Mycotoxins and the pet food industry: Toxicological evidence and risk assessment

2 authors, including:

Herman J Boermans
University of Guelph
103 PUBLICATIONS   **2,174** CITATIONS

Some of the authors of this publication are also working on these related projects:

Project   Environmental immunotoxicology View project

Mycotoxins and the pet food industry: Toxicological evidence and risk assessment

Herman J. Boermans [a,*], Maxwell C.K. Leung [b,1]

[a] Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada N1G 2W1
[b] Department of Animal and Poultry Science, Ontario Agricultural College, University of Guelph, Guelph, ON, Canada N1G 2W1

Abstract

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. The concepts of "risk assessment", using hazard identification, dose–response assessment, no observable adverse effect level (NOAEL), and lowest observed adverse effect level (LOAEL), should be applied to assess the risk and safety of mycotoxins in pet food, thereby instilling public confidence in the pet food industry.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Mycotoxin; Toxicology; Pet food; Risk assessment and safety

1. Introduction

Mycotoxin contamination in pet food poses a serious health threat to pets. Cereal grains and nuts are used as ingredients in commercial pet food for companion animals such as cats, dogs, birds, fish, reptiles and rodents. Cereal by-products may be diverted to animal feed even though they can contain mycotoxins at concentrations greater than raw cereals due to processing (Moss, 1996; Brera et al., 2006). Several mycotoxin outbreaks in commercial pet food have been reported in the past few years (Garland and Reagor, 2001; Stenske et al., 2006). Most outbreaks of pet mycotoxicosis, however, remain unpublished and may involve the death of hundreds of animals (MSNBC News Services, 2006).

The term "companion animal" implies the existence of a strong human–animal bond between pets and their owners (Adams et al., 2004). A pet is often regarded as a family member by its owner and a person may develop strong relationships with animals throughout his or her lifetime. Pet interactions and ownership have been associated with both emotional and physical health

benefits (Milani, 1996; Adams et al., 2004). The human–animal bond has resulted in over sixty four million American households in owning one or more pets, thereby creating a huge market for the pet food industry (APPMA, 2006). Dogs and cats continue to be the most popular pet to own, found in at least one out of three US households. The health problems of pets, are therefore more of an emotional concern as compared to a mainly financial concern in farm animals (Dunn et al., 2005; Milani, 1996).

2. Surveys of mycotoxins in pet food and outbreaks of mycotoxicoses in pets

There have been several published mycotoxin surveys of commercial pet foods (Table 1). Aflatoxins, ochratoxins, trichothecenes, zearalenone and fumonisins were detected in food for dogs, cats, birds, rodents, fish and horses with different prevalences across regions. Wild bird seed, for instance, has been found to be most contaminated among different pet food products (Henke et al., 2001).

There has been a recent review of individual cases involving mycotoxins in pets (Leung et al., 2006). Aflatoxins have been the most common cause of acute mycotoxin outbreaks in commercial dog food (Table 2). Corn is the usual source of aflatoxins in these cases. An ochratoxicosis outbreak was also reported in Korea with 3 known deaths. These available reports of acute mycotoxicosis,

* Corresponding author. Tel.: +1 519 824 4120x54984.
  E-mail addresses: hboerman@uoguelph.ca (H.J. Boermans),
chunkit@uoguelph.ca (M.C.K. Leung).
  [1] Tel.: +1 519 824 4120x56446.

Table 1
Some published mycotoxin surveys of commercial pet food

Location	Pet food surveyed	Mycotoxins detected	Reference
United Kingdom	100 pet food samples – 35 dry dog foods – 35 dry dog cat foods – 15 domestic bird foods –15 wild bird foods	Mycotoxins were detected in 16% of the samples, including 7 of 30 bird foods tested – Aflatoxin B_1: 2 samples with 2.1 and 370 μg/kg, respectively – Ochratoxin A: 10% of the samples; 1–7 μg/kg – Fumonisins B_1: 30% of the samples; 90–690 μg/kg	Scudamore et al. (1997)
Brazil	100 pet food samples –45 dog foods –25 cat foods –30 bird foods	– Aflatoxins were found in 6.7%, 4.0%, and 26.7 % of the dog, cat and bird food samples – Aflatoxin B_1 level of 19, 16 and 110 μg/kg, respectively.	Maia et al. (2002)
Portugal	60 dry pet food samples – 20 dog foods – 20 cat foods – 20 domestic bird foods	Mycotoxins were only detected in dog food – Aflatoxins: not detected – Ochratoxin A: 5 samples, 2.0–3.6 μg/kg – Fumonisin B_1: 3 samples 12.0–24.0 μg/kg – Deoxynivalenol: 3 samples, 100.0–130.0 μg/kg	Martins et al. (2003)
United States	142 wild bird seed samples	– Aflatoxin concentrations ranged from 0 to 2780 μg/kg. – 17% of the samples had aflatoxin concentrations greater than 100 μg/kg	Henke et al. (2001)
Brazil	123 pet food samples – 46 dog – 19 cat – 6 hamster – 26 horse – 3 bird – 3 rabbit	All samples contained one or more analyzed mycotoxin aflatoxins, ochratoxin, fumonisins, or zearalenone – 6 (4.9%) and 19 (15.5%) of the samples had more than 50 μg/kg of aflatoxins and zearalenone respectively.	Scussel et al. (2006)

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.

