0,989 (2,3-3.7) | - 5,894- 11, 894 | |

**Table 4.** Association with plasma sodium (Na) levels and Macrophage Migration Inhibitory Factor (MIF) gene -173 G/C polymorphism in children with dilated cardiomyopathy.

| MIF genotypes | Plasma Na level (mEq/L) Mean ± SD (min-max) | 95% CI | p |
|---|---|---|---|
| CC | 137,50 ±2,121 (136-139) | 118,44-156,56 | 0.042 |
| GC | 131,00±1,414 (130-132) | 118,29- 143,71 | |

## Discussion

Although the pathogenesis of CMP is not fully understood, cellular as well as humoral autoimmun responses are critically associated with the pathogenesis and progression of the disease. Furthermore, disturbances of the cellular and humoral immune system are frequently observed, and myocardial inflammation is one of the commonest mechanisms in cardiomyopathy [3]. Two of the postulated factors are; firstly, myocardial inflammation mediated by the effector cells of the immune system; and secondly locoregional effect of inflammatory mediators, released by the infiltrating lymphocytes, macrophages or endothelial cells [21]. Both MIF and MBL play several roles in innate and adaptive immune responses, and changes in levels of MBL and MIF are implicated as playing causative role in many disease states [4, 6, 22].

Better understanding of the molecular genetics underlying CMP may provide a means of early diagnosis, genotype-based therapy, and even prevention of the disease.

Mannose binding lectin deficiency is associated with susceptibility to infectious and autoimmun diseases and serum MBL levels vary substantially because of the variant alleles in exon 1 of the MBL2 gene, located on chromosome 10 in the humans [22]. In the present study, homozygosity for MBL variant allele was observed only two patients with dilated CMP and the A/A genotype was higher than the variant alleles in CMP patients, but these results were not statistically significant.

Messias-Reason et al, suggested that wild type variants of MBL2 gene was significantly higher in rheumatic hearth diseases [23]. Ramasawmy et al. reported that subjects homozygous for the wild type allele had a higher concentration of MBL than heterozygous subjects and than homozygous for the variant MBL2 alleles [24]. Schafranski et al showed that, genotypes associated with a higher level of MBL seem to represent a risk factor for the evolution of rheumatic carditis, and MBL play a substantial role in the progression of the disease to chronic form [25]. However, some studies indicated that there was either no association of MBL gene polymorphisms and systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), or even an increased risk for RA has been demonstrated in MBL insufficiency [8, 26]. In the present study, homozygosity for MBL variant allele was observed only two patients with dilated CMP and the A/A genotype was higher than the variant alleles in CMP patients, but these results were not statistically significant. These different results in literature highlight the need for further detailed studies to understand the exact role of MBL2 gene polymorphism in several diseases.

We investigated correlations of MBL genotypes with clinical and laboratory findings of the disease, and found that uric acid levels were higher in patients with MBL AA genotype than the other genotypes. Uric acid is a useful marker for the decompensation trigger in chronic heart failure, and might be related to inflammatory responses [27]. It has been shown that the left ventricular hypertrophy has an important potential to increase uric acid level [28]. Gullu et al. measured uric acid levels in idiopathic dilated cardiomyopthy, and they observed that serum uric acid levels are significantly higher in the lower coronary flow reverse group than in the higher coronary flow reverse group [29]. Interestingly, Garred et al reported that homozygous for wild type alleles in exon 1 of MBL2 gene were more likely to show evidence of persistent inflammation [30]. We suggest that the reason of increased uric acid level in our patients may be both inflammation and chronic heart failure.

Macrophage migration inhibitory factor plays an important role in the control of innate immun responses and promotes proinflammatory biological activities. Four polymorphisms of the human MIF gene (-794, -173, +254, +656) have been reported, and this polymorphisms would predispose affected hosts to altered susceptibility to or severity of inflammatory or infectious disease [4]. Patients with -173 C allele (that is, guanine-to-cytosine transition at position -173) had increased levels of MIF, and increased MIF concentrations had been associated with severe clinical manifestations, high severity scores, and poor outcome of inflammatory disease [4, 31, 32]. In the other studies, no association were found in genotype distributions of MIF -173 G/C polymorphism between ulcerative colitis, juvenile rheumatoid arthritis and healthy controls [14, 33]. However, Donn et al showed that MIF -173 C allele was associated with juvenile idiopathic arthritis [31, 32]. Moreover, MIF-173 C allele had a significantly greater number of joints with active arthritis and was associated with a poor response to glucocorticoids in patients with juvenile idiopathic arthritis [31]. Berdeli et al showed that, the MIF -173 C allele was a poor outcome predictor in JRA [14].

