

TABLE OF CONTENTS

TABLE OF CONTENTS.....	1
Jerky Pet Treat Investigation Testing Rationale and Results for October 1, 2013 – December 31, 2015	4
Introduction	4
1. Microbiological Testing	4
1.1. FY 2014 Rationale and Results	4
1.2. FY 2015-2016 Rationale and Results	5
2. Compositional Testing.....	5
2.1. FY 2014 Rationale.....	6
2.2. FY 2014 Results.....	7
2.2.1. Glycerol	7
2.2.2. Lysine.....	7
2.2.3. MSG	7
2.2.4. Sulfur	7
2.3. FY 2015 Rationale.....	7
2.4. FY 2015 Results.....	8
2.4.1. Glycerol	8
2.4.2. Sorbitol	9
2.4.3. Xylitol.....	9
2.4.4. Fructose.....	9
2.4.5. Potassium Sorbate.....	9
2.4.6. MSG	9
2.4.7. 3-monochloropropane-1,2-diol (3-MCPD)	9
2.4.8. DNA	10
2.5. FY 2016 Rationale.....	10
2.6. FY 2016 Results.....	10
2.6.1. Glycerol	10
2.6.2. Salt (NaCl).....	10
2.6.3. 3-MCPD	10
3. Chemical Toxicology Testing	11

3.1.	Chemical Toxicology Testing- Analytes List for October 1, 2013 – December 31, 2015.....	11
3.2.	Chemical Toxicology Testing-Rationale and Results	12
3.2.1.	General Screens for Toxic Compounds	12
3.2.2.	Metals and Elements.....	13
3.2.3.	Glycols	14
3.2.4.	Glycerol Metabolites	14
3.2.5.	Sugar Alcohols	15
3.2.6.	Other Organics	15
3.2.7.	Antibiotics, Antivirals, DEET, and other Drugs	16
3.2.8.	Biogenic Amines	18
3.2.9.	Mycotoxins	18
3.2.10.	Sulfonamide Herbicides	18
3.2.11.	Phorbol Esters: Jatropha curcas Toxins.....	19
3.2.12.	Additives/Preservatives.....	19
3.2.13.	Flavoring Agents.....	20
3.2.14.	Tanning Agents.....	20
3.2.15.	Illegal Dye Agents	21
3.3.	Chemical Toxicology Testing -Method development.....	21
3.3.1.	Maleic Acid	21
3.3.2.	Diglycolic Acid (DGA) and Glycolic Acid (GA).....	22
3.3.3.	Sulfite and Bisulfite	23
3.3.4.	Epichlorohydrin (ECH)	24
3.3.5.	Selected antibiotics/antivirals	24
3.3.6.	Bufotenin.....	25
3.3.7.	Chaconine.....	25
3.3.8.	Amikacin	26
3.3.9.	3-MCPD, and 3-MCPD esters.....	26
3.3.10.	Negative Ion liquid chromatography- mass spectrometry (LC-MS) method	27
3.3.11.	Glyceraldehyde and other glycerol breakdown products.....	27
3.3.12.	Compound Discoverer Software	28
4.	Evaluation of Jerky Pet Treat Irradiation.....	28
5.	Formaldehyde Testing.....	29

6.	Radioactivity Testing	29
7.	Viral Testing.....	29
8.	References.....	30

Jerky Pet Treat Investigation Testing Rationale and Results for October 1, 2013 – December 31, 2015

Introduction

The Center for Veterinary Medicine's (CVM) Veterinary Laboratory Investigation and Response Network (Vet-LIRN) continued testing jerky pet treat (JPT) products collected from consumers who reported pet illnesses. The specific types of tests we conducted from October 1, 2013 until December 31, 2015 include:

- Microbiological Testing
- Compositional Testing
- Chemical Toxicology Testing
- Evaluation of Jerky Treat Irradiation
- Formaldehyde Testing
- Radioactivity testing
- Viral Testing

This update includes a testing rationale for new analytes added after October 2013, as well as results for tests we conducted from October 2013 until December 31, 2015. The rationale for testing completed before October 2013 is available online (Vet-LIRN, JPT diagnostic test rationale and results, link:

<http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/UCM371485.pdf>).

1. Microbiological Testing

During Fiscal Year (FY) 2014 (October 2013 - September 2014), Vet-LIRN worked on a multi-phase project with one of the network laboratories to:

- a) culture and test JPT for the presence of *B. cereus*, *S. aureus*, and *C. perfringens*,
- b) validate diagnostic kits for the toxins produced by *B. cereus*, Shiga toxin-producing *E. coli*, *S. aureus*, and *C. perfringens*, and
- c) test the treats for the above-mentioned toxins.

Vet-LIRN did not initiate new projects for microbiological testing during FY 2015 (October 2014 - September 2015) or during the first quarter of FY 2016 (October 2015 - December 31, 2016).

1.1. FY 2014 Rationale and Results

As we mentioned in our 2013 rationale document (Vet-LIRN, JPT diagnostic test rationale and results, link:

<http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/UCM371485.pdf>) several bacterial enterotoxins can cause severe illness in humans and animals.

Product irradiation reduces the chance of bacterial growth after packaging. However, if bacteria produced toxins prior to irradiation, the toxins could potentially survive irradiation and be present in the final product. Enterotoxins are often heat-stable, but could potentially be destroyed by heating while drying. Since no validated methods for detecting these toxins in this type of food product exist, we purchased four commercially available enterotoxin kits (*B. cereus*, *E. coli* Shiga Toxin, *S. aureus*, *C. perfringens*) and asked our laboratory to validate them for JPT testing.

- a) The laboratory cultured 61 unopened bags of JPT (10 case-related samples, and 51 store-bought samples) for *B. cereus*, *C. perfringens* and *S. aureus*. Two samples tested positive for *B. cereus*, and all other samples were negative for all three microorganisms. Of the case-related samples, we cultured only unopened bags for bacteria. The laboratory did not culture open bag consumer samples due to possible contamination after opening.
- b) The laboratory successfully validated diagnostic kits for *E. coli* Shiga Toxin and *S. aureus* enterotoxins. The laboratory was unable to validate diagnostic kits for *B. cereus* and *C. perfringens* enterotoxins due to difficulties related to obtaining (*B. cereus*) and recovering (*C. perfringens*) kit controls.
- c) The laboratory tested 68 case-related and 21 store-bought samples for *E. coli* Shiga Toxin and *S. aureus* enterotoxins. All samples were negative.

1.2. FY 2015-2016 Rationale and Results

We continue submitting samples for microbiological testing on a case-by-case basis, after reviewing the consumer complaint and the patient's medical records. During FY 2015, we tested 1 case-related sample for molds and the following mycotoxins: Deoxynivalenol, Zearalenone, T-2 (trichothecene mycotoxin), HT-2 (fusarium mycotoxin), Aflatoxin B1, Ochratoxin, and Fumonisin B1. The sample was negative for molds and mycotoxins. As of December 31, 2015, no additional samples were submitted for microbiological testing.