3. Effects of mycotoxins on pet species

3.1. Aflatoxins

Aflatoxins are commonly found in corn, peanuts, cottonseed, milk, and tree nuts (Haschek et al., 2002). Aflatoxins B_1, B_2, G_1 and G_2 are four naturally-occurring forms of aflatoxins, with aflatoxin B_1 being the most potent, prevalent and carcinogenic (Puschner, 2002; IARC, 1993). After ingestion, aflatoxins are

absorbed and carried to the liver via the circulatory system. They are then converted by the liver into toxic reactive epoxides which bind covalently to intracellular macromolecules such as DNA, RNA and protein enzymes, resulting in damage to liver cells (Cullen and Newberne, 1994). 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 (Bohn and Razzai–Fazeli, 2005). 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

Table 2
Some major mycotoxin outbreaks in pet animals

Year	Location	Scale	Diet	Reference
1951	Southeastern United States	71 food poisoning cases of dogs (with several dead)	A brand of commercial dog food suspected to be made with contaminated corn	Bailey et al. (1959)
1987	Pretoria, South Africa	10 dogs dead with 1 acute, 7 sub-acute and 2 chronic cases	A brand of contaminated commercial dog food	Bastianello et al. (1987)
1998	United States	55 dogs dead with both acute and chronic cases	17 different formulations of commercial dog food made with two rail cars of non-uniformly contaminated corn in a milling plant in Texas in late-summer	Garland and Reagor (2001)
2005	United States	At least 100 dogs dead	19 different formulations of commercial dog food made with contaminated corn in a milling plant in South Carolina in summer	Stenske et al. (2006)
2006	Korea	3 dogs dead with renal failure	Fungal nephrotoxins in the diet, possibility ochratoxin and citrinin	Jeong et al. (2006)

much lower than the acute dose. Newberne and Wogan (1968) have experimentally induced malignant tumors in rats with a continual exposure of <1 mg aflatoxin B$_1$/kg feed. 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.

3.2. 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. There are four ochratoxin homologues — A, B, C and D. Ochratoxin A (OTA) is the most prevalent and, together with ochratoxin C, most toxic. Initial symptoms of ochratoxicosis observed in all species include anorexia, polydipsia, polyuria and dehydration, and are associated with renal damage.

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. This results in the degeneration of the proximal tubules and interstitial fibroses (Krogh, 1992). OTA is also known to bind with DNA molecules and induce renal tumors in animal models, although its carcinogenic mechanism remains controversial (Faucet et al., 2004; Mally et al., 2004).

Dogs show a high susceptibility to OTA, for example, a daily dose of 0.2 mg OTA/kg BW for 2 weeks or a single dose of 7.8 mg OTA/kg BW was fatal to young beagle dogs (Szczech et al., 1973). Clinical symptoms of the OTA poisoning included anorexia, weight loss, vomiting, tenesmus, bloody diarrhea, increased body temperature, tonsillitis, dehydration, and prostration. These findings were confirmed by a later study in which dogs showed similar symptoms at OTA doses between 0.2 and 3.0 mg/kg BW (Kitchen et al., 1977).

3.3. Trichothecenes

Trichothecenes are a family of *Fusarium* mycotoxins commonly found in corn, wheat, barley, as well as oats worldwide (Haschek et al., 2002). Over 150 trichothecenes have been identified to date, including deoxynivalenol (DON), nivalenol, diacetoxyscirpenol (DAS) and T-2 toxin (Pittet, 1998). Although not as toxic as DAS and T-2 toxin, DON is the most important and widespread trichothecene in cereal grains and animal feedstuffs, causing major economic loss in animal industries (Rotter et al., 1996). Trichothecenes are potent irritants and inhibitors of protein and DNA synthesis which interferes with cellular metabolic activities, ultimately leading to cell death. Rapidly dividing cells are particularly sensitive with the gastrointestinal and immune systems primarily affected in exposed animals. Typical clinical signs of trichothecene toxicosis include loss of appetite, vomiting, diarrhea, gastrointestinal hemorrhage, ataxia, and immune suppression (Smith, 1992).

The influence of trichothecenes on feed intake has been widely studied in a number of animals, including dogs. Hughes et al. (1999) investigated the feed refusal effect of DON on Brittany and Beagle dogs and cats in a two-week clinical feeding trial. It was estimated that 4.5 mg DON/kg pet food produced a significant decrease in feed intake, while vomiting was observed with the exposure of 8 mg DON/kg pet food. Cats were slightly more resistant than dogs. In a recent study, Leung et al. (in press) showed that the exposure of 3 mg DON/kg pet food caused significant weight loss and feed intake reduction in Beagle dogs. The study by Hughes et al. (1999) also showed that the dogs previously exposed to DON were able to preferentially select the uncontaminated diet.