Miller et al. demonstrated that MIF released from ischemic cardiomyocytes stimulates adenosine monophosphate-activated protein kinase (AMPK) activation and promotes glucose uptake, and thereby protects the hearth against ischaemia reperfusion injury [34]. Jian et al found that MIF protein is constitutively expressed by cardiomyocytes in vivo and is increased in the myocardium of infants with cyanotic

cardiac defects in myocardial biopsy materials [35]. Tereshchenko et al did not reveal an association of the myocardial infarction with the MIF-173 C allele polymorphism [36]. In the present study, homozygosity for MIF-173 C allele was observed only four patients with dilated CMP. Recently, it has been shown that presence of -173C allele indicates higher MIF levels [37], and cardiac inflammation (autoimmune, viral or post viral) has an important component in the pathogenesis of dilated CMP [38]. Therefore we suggest that, CC genotype in our patients may be partially responsible from inflammation in dilated CMP, and MIF polymorphism may contribute to MIF release from cardiomyocytes in children with CMP. However, we could not find any relationship between MIF genotypes and cardiac functions. Considering the limited number of our patients, we cannot say that MIF polymorphism does not modulate cardiac functions. Further detailed studies with large patient numbers are needed for this suggestion.

It has been shown that, plasma brain natriuretic peptide (BNP) concentrations were increased in various forms of heart disease with impaired left ventricular systolic function including cardiomyopathy [39]. Natriuretic peptides inhibit the transport of sodium and water in proximal tubules and block reabsorption of sodium [40]. In this study, plasma sodium levels were higher in patients with MIF CC genotype than the other genotypes. However, we could not conclude whether CC genotype of MIF has any effect on BNP with this study. This hypothesis needs further evaluation.

## Conclusion

This study is the first to investigate the MBL and MIF gene polymorphisms in Turkish children with CMP. We conclude that CC genotype of MIF (-173) gene may be a risk factor for CMP patients. However, further studies with larger samples are needed to address the exact role of this polymorphism in CMP.

## Competing Interests

The authors have declared that no competing interest exists.