2. Compositional Testing

During FY 2014, we tested 174 samples for the following analytes:

- glycerol,
- protein,
- fat,
- moisture,

- lysine,
- monosodium glutamate (MSG), and
- sulfur dioxide and total sulfites.

In FY 2015, we tested 173 samples for the following analytes:

- glycerol,
- protein,
- fat,
- moisture,
- sorbitol,
- xylitol,
- fructose,
- potassium sorbate,
- MSG, and
- 3-MCPD (3-monochloropropane-1,2-diol).

During FY 2015, we also tested 10 samples for DNA, to confirm species.

During the first quarter of FY 2016, we tested 79 samples for one or more of the following analytes:

- 3-MCPD,
- glycerol, and
- salt (NaCl).

2.1. FY 2014 Rationale

Since previous testing showed several products were mislabeled (Vet-LIRN 2013 rationale document, link:

<http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/UCM371485.pdf>), we submitted more samples for compositional testing. The FY 2013 (October 2013-September 2014) results for Lysine testing are reported below.

Several studies in rats showed that MSG exposure may cause an adverse effect on the renal function (Vinodini et al., 2010; Egbonu et al., 2010). Therefore, we tested several samples for MSG.

Sulfite preservatives can cause Thiamine (Vitamin B1) deficiency, and were associated with a number of deaths in dogs and cats (Studdert, 1991; Malik, 2005). JPT products could have higher sulfur levels if the chicken breast and/or sweet potatoes were treated/dipped in a sulfite containing solution that preserves the product's color. Therefore, we tested the treats for sulfur dioxide and total sulfites as possible causative agents of sulfite sensitivity.

2.2. FY 2014 Results

In FY 2014, we tested 174 samples for the following analytes:

2.2.1. Glycerol

Ten of 114 samples tested positive for glycerol, which was not a listed ingredient on the label. Some samples were correctly labeled as containing glycerol; however, the samples had very high glycerol contents. One product had a glycerol content of 200,000 ppm, which is equivalent to 20 percent of the product weight).

2.2.2. Lysine

We tested 9 samples for Lysine (7 of submitted samples were consumer case-related samples, 1 was a store-bought sample, and 1 sample was a control sample). The consumer case-related samples and the store-bought sample had similar Lysine concentrations when compared with the control.

2.2.3. MSG

We tested 11 samples for MSG (2 control, 6 consumer case-related, and 3 store-bought samples). The results did not indicate MSG was added to the treats. This method measures the total amount of glutamate and does not distinguish between glutamic acid, a naturally occurring substance in meat, and its sodium salt- MSG, not a naturally occurring substance in meat. Therefore, we tested control JPT (without added MSG) to use as a reference when comparing results and to help identify any jerky samples with large glutamate concentrations (which would indicate added MSG).

2.2.4. Sulfur

We tested 40 samples (37 consumer case-related and 3 control samples) for total sulfites and sulfur dioxide. All samples showed expected levels of these compounds. Compared to other JPT types, samples containing sweet potatoes showed higher amounts of total sulfites and sulfur dioxide, compounds which are natural components of sweet potatoes.

2.3. FY 2015 Rationale

Similar to FY 2014 testing, the FY 2015 compositional testing included various analytes within the following categories:

- nutrient analysis,
- food additives,

- food processing related contaminants, and
- DNA analysis (for species confirmation).

We tested 173 samples (86 store-bought, 71 case-related and 16 control samples).

Previous product testing showed that some of the products were mislabeled for glycerol; therefore, we continued testing the treats for this analyte. Additionally, the FY 2014 chemical toxicology testing for sugar alcohols (glycerol, sorbitol, xylitol) showed that some samples contained high sorbitol concentrations (up to 170,000 ppm). Sorbitol was not indicated on the package labeling for most of those samples. Because the chemical toxicology method for detecting sugar alcohols was semi-quantitative, we tested more samples for these analytes, using a different, quantitative method. The quantitative method gives more accurate information about the concentration of sorbitol.

3-MCPD is a food processing related contaminant. It was first detected in acid hydrolyzed vegetable protein (HVP), a seasoning ingredient in soy sauce and similar foods. Further studies showed that 3-MCPD might also occur in products other than HVP, such as thermally processed foods (e.g., bakery products, malt-derived products, cooked/cured fish or meat, and other products). In thermally processed foods, 3-MCPD is formed from lipids and salt, naturally present or added to the food, during manufacturing or cooking. 3-MCPD can also be formed from Epichlorohydrin (ECH) by hydrolysis, reaction using water to break bonds in a molecule (International Program on Chemical Safety, 1984), link: <http://www.inchem.org/documents/ehc/ehc/ehc33.htm>). Section 3 of this document has more details about [ECH](#) (Chemical Toxicology Testing section).

3-MCPD is primarily toxic to the kidney. Chronic oral exposure causes a nephropathy and tubular hyperplasia (Barocelli et al, 2011; Moris et al, 1980; Kluwe et al, 1983). Studies show that 3-MCPD is most often found in the aforementioned foods as an ester linked with fatty acids, e.g., mono- or di-esters (Svejkovská et al., 2004; Weisshaar, 2011; Crews et al., 2013). High levels of 3-MCPD esters are found in edible refined plant oils and fats, and composite foods containing these oils/fats. Lipases can release 3-MCPD from the esters *in vivo*, and a recent study (Abraham et al., 2013) supports equal oral bioavailability of 3-MCPD in the free form and in the ester form.

2.4. FY 2015 Results

- **Nutrients and food additives**

2.4.1. Glycerol

We tested 104 samples for glycerol (34 case-related, 62 store-bought, 8 control samples). Several store-bought products collected recently contained glycerol ranging from 83,000 ppm to 108,000 ppm, and glycerol was not on the package label. All case-related samples that tested

positive for glycerol had glycerol listed as an ingredient on the package label. Concentrations of protein, fat, and moisture were within expected ranges for the types of products tested.

2.4.2. Sorbitol

We tested 92 samples for sorbitol. Eighteen tested sorbitol positive, and 14 of those positive samples did not list sorbitol as an ingredient on the package label (8 store-bought and 6 case-related). The sorbitol concentrations in these 14 samples ranged from 7,890 ppm to 175,000 ppm. These samples were made by 8 different product brands.

2.4.3. Xylitol

We tested 92 samples for xylitol. All tested negative.

2.4.4. Fructose

We tested 25 samples for fructose. Six products tested positive at normal concentrations, ranging from 0.3 ppm to 7.4 ppm. Fructose is naturally found in certain foods such as sweet potatoes. Five of the positive samples listed sweet potatoes as an ingredient.