Feed refusal and vomiting, the most notable behavioral effects of trichothecenes, are possibly related to their influences on brain regional neurochemistry and feed intake regulation. There is strong evidence that serotonergic and α2-noradrenergic systems interact antagonistically through medial hypothalamic satiety mechanisms (Leibowitz and Shor-Posner, 1986). In a recent comparative study (Swamy et al., 2004), swine fed with trichothecene-contaminated grains showed a decrease in norepinephrine concentration and increases in serotonergic neurotransmitter concentrations, possibly affecting the antagonistic interaction between these two systems on feed intake. On the other hand, broiler chickens showed increases in both norepinephrine and serotonin concentrations and the antagonistic interaction between the two systems was therefore maintained. This comparison accounts for the species differences in *Fusarium* mycotoxin susceptibility. Similar mechanistic studies have not been performed in pet species.

The effects of trichothecenes are especially obvious in rapidly dividing cells of the intestinal mucosa and bone marrow. A single oral administration of T-2 toxin was sufficient to induce cell death in the intestinal crypt epithelium at a dose of 2.5 mg T-2/kg BW. A dose of 25 mg/kg BW caused extensive damage to intestinal villi (Li et al., 1997; Haschek et al., 2002). In another study, Coppock et al. (1989) administrated an intravenous dose of 0.5 mg DAS/kg BW to dogs and examined their hematological changes in the following 8 hours. Damage to bone marrow haematopoietic elements and an increase in deformed blood cells were observed. The vulnerability of gastrointestinal and immune systems to trichothecene toxicity accounts for the symptoms of gastrointestinal distress, bloody diarrhea and immune suppression in trichothecene poisoning.

3.4. Zearalenone

Zearalenone is an estrogenic *Fusarium* mycotoxin found in various cereal crops, most frequently in corn (Cheeke, 1998), causing hyperestrogenism and reproductive problems in all animal species, especially pigs (Bohn and Razzai–Fazeli, 2005). Low doses of zearalenone (∼ 1 mg/kg feed) may cause infertility, affecting ovulation, conception, implantation, fetal development, and the newborn's viability (Schweighardt, 1980; Price et al., 1993). Zearalenone has structural similarities to estrogen. It can bind to cytosolic estrogen receptors in target cells (Riley and Norred, 1996). The resultant receptor–zearalenone complex activates the transcription of estrogen-responsive genes, causing the translation of new proteins and expressing estrogenic effects upon the target cells. Most clinical signs of zearalenone poisoning are related to the hyperstimulation of estrogen-dependent tissues.

Both male and female dogs are affected by zearalenone toxicity. Hidy et al. (1977) gavaged dogs with 5 mg zearalenone/kg BW/day for 13 weeks. Reduced number of corpora lutea and arrested spermatogenesis were observed in female and male dogs, respectively. Although the dose used by Hidy et al. (1977) was much higher than normal dietary exposure, some recent studies suggested that low levels of zearalenone exposure can also produce significant toxic effects. Female dogs fed 200 μg zearalenone/kg BW/day for 7 days showed cell damage in ovaries; and edema and hyperplasia in oviducts and the uterus (Gajecka et al., 2004a). Dogs also demonstrated a reduction in serum antibodies and white blood cell levels after a 50-day dietary exposure to 25 and 50 μg zearalenone/kg BW/day (Gajecka et al., 2004b). Uterine edema and hyperplasia leads to pyometra, an infection of the uterus, a disease common in female dogs.

3.5. Fumonisins

Fumonisins are found in corn throughout the world with more than 15 homologues isolated, including fumonisin B_1, B_2, B_3, B_4, A_1 and A_2 (Rheeder et al., 1992; Haschek et al., 2002; Scott, 1993). Fumonisins B_1, B_2 and B_3 (FB_1, FB_2 and FB_3) are the most prevalent members and FB1 represents up to 70% of food-borne fumonisins. Fumonisins are poorly absorbed from the gut and can be inactivated by the gut microflora resulting in large species differences in susceptibility. Once they enter the blood circulation, fumonisins damage numerous organs in all species studied. These mycotoxins are structurally similar to sphingoid bases, inhibiting the activity of ceramide synthase to produce sphingolipids. The disruption of sphingolipid synthesis and metabolism in cells sequentially causes cell damage, apoptosis, necrosis and compensatory hyperplasia (Haschek et al., 2002; Wang et al., 1991). Since fumonisins also inhibit the function of L-type calcium channels, the cardiovascular system is also disturbed. While the liver and kidney are highly susceptible organs to acute fumonisin toxicity, the immune system is mainly affected in chronic fumonisin exposure.

The clinical effects of fumonisins vary between animal species but most toxicology investigations are done on species other than pets. Voss et al. (1998) reported an increased rate of hepatic and renal lesions in rats after a three-week dietary exposure to 32–49 mg FB_1/kg feed. The oral exposure of 1.75 mg FB_1/kg BW/day was found lethal in rabbits, resulting in renal and hepatic toxicity, leukoencephalomalacia, and cerebral hemorrhage (Bucci et al., 1996). Horses develop leukoencephalomalacia with a minimal dose of 5 mg FB_1/kg feed (Haschek et al., 2002). In a sublethal fumonisin poisoning study, pigs fed 20 mg FB_1/kg BW over 7 days showed the inhibition of pulmonary intravascular macrophage (Smith et al., 1996). In poultry, a relatively mycotoxin-resistant species, a 21-day dietary exposure of 200 mg FB_1/kg feed was found to reduce feed intake, weight gain as well as the relative weights of the liver, kidney, pancreas and proventriculus in newborne ducklings (Bermudez et al., 1995).