## References


1. Nugent AW, Daubeney DF, Chandras P, et al. National Australian Childhood Cardiomiyopathy Study. The epidemiology of childhood cardiomiyopathy in Australia. N Engl J Med. 2003; 348: 1639-46.
2. Lipshultz SE, Sleeper TA, Towbin JA, et al. The incidence of pediatric cardiomiyopathy in two regions of the United States. N Engl J Med. 2003; 348: 1647-55.
3. Maisch B, Richter A, Sandmoller A, BMBF – Herth Failure Network. Inflammatory dilated cardiomyopathy (DCMI). Herz 2005; 30: 535-44.
4. Renner P, Roger T, Calandra T. Macrophage migration inhibitory factor: Gene polymorphisms and suspectibility to inflammatory diseases. Clin Infect Dis. 2005; 41: 513-9.
5. Ruskamp JM, Hoekstra MO, Rovers MM, et al. Mannose-binding lectin and upper respiratory tract infections in children and adolescents. Arch Otolaryngol Head Neck Surg. 2006;132: 482-6.
6. Turner MW. The role of mannose-binding lectin in health and disease. Mol Immunol. 2003; 40: 423-9.
7. Garred P, Madsen HO, Halberg P, et al. Mannose-binding lectin polymorphisms and susceptibility to infection in systemic lupus erythematosus. Arthritis Rheum 1999; 42: 2145-52.
8. Ip WK, Lau L, Chan SY, et al. Mannose-binding lectin and rheumatoid arthritis in Southern Chinese. Arthritis Rheum. 2000; 43: 1679-87.
9. Zou C, La Bonte LR, Pavlov VI, et al. Murine hyperglycemic vasculopathy and cardiomyopathy: whole-genome gene expression analysis predicts cellular targets and regulatory networks influenced by mannose binding lectin. Front Immunol. 2012; 3:15.
10. Pavlov VI, La Bonte LR, Baldwin WM, et al. Absence of mannose-binding lectin prevents hyperglycemic cardiovascular complications. Am J Pathol. 2012; 180:104-12.
11. Busche MN, Walsh MC, McMullen ME, et al. Mannose-binding lectin plays a critical role in myocardial ischaemia and reperfusion injury in a mouse model of diabetes. Diabetologia. 2008; 51: 1544-51.
12. Schafranski MD, Ferrari LP, Scherner D, et al. High-producing MBL2 genotypes increase the risk of acute and chronic carditis in patients with history of rheumatic fever. Mol Immunol. 2008; 45: 3827-31.
13. Yu XY, Chen HM, Liang JL, et al. Hyperglycemic myocardial damage is mediated by proinflammatory cytokine: macrophage migration inhibitory factor. PLoS One. 2011; 25:e16239.
14. Berdeli A, Ozyürek AY, Ulger Z, et al. Association of macrophage migration inhibitory factor gene -173G/C polymorphism with prognosis in Turkish children with juvenile rheumatoid arthritis. Rheumatol Int. 2006; 26: 726-31.
15. Lan HY, Yang N, Nikolic- Paterson DJ, et al. Expression of macrophage migration inhibitory factor in human glomerulonephritis. Kidney Int. 2000; 57: 499-509.
16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16: 1215.
17. Vardar F, Pehlivan S, Onay H, et al. Association between mannose binding lectin polymorphisms and predisposition to bacterial meningitis. Turk J Pediatr. 2007; 49: 270-3.
18. Akcali A, Pehlivan S, Pehlivan M, et al. Association of macrophage migration inhibitory factor gene promoter polymorphisms with multiple sclerosis in Turkish patients. J Int Med Res. 2010; 38: 69-77.
19. [Internet] Strom TM, Wienker TF. Hardy-Weinberg equilibrium online analysis program. http:/ihg.gsf.de/cgi-bin/hw/hwa2.pl
20. Col-Araz N, Pehlivan S, Baspinar O, et al. Association of macrophage migration inhibitory factor and mannose-binding lectin-2 gene polymorphisms in acute rheumatic fever. Cardiol Young. 2012; [Epub ahead of print].
21. Pankuweit S, Ruppert V, Maisch B. Inflammation in dilated cardiomyopathy. Herz 2004; 29: 788-93.
22. Worthley DL, Bardy PG, Mullighan CG. Mannose-binding lectin: biology and clinical implications. Intern Med J. 2005; 35: 548-55.
23. Messias Reason LJ, Schafranski MD, Jensenius JC, et al. The association between mannose-binding lectin gene poymorphism and rheumatic hearth disease. Hum Immunol. 2006; 67: 991-8.
24. Ramasawmy R, Spina SG, Fae KC, et al. Association of mannose-binding lectin gene polymorphism but not of mannose-binding serine protease 2 with chronic severe aortic regurgitation of rheumatic etiology. Clin Vaccine Immunol. 2008; 15: 932-6.
25. Schafranski MD, Ferrari LP, Scherner D, et al. High-producing MBL2 genotypes increase the risk of acute and chronic carditis in patients with history of rheumatic fever. Mol Immunol. 2008; 45: 3827-31.
26. Horiuchi T, Tsukamoto H, Morita C, et al. Mannose-binding lectin (MBL) gene mutation is not a risk factor for systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Japanese. Genes Immun. 2000; 1: 464-6.
27. Leyva F, Anker SD, Godsland IF, et al. Uric acid in chronic heart failure: A marker of chronic inflammation. Eur Hearth J. 1998; 19: 1814-22.
28. Kurata A, Shigematsu Y, Higaki J. Sex-related differences in relations of uric acid to left ventricular hypertrophy and remodeling in Japanese hypertensive patients. Hypertens Res. 2005; 28: 133-9.
29. Gullu H, Erdogan D, Caliskan M, et al. Elevated serum uric acid levels impair coronary microvascular function in patients with idiopathic cardiomiyopathy. Eur J Hearth Fail. 2007; 9: 466-8.
30. Garred P, Madsen HO, Marquarth H, et al. Two edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study. J Rheumatol. 2000; 27: 26-34.