2.4.5. Potassium Sorbate

We tested 20 samples for potassium sorbate (10 case-related, 9 store-bought, and 1 control sample). Seven samples tested positive (4 case-related and 3 store-bought). The potassium sorbate concentrations ranged from 400 ppm to 4,900 ppm, which are within acceptable concentrations.

2.4.6. MSG

We tested 2 store-bought samples for added MSG. The results did not indicate MSG was added.

- **Food Processing Related Contaminants (3-MCPD)**

2.4.7. 3-monochloropropane-1,2-diol (3-MCPD)

A commercial laboratory tested 20 samples for 3-MCPD (4 control samples and 16 case-related samples). Seven case-related samples tested positive at concentrations ranging from 0.049 ppm to 0.352 ppm. In order to evaluate a larger number of samples, we began working on method development for this analyte in FY 2015 (more details available in [Section 3: Chemical Toxicology Testing](#)). The review of available scientific literature currently does not suggest these 3-MCPD levels would cause illness in pets. We continue to test more samples and evaluate this information.

- **DNA analysis**

2.4.8. DNA

We tested 10 chicken JPT samples (9 store-bought and 1 control sample) for the presence of chicken meat, which would confirm the package labeling statement. Testing confirmed chicken meat in all samples.

2.5. FY 2016 Rationale

Since several samples previously tested positive for 3-MCPD, we continue testing samples for this analyte, in order to better understand the importance of these findings. A commercial laboratory is performing the testing until method development is completed. We continue submitting samples for nutrient analysis and food additives/preservatives. Since our previous nutrient analysis did not include NaCl, we added this analyte to our testing list. This analysis was undertaken to better characterize the composition of the treats, as some contaminants, such as 3-MCPD, can be formed by interacting with NaCl during food processing

As of December 31, 2015, we tested 79 samples (65 case-related, 11 store-bought, and 2 control samples).

2.6. FY 2016 Results

- **Nutrients and Food Additives**

2.6.1. Glycerol

We tested 23 samples for glycerol (11 case-related, 11 store-bought, 1 control sample). All positive samples had glycerol listed as an ingredient on the product packaging. Concentrations of protein, fat, and moisture were within expected ranges for the types of products tested.

2.6.2. Salt (NaCl)

We tested 55 samples for NaCl (53 case-relates and 2 control samples). All samples were positive for NaCl with concentrations ranging from 0.1 ppm to 4 ppm.

- **Food Processing Related Contaminants (3-MCPD)**

2.6.3. 3-MCPD

A commercial laboratory tested 24 samples for 3-MCPD (12 case-related samples, 11 store-bought samples, and 1 control sample). Five case-related samples tested positive with concentrations ranging from 0.029 ppm to 0.143 ppm. Five store-bought samples tested positive with concentrations ranging from 0.03 ppm to 0.04 ppm. Review of the current scientific literature does not suggest that these levels would cause pet illness.

3. Chemical Toxicology Testing

We continued testing the treats for some of previously selected analytes (Vet-LIRN, JPT diagnostic test rationale and results, link:

<http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/UCM371485.pdf>). We also added new specific analytes of interest. As done previously, analyte selection was based on its potential to cause renal disease, Fanconi syndrome, or gastrointestinal signs (reported in many of the consumer complaints). We removed some analytes from the testing list based on the testing results (negative, or positive in concentrations, not considered to be harmful). For example, phorbol esters, tanning agents, and illegal dye agents were removed from the testing list based on continued negative testing results. The chemical toxicology testing analytes-list below shows which methods and analytes were included or removed from our testing plan.

In FY 2014, we tested 71 samples (11 store-bought and 60 case-related samples). In FY 2015, we tested 50 JPT samples and began development of additional methods (tests) for analytes of interest. Samples were screened for a variety of analytes using multiple analytical methods, including gas chromatography-mass spectrometry (GC-MS), and high-resolution liquid chromatography-mass spectrometry screen (LC/MS screen). The list of analytes for method development is provided in a separate [section](#) of this document. In FY 2016, we plan to test 75 JPT samples, expand several existing methods, and develop additional tests for analytes of interest. As of December 31, 2015, we submitted 50 jerky pet treat samples under this contract, and requested additional method expansions and method developments.

3.1. Chemical Toxicology Testing- Analytes List for October 1, 2013 – December 31, 2015

- General screens for toxic compounds: restricted list
- Metals and Elements: various metals and elements including heavy metals (boron, fluoride, antimony, and cesium added in FY 2015)
- Glycols: diethylene glycol-DEG; ethylene glycol-EG; propylene glycol-PEG; dihydroxyacetone (DHA); and 1,3 propanediol
- Glycerol metabolites: glycolic acid (see [Section 3.3 Method Development List](#)), diglycolic acid ([Section 3.3 Method Development List](#)), tartronic acid (added in 2014), glyoxylic acid (added in 2014), lactic acid (removed from testing list in 2013), glyceraldehyde (see [Section 3.3 Method Development List](#), added in 2015)

- Sugar alcohols: xylitol, sorbitol, and glycerol
- Other organics: oxalic acid, paraquat, aristolochic acid, aristolactam (added in 2014), and hexachlorobutadiene (removed from testing list in 2013)
- Antibiotics: ampicillin, cephalixin, cloxacillin, penicillin G, ceftiofur, sulfonamides (sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethazine, sulfapyridine, sulfaquinoxaline, sulfathiazole), tylosin, enrofloxacin, sarafloxacin, gatifloxacin, azitromycin (see [Section 3.3 Method Development List](#), added in 2015), and amikacin (see [Section 3.3 Method Development List](#), added in 2016)
- Antivirals: amantadine, arbidol, oseltamivir, oseltamivir acid, ribavirin, rimantadine (added in 2014), and ritanovir (see [Section 3.3 Method Development List](#), added in 2015)
- Other drugs: quinocetone, forensic drug screen (list-restricted information), and monensin and other anticoccidial drugs added in 2014 (salinomycin, narasin and lasalocid)
- Biogenic amines: putrescine, cadaverine, histamine, agmatine, spermidine, and spermine (all removed from testing list in 2013)
- Mycotoxins: aflatoxin B1, diacetoxyscirpenol (DAS), roquefortine C, ochratoxin, citrinin (added in 2014), and other toxic metabolites produced by molds (4-*Ipomeanol*, added in 2014)
- Sulfonamide herbicides: asulam and pyroxsulam (added in 2014)
- Phorbol esters: *Jatropha curcas* toxins (removed from testing list in 2013)
- Additives/preservatives: nitrites (removed from testing list in 2013), sulfites (added in 2014, see [Section 3.3 Method Development List](#)), bisulfites (added in 2014, see [Section 3.3 Method Development List](#)), and sulfate (added in 2014))
- Flavoring agents: malic acid, maleic acid (see [Section 3.3 Method Development List](#)), and fumaric acid
- Tanning agents: tannic acid and gallic acid (both removed from testing list in 2013)
- Illegal dye agents: Auramine, Bixin, Butter Yellow, Fast Garnet, Metanil Yellow, Orange II, Orange Oil SS, Para Red, Rhodamine B, Sudan Black B, Sudan I-IV G, Sudan Orange, Sudan Red 7B, Sudan Red B, Sudan Red G, and Toluidine Red (all removed from testing list in 2013)
- Epichlorohydrin (added in 2015, see method development list), 3-MCPD, and 3-MCPD esters (added in 2016, see [Section 3.3 Method Development List](#))
- Bufotenin: added in 2015, see [Section 3.3 Method Development List](#)
- Chaconine: added in 2015, see [Section 3.3 Method Development List](#) Negative Ion LC-MS method: added in 2015, see [Section 3.3 Method Development List](#)
- Compound Discoverer Software: added in 2016, see [Section 3.3 Method Development List](#)