3.6. Fusaric acid

Fusaric acid is a potent inhibitor of dopamine-β-hydroxylase, suppressing norepinephrine synthesis in the brain. In addition to the hypotensive and vomiting effects, fusaric acid also increases brain serotonin concentrations in a manner similar to other *Fusarium* mycotoxins, such as DON and T-2 toxin, and therefore this mycotoxin interaction increases overall toxicity of natural mixtures of these mycotoxins. Since fusaric acid is widely produced by *Fusarium* fungi, it is possibly the most common *Fusarium* mycotoxin found in animal feedstuffs (Bacon et al., 1996).

The overt toxicity, immunotoxicity and hypotensive effects of fusaric acid on dogs were demonstrated by some early clinical feeding trials. A series of experiments were conducted by Matsuzaki et al. (1976a,b) who fed young Beagle dogs 50 mg fusaric acid calcium salt (FA-Ca)/kg BW for 30 days and 6 months, respectively. Low appetite, vomiting, suppressed weight gain and hypotension were observed. A six-month exposure of 100 mg FA-Ca/kg BW was lethal to the Beagles, causing significant hypotension and gastrointestinal, hepatic and pneumonic bleeding. Daily doses of 20 mg FA-Ca/kg BW had, however, no effect in general to the Beagles despite a six-month dietary exposure.

4. Multiple simultaneous mycotoxin exposure

Multiple mycotoxin exposure is common in the natural situation. There are four categories describing possible mycotoxin interactions (Eaton and Klaassen, 2001).

(1) Additivism — the combined effects of the mycotoxins is equal to the sum of each of the mycotoxins given alone.
(2) Synergism — the combined effects of the mycotoxins are much greater than the sum of the effects of each mycotoxin alone.
(3) Potentiation — one mycotoxin has no toxic effect on an organ but when added to another mycotoxin makes that mycotoxin much more toxic.
(4) Antagonism — two mycotoxins administered together interfere with each other's actions reducing the toxic effect.

Chemical interactions occur due to alterations in absorption, protein binding, biotransformation, and excretion. Mycotoxins that have a common site of action present the greatest opportunity for a cumulative toxic effect. A toxicological potentiation between DON and fusaric acid, for instance, has been demonstrated in 8 kg piglets where DON toxicity was augmented when fusaric acid was added in diet (Smith et al., 1997). In another study, it was found that chickens fed combinations of DON and T-2 toxin from hatching to three weeks had significantly reduced body weight gain (Kubena et al., 1990). This variable was not reduced, however, when either DON or T-2 toxin were fed singly, thereby suggesting a synergistic interaction. The toxicity of a particular mycotoxin, therefore, depends on not only its own concentration but also the presence of other mycotoxins.

5. Determination of risk and safety

"Risk assessment" is the systematic scientific characterization of potential adverse effects resulting from exposure to hazardous agents (NRC, 1993; Faustman and Omenn, 2001). Risk is the

probability that a substance will produce a toxic effect. Risk involves two components: toxicity and exposure. Thus mycotoxins of relatively low toxicity may pose significant risks if exposure is great, frequent, and long. Conversely, mycotoxins of high toxicity, such as aflatoxins, may pose virtually no risk if exposure can be substantially reduced. "Exposure assessment" determines what type, levels, and duration of exposures are expected. Although the exposure of pet animals to mycotoxins in grain-based pet food is generally low, it is unavoidable and occurs throughout the entire lifespan of the animal.

The toxicity of a substance is dependent on its chemical, physical and biological properties. Often referred to as "hazard", toxicity is an inherent property of the compound and the animal being exposed (Faustman and Omenn, 2001). The objectives of classical mammalian toxicity studies developed for risk assessment are as follows:

(1) Hazard identification — determine the kinds of adverse effects
(2) Dose–response assessment — determine the potency or sensitivity of effects
(3) No observable adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL).

Toxicological information on mycotoxins is important because it allows us to judge the relative risk that may result from exposure to these toxic substances. Today's short-term repeat dose toxicity testing is used to derive symptoms as the main objective with mortality as a secondary objective (Paine and Marrs, 2000). From this data, LD_{50} can be calculated as an indicator of acute response but LD_{50} alone gives little information on chronic response. Sub-chronic toxicity studies of 90 day exposures are used to determine the chemical dose an animal can consume daily without any demonstrable effect (NOAEL), and to characterize the effects of the chemical when administered at doses above the NOAEL. Chronic toxicity studies measure the effect of doses below the NOAEL on the normal life span of the animal. Chronic studies are often used to determine if the substance causes "delayed" effects on reproduction, development or cancer. One dose in chronic studies should cause subtle signs of toxicity such as reduced weight gain or a minor physiological response. This dose is defined as the "lowest adverse effect level" (LOAEL). These classical toxicity studies using dosages in both effects and no effect range are designed to derive data to be applied to risk determination.