31. De Benedetti FD, Meazza C, Vivarelli M, et al. The British Paediatric Rheumatology Study Group. Functional and prognostic relevance of the -173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2003; 48: 1398-407.
32. Donn R, Alourfi Z, De Benedetti F, et al. The British Paediatric Rheumatology Study Group. Mutation screening of the macrophage migration inhibitory factor gene. Positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. Arthritis Rheum. 2002; 46: 2402-9
33. Nohara H, Okayama N, Inoune N, et al. Association of the 173G/C polymorphism of the macrophage migration inhibitory factor gene with ulserative colitis. J Gastroenterol. 2004; 39: 242-6.
34. Miller EJ, Li J, Leng L, et al. Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic hearth. Nature 2008; 451: 578-82
35. Jian Z, Li JB, Ma RY, et al. Increase of macrophage migration inhibitory factor (MIF) expression in cardiomyocytes during chronic hypoxia. Clin Chim Acta. 2009; 405:132-8.
36. Tereshcenko IP, Petrkova J, Mrazek F, et al. The macrophage migration inhibitory factor (MIF) gene polymorphism in Czech and Russian patients with myocardial infarction. Clin Chim Acta. 2009; 402: 199-202.
37. Xie Q, Wang SC, Bian G, et al. Association of MIF -173G/C and MBL2 codon 54 gene polymorphisms with rheumatoid arthritis: a meta-analysis. Hum Immunol. 2012; [Epub ahead of print]
38. Pankuweit S, Ruppert V, Maisch B. Inflammation in dilated cardiomyopathy. Herz 2004; 29: 788-93.
39. Bielecka-Dabrowa A, Wierzbicka M, Dabrowa M, et al. New methods in laboratory diagnostics of dilated cardiomiyopathy. Cardiol J. 2008; 15: 388-95.
40. Bettencourt PM. Clinical usefulness of B-type natriuretic peptide measurement: present and future perspectives. Hearth 2005; 91: 1489-94.

EXPERT REVIEWS

# Nutritional and micronutrient determinants of idiopathic dilated cardiomyopathy: diagnostic and therapeutic implications

*Expert Rev. Cardiovasc. Ther.* 9(9), 1161–1170 (2011)

Victor Marinescu[†1] and Peter A McCullough[2]

[1]*Department of Medicine, William Beaumont Hospital, Royal Oak, MI 48073, USA*
[2]*St John Providence Health System, Providence Park Heart Institute, Novi, MI 48374, USA*
[†]*Author for correspondence:*
*Tel.: +1 248 885 4197*
*Fax: +1 248 453 5879*
*victor.marinescu@beaumont.edu*

Idiopathic dilated cardiomyopathy (IDCM) is the term used to describe a group of myocardial diseases of unknown cause whose common clinical presentation is heart failure. The prevalence of IDCM is estimated to be between 7 and 13% of patients with systolic heart failure. Throughout medical history, several nutrient-deficient states have been identified as the root cause of IDCMs, Keshan's disease being one such example, where selenium deficiency-induced heart failure is now well documented. This raises the question of whether a micro- or macro-nutrient imbalance can provide the milieu for inefficient energy expenditure and cardiac metabolism in the context of IDCMs, either causing or exacerbating the condition. To date, there is insufficient evidence in the literature to support this theory, although numerous studies suggest a link between nutrient deficiencies, inefficient energy expenditure and subsequent heart failure. Given the unique metabolic needs of the failing heart, the role of micronutrient testing and supplementation in IDCMs warrants further well-designed studies.

B4

 10.1586/ERC.11.95  ISSN 1477-9072 

# B4

# B4

B4

# B4

B4

# B4

B4

# B4

B4