3.2. Chemical Toxicology Testing-Rationale and Results

3.2.1. General Screens for Toxic Compounds

Rationale

As previously indicated (2013 rationale document), consumers report many different clinical signs in their animals. We used a test that screens for a variety of toxic chemicals. After extraction, the samples are run on a GC/MS instrument, which compares the samples' results to a library of results for known chemicals. The list of toxic chemicals we tested for is confidential.

Results

In FY 2014, we tested 71 samples. Multiple analytes tested positive in very low concentrations (not considered to be related to reported illnesses), including nicotine (3 samples) and creosol (2 samples). We are not providing the complete list of all positive analytes; however, samples were negative for the various known toxic chemicals in the screen. In FY 2015, we tested 50 samples. Similar to FY 2014, multiple samples tested positive for a number of analytes. We are still evaluating the significance of these findings. However, it is unlikely that any of the detected analytes were the root cause of the reported animal illnesses. For example, some of the positive analytes include monensin, lasalocid, azitromycin, bufotenin, amantadine, carbofuran, and others in very low concentrations. Additionally, many of detected analytes are naturally found in foods (e.g., malic acid, glycolic acid, oxalic acid), and some of them are approved food additives (e.g., glycerol, propylene glycol, sorbitol). As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

3.2.2. Metals and Elements**Rationale**

As previously reported (2013 rationale document), in 2013 we sent 40 additional samples for a Metals Screen 3 (Vet-LIRN code for a testing laboratory) test, which includes sulfur. Sulfur can enter the food production chain in several ways and can cause animal hypersensitivity responses if in sulfite form (sulfite, bisulfite, metabisulfite, and sulfur dioxide). We used the metals-elemental analysis as a first test to screen samples for these compounds.

In FY 2015, we submitted 25 samples (24 case-related and 1 control sample) to 2 different laboratories (Metals Screen 1 and Metals Screen 2). We wanted to determine concentrations of various metals and elements and to compare each laboratory's findings. Because previous testing did not include boron (B), fluoride (F), antimony (Sb), and cesium (Cs), we added these elements to the testing list. Metals Screen 1 looked for 47 metals and elements (Ag, Al, As, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Ge, Hg, Ho, In, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sm, Sn, Sr, Th, Ti, Tl, Tm, U, V, W, Yb, Zn, Zr). Metals Screen 2 looked for 21 metals and elements (As, Ba, Cd, Ca, Cr, Co, Cu, Pb, Mg, Mn, Mb, P, K, Se, Na, S, V, Zn, Sb, Cs, F).

As of December 31, 2015, we have not submitted additional samples for FY 2016.

Results

In FY 2014, testing by Metals Screen 3 (As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, Pb, S, and Zn) was completed for 40 samples submitted in 2013 (37 consumer complaint cases samples and 3 control samples). None of the samples tested positive at toxic concentrations.

In FY 2015, Metals Screens 1 and 2 did not show increased levels of metals and elements. On the contrary, the results showed low levels (levels below the AAFCO dog food minimum) of several elements: Ca, Cu, Mn, and Zn. These low levels could potentially cause a nutrient deficiency, if the diet consists of primarily jerky treats. Jerky pet treats should not be substituted for a balanced diet and are intended to be fed only occasionally and in small quantities.

3.2.3. Glycols

Rationale

We continued testing the treats for diethylene glycol (DEG), ethylene glycol (EG), and propylene glycol (PG). Please see our 2013 rationale document for further explanation. This section also includes treat testing results for Dihydroxyacetone (DHA) and 1,3 Propanediol.

Results

In FY 2014, we tested 71 samples for these analytes. Two case-related samples tested positive for EG in very small amounts. We believe these small amounts did not cause the reported symptoms in dogs. Several samples tested positive for PG, including 11 case-related samples without PG listed as an ingredient on the package label. Concentrations of PG in those samples ranged from 20 ppm to 35,000 ppm. These concentrations are not considered high. In addition, 2 case-related samples tested positive for DHA, and 1 case-related sample tested positive for 1,3 propanediol, both in very small amounts.

In FY 2015, we tested 50 samples for these analytes. Several samples tested positive for PG. Because of the positive PG results, we continue testing for these analytes. As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

3.2.4. Glycerol Metabolites

Rationale

As reported in our 2013 rationale document, in 2013 we began working on several method developments for glycerol metabolites (Figure 1; Gil et. al., 2011). Consumer complaints report clinical signs very similar to those associated with antifreeze (EG) poisoning. The body converts EG and DEG into calcium oxalate and other metabolites which can cause renal failure. We are exploring the possibility that during jerky pet treat manufacturing, irradiation, in combination with other factors, could produce toxic metabolites from glycerol. We have identified several analytes for method development: glycolic acid (GA), diglycolic acid (DGA), tartronic acid, glyoxylic acid, and glyceraldehyde.

Results

In FY 2014, we tested 71 samples for GA and DGA, using a preliminary method. Multiple samples tested positive for GA with trace amounts (59 case-related and 10 store-bought samples). One store-bought sample tested positive for DGA with trace amounts. In FY 2015, we requested further method development for these analytes to increase test sensitivity. Method development was completed, and we tested 50 samples for these analytes during FY 2015. Method development for the remaining analytes (tartronic acid, glyoxylic acid, and glyceraldehyde) began in FY 2015. Please see Method Development section of this document for more information. As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

3.2.5. Sugar Alcohols

Rationale

As we mentioned in our 2013 rationale document, glycerol can be converted to xylitol (Zhang et al., 2011). Xylitol administration was associated with calcium oxalate crystals in the kidney of a human patient (Evans, 1973) and with liver failure in dogs (Dunayer, 2006). Sorbitol is a sugar substitute, and testing is performed to establish if it is being added to jerky pet treats without proper labeling.