Toxicity testing has made great strides, ever increasing our ability to detect sensitive toxic endpoints. Routine haematology, blood biochemistry, histology, and cytology are being supplemented by sophisticated diagnostic equipment including ultrasound imaging, magnetic resonance imaging (MRI), and electron microscopy. New technologies are extremely sensitive at detecting effects at sub-clinical dosages. Molecular techniques (e.g. DNA Microarray) detect alterations at the molecular level and help elucidate modes of action. Toxic endpoints should, however, have a level of clinical significance. What should be the most sensitive toxicological parameter may soon have to be answered.

Most mycotoxin research has been designed to investigate toxic effects and therefore dosages used are in the toxic range. In such experiments the lowest experimental dose causing a toxic effect may be far greater than the threshold of the adverse effect and therefore overestimates the true LOAEL (Fig. 1). Furthermore, if an experimental dose falls into the no-effect range, it may be far below the threshold dose and therefore greatly underestimates the true NOAEL. These factors introduce variability and uncertainty in the estimation of NOAEL and LOAEL (USEPA, 1995).

As for all risk assessments, pet health risk assessment requires data on toxicity and exposure. Pet species are seldom used for toxicity studies and therefore data obtained from other species are used in the risk assessment for pets. This results in a level of uncertainty when extrapolating toxicity data from experimental animals to pet species (Faustman and Omenn, 2001).

The process of human health risk assessment (Covello and Merkhofer, 1993) can be applied to the risk determination in animals. In order to estimate the risk associated with mycotoxin exposure, we need to determine the dose a pet can consume in the food on a daily basis for their entire life with no adverse effect (i.e. NOAEL). This level can then be divided by an appropriate safety factor. Safety Factors (SF) (i.e. uncertainty factors) are numerical values applied to the NOAEL or other effect levels to account for any uncertainty in the data. The quotient derived is referred to as
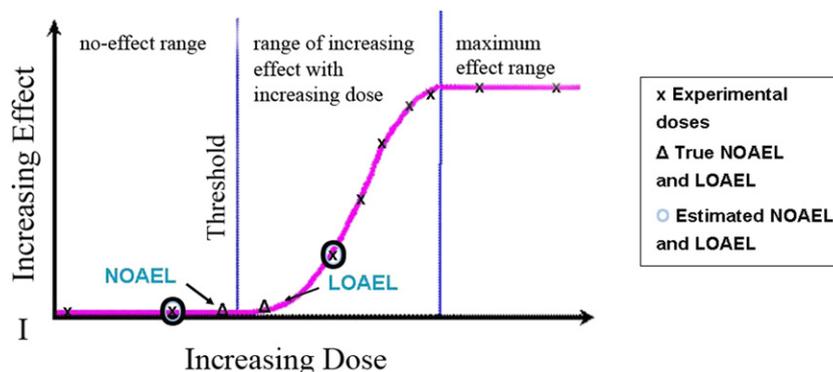

Fig. 1. Illustration of the calculation of the NOAEL and LOAEL using a dose–response curve.

the acceptable daily intake or ADI. A pet ADI could be determined as follows:

$$ADI(\text{mg mycotoxin/kg BW}) \text{ pet specific} = \frac{NOAEL_{Rodent}}{SF_{10}}.$$

Uncertainty includes species extrapolation, the nature and severity of effect, differences in pet breeds, and variability in LOAEL estimation. SF's could be adjusted according to epidemiological data on pets. Human SF numbers are often selected as a factor of 10, 100, or 1000 (Table 3) and set by the World Health Organization/Food and Agriculture Organization (WHO/FAO). Pet food SF's could be set by the pet food industry to apply standard safety guidelines.

This process of combining the qualitative and quantitative aspects of toxicity and exposure to derive a quantitative level of risk is called "risk characterization" (Faustman and Omenn, 2001). To complete the process a safe pet dietary level (SPDL) can be determined using a pet specific food factor (FF). An example calculation is given for aflatoxin:

LOAEL$_{Dog}$ 1.2 µg/kg BW (hepatotoxicity) SF: 10
                (severity of effect)
Food factor 20 g/kg BW
Average BW 10 kg BW

$$SPDL = \frac{1.2 \ \mu g/kgBW \times 10 \ kgBW}{10 \times (20 \ g/kg \ BW \times 10 \ kg \ BW)}$$
$$= 6 \ \mu g \ \ aflatoxin/kg \ pet \ food.$$

The food factor is the amount of food consumed daily and accounts for the differences in the quantity of food consumed by different animal species. A safe pet dietary level would be equivalent to the human maximal permissible (tolerance) level in foods (MPL).

Risk management refers to the process by which policy actions are chosen to control hazards identified in the risk assessment/risk characterization processes (Faustman and Omenn, 2001; Covello and Merkhofer, 1993). Calculation of SPDL would provide producers with a pet specific maximal permissible (tolerance) level of mycotoxin in food. Managers would consider scientific evidence and risk estimates along with processing, engineering, economic, social and political factors

in evaluating alternative options. This comprehensive approach would instil public confidence in the pet food industry.