Results

More work is needed on method development to improve accuracy for these analytes. At this point, our method is semi-quantitative. This method is different from a quantitative method performed by a different, commercial lab. We provided the results for glycerol, sorbitol, and xylitol in the [Compositional Testing Section](#). In FY 2014, we tested 71 samples. One case-related sample tested positive for xylitol (170 ppm). Ten case-related samples tested positive for sorbitol, and for 7 JPT samples, sorbitol was not on the package label. Sorbitol concentrations for the 7 samples ranged from 300 ppm to 170,000 ppm. Multiple samples tested positive for glycerol. In FY 2015, we tested 50 samples. Six case-related samples tested positive for sorbitol, and none had sorbitol on the package label. Sorbitol concentrations for those samples ranged from 100 ppm to 53,000 ppm. Multiple samples tested positive for glycerol. None of the samples tested positive for xylitol. As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

3.2.6. Other Organics

Rationale

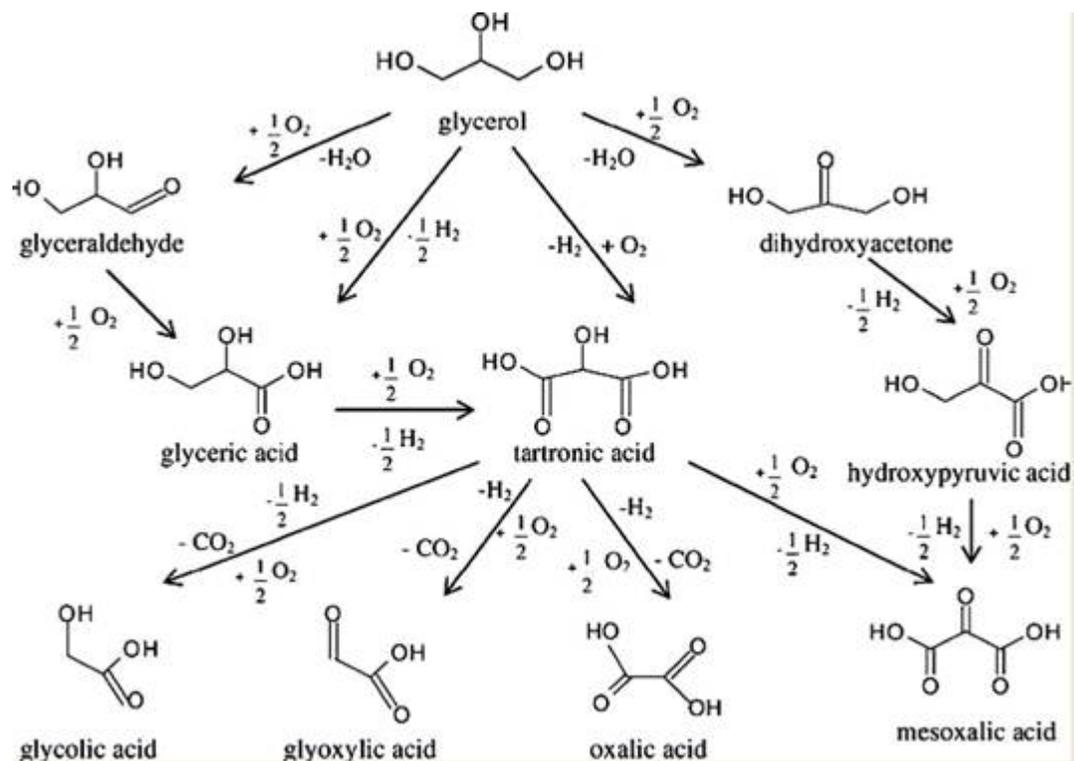
Rationale for this group of analytes (oxalic acid, paraquat, aristolochic acid, and aristolactam), can be found in our 2013 rationale document. Aristolactam is the only new analyte added to this group since the last update. We added aristolactam because it is a metabolite of aristolochic acid (Stiborová et al., 1999; Mix et al., 1982).

Results

In FY 2014, Lab 1 tested 71 samples for aristolochic acid, aristolactam, paraquat, and oxalic acid (11 store-bought and 60 case-related samples). All samples were negative for aristolochic acid, aristolactam, and paraquat. Forty-five case-related samples and 1 store-bought sample were positive for oxalic acid. Oxalic acid concentrations ranged from 50 ppm to 1,800 ppm. Lab 2 tested 20 case-related and 2 store-bought samples for aristolochic acid and aristolactam. All samples tested negative for aristolochic acid and aristolactam.

In FY 2015, Lab 1 tested 50 samples for these analytes. All samples were negative for aristolochic acid, aristolactam, and paraquat. Twelve case-related samples were positive for oxalic acid. Oxalic acid concentrations ranged from 52 ppm to 610 ppm. As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

Figure 1. (Gil et al., 2011)



3.2.7. Antibiotics, Antivirals, DEET, and other Drugs

Rationale

Rationale for the antibiotics testing can be found in our 2013 rationale document. All testing for FY 2014 testing period (71 samples) was completed by Lab 1 using an Exactive screen. Testing for FY 2015 was completed by Lab 1 (50 samples) and Lab 2 (The New York State Department of Agriculture and Markets Laboratory-NYSDAM), which tested 9 samples. Lab 1's screen included

multiple analytes listed in Chemical Toxicology Testing-Analytes List. Lab 2's screen included the following analytes: sulfaclozine, sulfaquinoxaline, sulfamethoxazole, trimethoprim, enrofloxacin, tilmicosin, amantadine, and DEET (active ingredient in insect repellents). Amantadine is an FDA-approved antiviral drug for use in people and for use (extra-label) to control pain in dogs. In 2006, FDA prohibited use of amantadine in poultry, to preserve its effectiveness for preventing and treating influenza A in humans. We tested the treats for these drugs after several newspapers reported finding amantadine in poultry meat in China, in December of 2012 (Link 1: <http://www.investors.com/antibiotics-in-yum-chicken-within-legal-limits/?ven=nrelatecp>; Link 2: <http://money.cnn.com/2014/07/21/news/companies/kfc-mcdonalds-china/>). Since October 2013, FDA added testing for antiviral drugs to its testing list, after some historically collected treats tested positive for amantadine.

Rationale for antimicrobial and anticoccidial drug testing can be found in our 2013 rationale document. In 2013, we tested treats for quinocetone (antimicrobial drug) and monensin (antibiotic used to control coccidia, a gastrointestinal parasite in poultry). During FY 2014, we added salinomycin, narasin, and lasalocid to the screen, expanding our search for anticoccidial drugs. We also increased sensitivity of the screen (from a reporting level 50 ppb to 20 ppb), allowing us to detect more positive samples.

As of December 31, 2015, 25 samples were submitted to Lab 1 for FY 2016.