6. Regulatory issues and preventative strategies

Considering that the intrinsic toxicological properties of a chemical cannot be altered, regulatory agencies consider exposure mitigation the only meaningful opportunity for risk reduction (NRC, 1993). Government regulations of mycotoxin contamination, however, are often compromised by the analytical detection limits, regional prevalence, as well as trade relationships amongst different countries instead of fulfilling the scientific approach of risk assessment and safety determination (Leung et al., 2006). Scientifically based regulations for the acceptable limit of mycotoxins in pet food would be beneficial. Strict regulations, however, would create greater competition with the human food chain resulting in increased pet food costs and decreased industry profits. It is also possible that the avoidance of severe regulations will promote mycotoxin outbreaks.

Pet food amelioration is often considered a practical solution for mycotoxin contamination. Food processing techniques such as sieving, washing, pearling, ozonation, and acid-based mold inhibition can reduce the mycotoxin content of cereal grains (Leung et al., 2006). Dietary supplementation with large neutral amino acids, antioxidants, and omega-3 polyunsaturated fatty acids as well as inclusion of mycotoxin-sequestering agents and detoxifying microbes may ameliorate the harmful effects of mycotoxins in contaminated pet food. Amelioration of pet food, however, should be used as an additional safety factor but not to replace the sound application of risk and safety determination.

The public has recently begun a shift to organic pet foods. The public perception is that organic foods are safer due to the lack of pesticide residues. In the case of mycotoxins, however, the avoidance of insecticides and fungicides may result in increased crop pest damage, fungal growth and mycotoxin production.

7. Conclusion

Mycotoxin contamination in pet food poses a serious health threat to pets. The health problems of pets are of a highly emotional concern and pet food safety is the responsibility of the pet food industry. Risk and safety determination is needed and must address many issues including sensitivity of toxic endpoints, multiple mycotoxin exposure, and pet food amelioration. More pet specific research is needed to better address the pet mycotoxin problem.

Table 3
US EPA 1995 guidelines for Safety Factors (SF) in assessing chemical risks in humans and possible values for pets

From	To	Human SF	Pet SF
Average	Sensitive individual	=10	5
Research animal	Human or Pet	=10	10
LOAEL	NOAEL	=10	5
Sub-chronic	Chronic	=10	5
LD50	NOEL		1000
Data Base *		0–10	0–5

* Uncertainties include data inadequacies, inconsistent finding across species, multiple variable health effects, reversibility of effects, and mechanism of effects across species.

References

Adams, C.L., Conlon, P.D., Long, K.C., 2004. Professional and veterinary competencies: addressing human relations and the human–animal bond in veterinary medicine. J. Vet. Med. Educ. 31, 66–71.

American Pet Products Manufacturers Association (APPMA). National Pet Owners Survey 2005/2006. Greenwich, USA.

Bacon, C.W., Porter, J.K., Norred, W.P., Lesile, U., 1996. Production of fusaric acid by *Fusarium* species. Appl. Environ. Microbiol. 62, 4039–4043.

Bailey, W.S., Groth, A.H., 1959. The relationship of hepatitis X in dogs and moldy corn poisoning of swine. J. Am. Vet. Med. Assoc. 134, 483–486.

Bastianello, S.S., Nesbit, J.W., Williams, M.C., Lange, A.L., 1987. Pathological findings in a natural outbreak of aflatoxicosis in dogs. Onderstepoort J. Vet. Res. 54, 635–640.

Bermudez, A.J., Ledoux, D.R., Rottinghaus, G.E., 1995. Effects of *Fusarium moniliforme* culture material containing known levels of fumonisin B_1 in ducklings. Avian Dis. 39, 879–886.

Bohn, J., Razzai–Fazeli, E., 2005. Effects of mycotoxins on domestic pet species. In: Diaz, D. (Ed.), The Mycotoxin Blue Book. Nottingham University Press, Nottingham, UK, pp. 77–92.

Brera, C., Catano, C., de Santis, B., Debegnach, F., de Giacomo, M., Pannunzi, E., Miraglia, M., 2006. Effect of industrial processing on the distribution of aflatoxins and zearalenone in corn-milling fractions. J. Agric. Food Chem. 54, 5014–5019.

Bucci, T.J., Hansen, D.K., LaBorde, J.B., 1996. Leukoencephalomalacia and hemorrhage in the brain of rabbits gavaged with mycotoxin fumonisin B_1. Nat. Toxins 4, 51–52.

Cheeke, P.R., 1998. Natural Toxicants in Feeds and Poisonous Plants, 2nd ed. Interstate Publishers, Danville, United States, pp. 116–122.

Coppock, R.W., Hoffmann, W.E., Gelberg, H.B., Bass, D., Buck, W.B., 1989. Hematologic changes induced by intravenous administration of diacetoxyscirpenol in pigs, dogs, and calves. Am. J. Vet. Res. 50, 411–415.

Covello, V.T., Merkhofer, M.W., 1993. Risk Assessment Methods. Approaches for assessing health and environmental risk.

Cullen, J.M., Newberne, P.M., 1994. Acute Hepatotoxicity of Aflatoxins, In The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance. In: Eaton, D.L., Groopman, J.D. (Eds.), Academic Press, San Diego, CA, pp. 3–26.

Dunn, K.L., Mehler, S.J., Greenberg, H.S., 2005. Social work with a pet loss support group in a university veterinary hospital. Soc. Work Health Care 41, 159–170.

Eaton, D.L., Klaassen, C.D., 2001. Principles of Toxicology. In: Klaassen, C.D. (Ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, USA, pp. 11–34.

Faucet, V., Pfohl-Leszkowicz, A., Dai, J., Castegnaro, M., Manderville, R.A., 2004. Evidence for covalent DNA adduction by ochratoxin A following chronic exposure to rat and subacute exposure to pig. Chem. Res. Toxicol. 17, 1289–1296.

Faustman, E.M., Omenn, G.S., 2001. Risk assessment. In: Klaassen, C.D. (Ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, USA, pp. 83–104.

Gajecka, M., Jakimiuk, E., Polak, M., Otrocka-Domagala, I., Janowski, T., Zwierzchowski, W., Obremski, K., Zielonka, L., Apoznanski, J., Gajecki, M., 2004a. Zearalenone applied per os provides adverse effects in structure of chosen parts of bitch reproductive system. Pol. J. Vet. Sci. 7, 59–66.

Gajecka, M., Jakimiuk, E., Skorska-Wyszynska, E., Zielonka, L., Polak, M., Paluszewski, A., Rybarczyk, L., 2004b. Influence of zearalenone mycotoxicosis on selected immunological, haematological and biochemical indexes of blood plasma in bitches. Pol. J. Vet. Sci. 7, 175–180.

Garland, T., Reagor, J., 2001. Chronic canine aflatoxicosis and management of an epidemic. In Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium. In: deKoe, W., Samson, R., van Egmond, H., Gilbert, J., Sabino, M. (Eds.), Ponsen & Looven, Wageningen, Netherlands, pp. 231–236.

Haschek, W.M., Voss, K.A., Beasley, V.R., 2002. Selected mycotoxins affecting animal and human health. In Handbook of Toxicologic Pathology, In: Haschek, W.M., Roussex, C.G., Wallig, M.A. (Eds.), 2nd ed. Academic Press, New York, NY, pp. 645–698.

Henke, S.E., Gallardo, V.C., Martinez, B., Balley, R., 2001. Survey of aflatoxin concentrations in wild bird seed purchased in Texas. J. Wildl. Dis. 37, 831–835.

Hidy, P.H., Baldwin, R.S., Greasham, R.L., Keith, C.L., McMullen, J.R., 1977. Zearalenone and some derivatives: production and biological activities. Adv. Appl. Microbiol. 22, 59–82.

Hughes, D.M., Gahl, M.J., Graham, C.H., Grieb, S.L., 1999. Overt signs of toxicity to dogs and cats of dietary deoxynivalenol. J. Anim. Sci. 77, 693–700.

International Agency for Research on Cancer — World Health Organization (IARC), 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, IARC Working Group, vol. 56. World Health Organization, Lyon, France, pp. 467–488.

Jeong, W.I., Do, S.H., Jeong, D.H., Chung, J.Y., Yang, H.J., Yuan, D.W., Hong, I.H., Park, J.K., Goo, M.J., Jeong, K.S., 2006. Canine renal failure syndrome in three dogs. J. Vet. Sci. 7, 299–301.

Kitchen, D.N., Carlton, W.W., Tuite, J., 1977. Ochratoxin A and citrinin induced nephrosis in Beagle dogs. I. Clinical and clinicopathological features. Vet. Pathol. 14, 154–172.

Krogh, P., 1992. Role of ochratoxin in disease causation. Food Chem. Toxicol. 30, 213–224.

Kubena, L.F., Harvey, R.B., Huff, W.E., Corrier, D.E., Phillips, T.D., Rottinghaus, G.E., 1990. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci. 69, 1078–1086.

Leibowitz, S.F., Shor-Posner, G., 1986. Brain serotonin and eating behaviour. Appetite 7, 1–14.

Leung, M.C.K., Diaz-Llano, G., Smith, T.K., 2006. Mycotoxins in pet food: a review on worldwide prevalence and preventative strategies. J. Agric. Food Chem. 54, 9623–9635.

Leung, M.C.K., Smith, T.K., Karrow, N.A., Boermans, H.J., in press. Effects of feeding diets naturally contaminated with *Fusarium* mycotoxins on feed intake, body weight, hematology, and nutrient digestibility of mature beagles. Am. J. Vet. Res.

Li, G., Shinozuka, J., Uetsuka, K., Nakayama, H., Doi, K., 1997. T-2 toxin-induced apoptosis in intestinal crypt epithelial cells of mice. Exp. Toxicol. Pathol. 49, 447–500.

Maia, P.P., Pereira Bastos de Siqueira, M.E., 2002. Occurrence of aflatoxins B_1, B_2, G_1 and G_2 in some Brazilian pet foods. Food Addit. Contam. 19, 1180–1183.

Mally, A., Zepnik, H., Wanek, P., Eder, E., Dingley, K., Ihmels, H., Volkel, W., Dekant, W., 2004. Ochratoxin A: lack of formation of covalent DNA adducts. Chem. Res. Toxicol. 17, 234–242.

Martins, M.L., Martins, H.M., Bernardo, F., 2003. Fungal flora and mycotoxins detection in commercial pet food. Rev. Port. Ciênc. Vet. 98, 179–183.