Results

In FY 2014, Lab 1 tested 71 samples for multiple analytes listed in the analytes list, including amantadine and antibiotics. Amantadine was detected in 27 case-related samples, all of which were imported products. None of the samples were positive for the remaining antivirals (arbidol, oseltamivir, oseltamivir acid, ribavirin, and rimantadine) or for any antibiotics from the analytes list. We do not believe amantadine contributed to the dog illnesses. The known side effects or adverse events associated with amantadine do not correlate with the symptoms reported in jerky pet treat-related cases. Traces of monensin were detected in 16 samples (5 case-related and 11 store-bought samples). All samples were negative for quinocetone.

In FY 2015, Lab 1 tested 50 samples for multiple analytes listed in the analytes list, including amantadine and antibiotics. The antibiotic azithromycin was detected in 7 samples, and amantadine was found in 9 samples (all products were imported). None of the samples were positive for the remaining antivirals (arbidol, oseltamivir, oseltamivir acid, ribavirin, and rimantadine), or for any antibiotics from the analytes list. In FY 2015, Lab 2 (NYS DAM) tested 9 samples (80 pieces from 5 store-bought samples, 3 case-related samples, and 1 control sample). As with our previous testing in 2013, Lab 2 tested 5-10 pieces from each sample to evaluate the variability among different pieces within a single bag. Results showed very low (parts per billion) residues in some of the samples. Sulfaquinoxaline (tolerance 0.1 ppm) was found in 1 store-bought sample (8/10 pieces). The highest concentration detected was 0.078 ppm. Sulfaclozine (zero tolerance) was found in 2 samples (11/15 pieces). One of the samples was store-bought, and the other was case-related. The highest concentration detected was 0.102 ppm. Amantadine was found in 1 store-bought sample (10/10 pieces). The highest

concentration was 0.036 ppm. Tilmicosin was found in 1 case-related sample (1/10 pieces). The highest concentration was 0.0056 ppm. DEET was found in 1 store-bought sample (1/10 pieces). The highest concentration was 0.0041 ppm. The analyte levels found in these products are unlikely to cause the clinical signs reported in affected dogs.

As of December 31, 2015, testing is pending for 25 samples submitted to Lab 1, in FY 2016.

3.2.8. Biogenic Amines

Since previous testing for this group of analytes did not find elevated levels in JPT, we removed biogenic amines from our testing list.

3.2.9. Mycotoxins

Rationale

As mentioned in the 2013 rationale document (link: <http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/UCM371485.pdf>), several consumers reported the jerky treats looked moldy. Molds can produce a variety of toxicants reported to cause both renal and gastrointestinal disturbances in humans and domestic animals.

In FY 2014, in addition to some previously tested mycotoxins, we began testing for citrinin due to its nephrotoxic properties, both in humans and animals (Flajs et al., 2009; Kirby et al., 1987). Citrinin naturally occurs in cereal grains, including corn, which is one of the main ingredients in poultry diets. Dogs could potentially be exposed to citrinin by eating chicken meat (jerky treats) contaminated with this mycotoxin. Dogs given combined doses of citrinin and ochratoxin A had degeneration and necrosis of proximal and distal renal tubules (Kitchen et al., 1977).

Since many consumer complaints FDA receives involve treats containing sweet potatoes, in FY 2014, we looked for 4-ipomeanol. This mycotoxin is toxic to the liver and lungs (Chen et al., 2006; Gram, 1989), and can often be found in sweet potatoes infected with the fungus, *Fusarium solani*.

Results

In FY 2014, we tested 71 samples. Only 1 case-related sample tested positive for 4-ipomeanol at very low concentrations (10 ppb-50 ppb). All other samples were negative for the remaining mycotoxins. In FY 2015, we tested 50 samples for mycotoxins. All samples were negative. As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

3.2.10. Sulfonamide Herbicides

Rationale

Sulfonamides are one of the most widely used antibacterial agents in veterinary medicine. However, in some of the cases, animals (including dogs) can experience adverse reactions, such as allergies or other toxic effects. Two of the most frequently reported symptoms related to JPT ingestion are nausea and vomiting. These symptoms may occur when high sulfonamide concentrations disturb the normal digestive tract micro-floral balance and vitamin B synthesis (Boothe, 2012). We tested the treats containing sweet potatoes for asulam and pyroxsulam, two of the most widely used sulfonamide herbicides. We hypothesize that some of digestive disturbances in dogs could potentially be due to sulfonamide sensitivity if those herbicides were used on sweet potatoes.

Results

In FY 2014, we tested 71 samples. All tested negative for these analytes. In addition, in FY 2015, we tested 40 samples. All tested negative. As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

3.2.11. Phorbol Esters: *Jatropha curcas* Toxins

Rationale

As we previously reported (2013 rationale document), in 2012, we began investigating toxins from the plant *Jatropha curcas*. This part of our investigation led to FDA's notification to industry regarding products using oils, glycerin, or protein that were derived from the *Jatropha* plant (FDA, 2012; link: <http://www.fda.gov/ForIndustry/IndustryNoticesandGuidanceDocuments/ucm391133.htm>). Glycerol could potentially be contaminated with these toxins if the source of glycerol was from biodiesel production using *Jatropha* oil.

Results

CVM initiated method development for *Jatropha curcas* toxins in 2012. Because there are no commercially available standards, testing for these toxins required synthesizing standards from *Jatropha curcas* oil and plant seeds. No such toxins were found using a variety of preliminary methods (Nishshanka et al., 2016, prepress link: <http://authors.elsevier.com/a/1SoHT5a~s46CQU>). In 2014, FDA commissioned a private-sector partner to study the *Jatropha* supply chain in Malaysia and Indonesia and to assess potential threats and vulnerabilities. This study showed that *Jatropha* production appears to be minimal in these regions, despite reports of production on the internet (FDA, 2014, link: <http://www.fda.gov/ForIndustry/IndustryNoticesandGuidanceDocuments/ucm391140.htm>). We are not pursuing additional method development or testing of JPT for these analytes.

3.2.12. Additives/Preservatives

Rationale

The rationale for this testing was detailed in our 2013 rationale document. Briefly, sulfonamide sensitivity potentially could be due to sulfite sensitivity. Higher sulfur levels in JPT could be caused by dipping chicken breasts and/or sweet potatoes in a sulfite-containing solution to

preserve the products' color.

Results

As mentioned in the [Compositional Testing Section](#), in FY 2014, a commercial lab tested 40 samples for sulfur dioxide and sulfites. The samples tested within normal ranges for the JPT products. In FY 2015, one of our network laboratories (Lab 1) began method development for sulfites, sulfates, and bisulfite. More details about the progress of method development are provided in Method Development section of this document.