Matsuzaki, M., Yoshida, A., Akutsu, S., Tsuchida, M., Okuyama, D., 1976a. Studies on toxicity of fusaric acid-Ca. IV. Chronic toxicity in dogs. Jpn. J. Antibiot. 29, 518–542.

Matsuzaki, M., Yoshida, A., Tsuchida, M., Sekino, M., Asano, M., 1976b. Studies on toxicity of fusaric acid-Ca. III. Subacute toxicity. Jpn. J. Antibiot. 29, 491–517.

Milani, M.M., 1996. The importance of the human–animal bond. J. Am. Vet. Med. Assoc. 209, 1064–1065.

Moss, M.O., 1996. Mycotoxic fungi, In: Eley, A.R. (Ed.), Microbial Food Poisoning, 2nd ed. Chapman & Hall, New York, NY, pp. 75–93.

MSNBS News Services. Toxic pet food may have killed dozens of dogs. http://www.msnbc.msn.com/id/10771943/ - Last visited on November 27, 2006.

National Research Council (NRC), 1993. Issues in Risk Assessment. National Academy Press, Washington, DC, USA.

Newberne, P.M., Wogan, G.N., 1968. Sequential morphologic changes in aflatoxin B carcinogenesis in the rat. Cancer Res. 28, 700–781.

Paine, A.J., Marrs, T.C., 2000. The design of toxicological studies, In: Ballantyne, B., Marrs, T., Syversen, T. (Eds.), General and Applied Toxicology, 2nd ed. MacMillan References Texts Ltd, London, United Kingdom, pp. 321–334.

Pittet, A., 1998. Natural occurrence of mycotoxins in foods and feeds — an updated review. Rev. Med. Vet. 149, 479–492.

Price, W.D., Lowell, R.A., McChesney, D.G., 1993. Naturally occurring toxins in feedstuffs. J. Anim. Sci. 71, 2556–2562.

Puschner, B., 2002. Mycotoxins. Vet. Clin. North Am., Small Anim. Pract. 32, 409–419.

Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S., van Schalkwyk, D.J., 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82, 353–357.

Riley, R.T., Norred, W.P., 1996. Mechanism of mycotoxicity. In: Howard, D.H., Miller, J.D. (Eds.), The Mycota, vol. VI. Springer-Verlag, New York, United States, p. 193.

Rotter, B.A., Prelusky, D.B., Pestka, J.J., 1996. Toxicology of deoxynivalenol (vomitoxin). J. Toxicol. Environ. Health 48, 1–34.

Schweighardt, H., 1980. Mikrobiell bedingte Ernährungsschäden beim Geflügel und deren Nachweis. Wien. Tierärztl. Monatsschr. 67, 316–318.

Scott, P.M., 1993. Fumonisins. Int. J. Food Microbiol. 18, 257–270.

Scudamore, K.A., Hetmanski, M.T., Nawaz, S., Naylor, J., Rainbird, S., 1997. Determination of mycotoxins in pet foods sold for domestic pets and wild birds using linked-column immunoassay clean-up and HPLC. Food Addit. Contam. 14, 175–186.

Scussel, V.M., Glordano, B.N., Simao, V., Rocha, M.W., dos Reis, L.F.C., Xavier, J.J.M., 2006. Mycotoxin evaluation in pet food by liquid chromatography-tandem mass spectrometry. The World Mycotoxin Forum, Cincinnati, USA, p. 11.

Smith, T.K., 1992. Recent advances in the understanding of *Fusarium* trichothecene mycotoxicoses. J. Anim. Sci. 70, 3989–3993.

Smith, G.W., Constable, P.D., Smith, A., Bacon, C.W., Meredith, F.I., Wollenberg, G.K., Haschek, W.M., 1996. Effects of fumonisin-containing culture material on pulmonary clearance in swine. Am. J. Vet. Res. 57, 1223–1248.

Smith, T.K., McMillan, E.G., Castillo, J.B., 1997. Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. J. Anim. Sci. 75, 2184–2191.

Stenske, K.A., Smith, J.R., Newman, S.J., Newman, L.B., Kirk, C.A., 2006. Aflatoxicosis in dogs and dealing with suspected contaminated commercial foods. J. Am. Vet. Med. Assoc. 228, 1686–1691.Swamy, H.V., Smith, T.K., MacDonald, E.J., 2004. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on brain regional neurochemistry of starter pigs and broiler chickens. J. Anim. Sci. 82, 2131–2139.

Szczech, G.M., Carlton, W.W., Tuite, J., 1973. Ochratoxicosis in Beagle dogs. I. Clinical and clinicopathological features. Vet. Pathol. 10, 135–154.

United States Environmental Protection Agency (USEPA), 1995. The use of the benchmark dose approach. Health Risk Assessment. USEPA, Washington, DC, USA.

Voss, K.A., Plattner, R.D., Riley, R.T., Meredithe, F.I., Norred, W.P., 1998. In vivo effect of fumonisin B_1-producing and fumonisin B_1-nonproducing *Fusarium moniliforme* isolates are similar: fumonisin B_2 and fumonisin B_3 cause hepato- and nephrotoxicity in rats. Mycopathologia 141, 45–48.

Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T., Merrill, A.H., 1991. Inhibition of sphingolipid biosynthesis by fumonisins. J. Biol. Chem. 266, 14486–14490.