3.2.13. Flavoring Agents

Rationale

We continued testing flavoring agents included in testing from previous years, since some have shown toxic effects. Maleic acid can cause a Fanconi-like syndrome in dogs after intravenous administration (Al-Bander et al., 1982, 1985). Fumaric acid is approved in the U.S. by the USDA's Meat and Poultry Inspection Division as a curing accelerator used only in combination with curing agents to accelerate color fixing in cured, comminuted meat; meat food; poultry, or poultry products, at a level of 0.065 percent (or 1 oz. per 100 lbs.) by weight of the meat, meat by-products, poultry, or poultry by-products before processing (The Food Chemical News Guide, 2001). Upon heating, fumaric acid converts to the irritant maleic anhydride, which then hydrolyzes to maleic acid. We also wanted to see if these additives have been added to JPT without proper labeling.

Results

In FY 2014, we tested 71 samples (11 store-bought and 60 case-related samples) for malic acid, maleic acid, and fumaric acid. Forty-six samples tested positive for malic acid (35 case-related and 11 store-bought samples). The concentrations ranged from 50 ppm to 16,100 ppm. Maleic acid was not detected in any of the samples. The reporting limit for this maleic acid method was 50 ppm. The reporting limit is the smallest concentration or amount of analyte that can be reported by a laboratory. However, in FY 2015 we began developing a new method that is able to detect lower concentrations of maleic acid in treats. In addition, 8 samples (7 case-related and 1 store-bought) tested positive for fumaric acid (50-180 ppm).

In FY 2015, we tested 50 samples. None of the samples tested positive for Fumaric acid. Malic acid was detected in 44 samples (36 case-related, 7 store-bought, and 1 control sample), in concentrations ranging from 99 ppm to 970 ppm. Results for maleic acid are provided in Method Development section.

As of December 31, 2015, testing is pending for 25 samples submitted in FY 2016.

3.2.14. Tanning Agents

Since previous samples tested negative for this group of analytes, we removed them from our testing list.

3.2.15. Illegal Dye Agents

Since previous samples tested negative for this group of analytes, we removed them from our testing list.

3.3. Chemical Toxicology Testing -Method development

In addition to the existing screens, during FY 2015 and FY 2016, we requested method development for several new and existing analytes. We requested method development for existing analytes to improve method sensitivity, allowing us to better evaluate potential toxic effects.

The list of analytes for method development in FY 2015 and FY 2016 included:

- Maleic Acid
- Diglycolic Acid and Glycolic Acid-
- Sulfite and Bisulfite
- Epichlorohydrin (ECH)
- Selected antibiotics/antivirals
- Bufotenin
- Chaconine
- Glyceraldehyde and other glycerol breakdown products
- Negative Ion LCMS method
- Compound Discoverer Software
- Amikacin
- 3-MCPD and 3-MCPD esters

Following method developments have been completed:

3.3.1. Maleic Acid

Rationale

Maleic acid is an unapproved food additive. Occasional consumption of maleic acid at low levels does not pose any significant health risk; however, long-term consumption or experimental exposure to high levels can cause kidney damage or Fanconi syndrome (Gmaj et al., 1973; Al-Bander et al., 1982, 1985; Worthen, 1963; Hoppe et al., 1976; Bank et al., 1986, Brewer et al., 1993). Recent findings (in May 2013) of maleic acid in foods, such as tapioca starch, tapioca

balls, rice noodles, and hotpot ingredients, caused the recall of many starch-based food products in Asia (Link 3: <http://www.fda.gov.ph/advisories/food/76474-fda-advisory-on-maleic-acid>). Starch is listed as an ingredient on the labeling of some jerky treat products. We began testing treats for maleic acid in 2012, after a Vet-LIRN's network laboratory (Lab 1) developed a method for this analyte. The reporting limit of the method was 50 ppm. All treat samples tested by this method were negative for the presence of maleic acid. A review of available literature indicated that dogs might be more sensitive to maleic acid exposure than previously thought (Berliner et al., 1950; Everett et al., 1993). In order to better evaluate potential toxic effects, we requested further method development for this analyte, with the goal to improve method sensitivity. The laboratory developed a method 100 times more sensitive and can now detect concentrations of 0.5 ppm instead of 50 ppm.

Results

During FY 2015 testing, Maleic acid was found in 6 out of 50 samples (5 case-related and 1 control sample), with concentrations ranging from trace to 21 ppm. The significance of these findings is under evaluation.

3.3.2. Diglycolic Acid (DGA) and Glycolic Acid (GA)

Rationale

DGA and GA were analytes of interest for our investigation since 2013, when we conducted preliminary testing of JPT. A request for further method development came after preliminary results showed that multiple samples tested positive for GA in trace amounts (69 of 71 samples), and 1 sample tested positive for DGA in trace amounts.

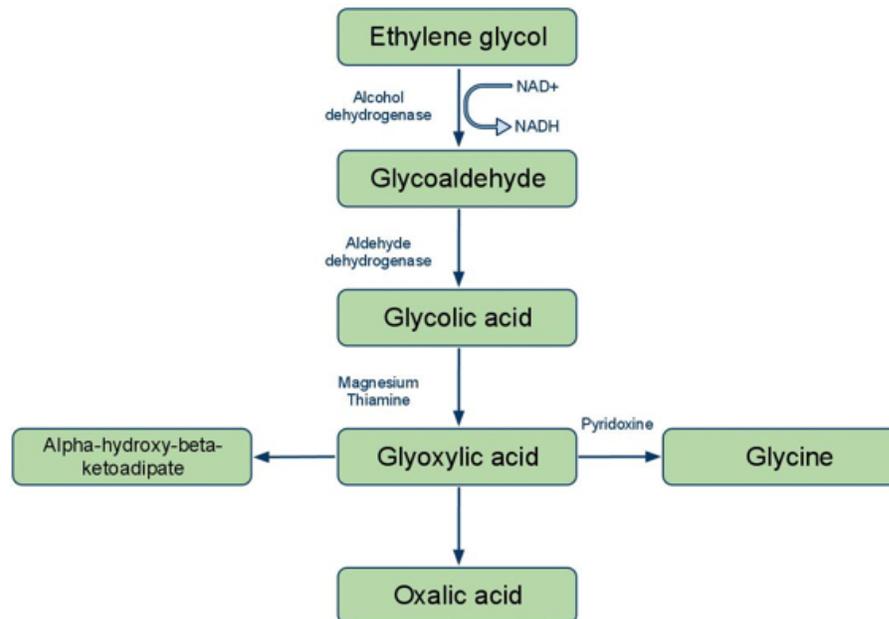
We previously reported that the clinical signs from the consumer complaints are very similar to those associated with antifreeze (EG and DEG) poisoning. Historically, DEG was inappropriately substituted in pharmaceutical preparations for nontoxic constituents, causing multiple epidemics of human poisoning with high mortality rates. Most documented cases of DEG poisoning were epidemics where DEG was substituted for the more expensive, but nontoxic, glycols and/or glycerol. The hallmark of DEG toxicity is acute renal failure. DGA is one of the main nephrotoxic metabolites in DEG poisoning, inducing necrosis in human proximal renal tubule cells in vitro (Landry et al., 2011; Besenhofer et al., 2011).

GA naturally occurs in many foods, including fruit and plants with a high sugar content. GA is also a metabolite of EG, which is converted to calcium oxalate after ingestion (Figure 2). Calcium oxalate is the main metabolite causing renal toxicity from EG (Guo et al, 2007). In 2013, we explored the possibility that during jerky pet treat manufacturing, irradiation, in combination with other factors, could cause glycerol to produce toxic metabolites of EG. The liver may then convert those metabolites to calcium oxalate.

Results

Method development for these analytes has been completed. The reporting limit for GA was lowered to 100 ppm with the sensitivity increased 2 times, and the reporting limit for DGA was lowered to 100 ppm with the sensitivity increased 5 times. For comparison, reporting limits of the initial methods were 200 ppm for GA and 500 ppm for DGA. During FY 2015 testing, all 50 samples tested positive for GA in trace amounts, and they were all negative for DGA.

Figure 2. Ethylene glycol poisoning



(Source: <https://kchemimage.wordpress.com/2015-answers-for-mays-clinical-image/>)

3.3.3. Sulfite and Bisulfite

Rationale

Sulfites are associated with human food intolerance symptoms. They are used as food additives and are naturally found in some foods. The most commonly used sulfite and sulfur containing food additives are sulfur dioxide (SO₂), potassium bisulfite or potassium metabisulfite, sodium bisulfite, sodium metabisulfite, and sodium sulfite. In addition to causing food intolerance (food allergy), sulfites also destroy thiamine (Vitamin B1). In Australia, a number of pet cats and dogs died from thiamine deficiency due to a steady diet of pet meat containing unlisted sulfites (Malik et al., 2005; Singh et al., 2005). Our testing for metals in the past showed that some case-related JPT samples (including sweet potato type treats) had higher (but still within the normal range for complete feedstock feeds) sulfur levels. We hypothesized that higher sulfur levels in the jerky treats may be caused by dipping chicken breasts and/or sweet potatoes in a sulfite/bisulfite containing solution to preserve the products' color.

Results

The sulfite/bisulfite method has been successfully developed and validated. The reporting limit was established at 5 ppm for “sulfites.” The method converts sulfite, bisulfite, and meta-bisulfite to a common form that is then derivatized with formaldehyde. The individual forms of sulfite are not distinguishable by this method. Because the formally accepted AOAC method utilized by FDA labs reports the values of sulfites as “ppm SO₂”, the results were reported in those units as well. All 50 jerky treat samples have been analyzed by this method. Five case-related samples tested positive for sulfites in concentrations ranging from 7.3 ppm to 120 ppm. The same samples were reported positive for SO₂, in concentrations ranging from 5.8 ppm to 96 ppm. Three of 5 positive samples were sweet potato fries type products, with sodium metabisulfite listed on a labeling. We are planning to test more samples for these analytes.

3.3.4. Epichlorohydrin (ECH)

Rationale

ECH is a highly reactive compound, primarily used in the production of epoxy resins used in coatings, adhesives, and plastics. ECH is also used in production of synthetic glycerol, textiles, paper, inks and dyes, solvents, surfactants, and pharmaceuticals. Water causes the hydrolysis of ECH to 3-MCPD (Gaca et al, 2011). ECH may also be formed as a byproduct during some types of glycerol production.

Results

The work was initiated and a preliminary method for ECH was developed. Due to the derivatization of ECH during the extraction phase, this method will be merged with the 3-MCPD method development, as a part of FY 2016 testing. In addition, a separate method will need to be developed for 3-MCPD esters. Initial testing for 3-MCPD was completed by a different laboratory (please see Compositional Testing Section for testing results).

3.3.5. Selected antibiotics/antivirals

Rationale

The majority of JPT products related to consumer complaint reports were imported from China. Although China’s poultry production sector shows a major transition towards development and growth, there are still concerns; a major one is poor farming practices. Some poultry suppliers may use antibiotics or antiviral drugs unapproved in the United States for use in poultry. Meat from these animals may be used for human consumption or for the production of JPT. For example, there was KFC’s “instant chicken scandal” (dubbed “instant” because of the chemicals added to chicken feed in order to make chicken grow faster), which involved addition of 18 different chemicals to chicken feed (eFeedlink, link: <http://www.efeedlink.com/contents/12-20-2012/f83bd748-ca03-41be-8801-6087b68032f4-e931.html>)

Based on this report, and several others, we expanded our testing list of antibiotics and antivirals. The planned list of analytes included: azithromycin, erythromycin, streptozotocin, doxycycline, florfenicol, and amikacin (antibiotics), and ritanovir and acyclovir (antivirals).

Results

Method expansion was successfully completed for azithromycin (antibiotic) and ritanovir (antiviral). We tested 50 JPT samples for azithromycin and ritanovir. Seven case-related samples tested positive for azithromycin, and none tested positive for ritanovir. We do not consider this finding a root cause of the reported animal illness. However, the results are concerning because azithromycin is not approved for use in poultry in the U.S. A list of analytes that could not be incorporated into existing screens is provided in section 3.3.8.

3.3.6. Bufotenin

Rationale

Bufotenin is an alkaloid found in the skin of some toad species, mushrooms, higher plants, and mammals. The name bufotenin originates from the “Bufo” genus of toads, which includes several species of psychoactive toads. Extracts of toad venom containing bufotenin and other bioactive compounds were used for centuries in some traditional Chinese medicines (Xie, 2002). Bufotenin is regulated as a Schedule I drug by the Drug Enforcement Administration (DEA) at the federal level in the U.S., and is therefore illegal to buy, possess, or sell (Title 21 Code of Federal Regulations, DEA Drug Code 7433, link: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1305&showFR=1>). We added this substance to the list of analytes after sources indicated it may be used by some Chinese poultry manufacturers to treat avian influenza and other respiratory diseases (Link 4: <http://www.google.com/patents/CN1562301A?cl=en>, Link 5: <http://www.google.com/patents/CN102920890B?cl=en>).

Results

Method expansion for bufotenin was successfully completed. Out of 50 tested samples, 1 was positive. We do not consider this finding a root cause of the reported animal illness. However, this finding confirms our suspicions of illegal farming practices by some poultry farmers in China.

3.3.7. Chaconine

Rationale

Chaconine and solanine are the main glycoalkaloids naturally present in potatoes. They contribute to a potato’s flavor, but at higher concentrations cause bitterness and toxicity to humans and animals (Korpan et al., 2004). The potato alkaloids affect the nervous system by interfering with the body’s ability to regulate acetylcholine, a neurotransmitter that conducts nerve impulses. Potato glycoalkaloids also disrupt cell membranes. Symptoms of toxicity include headache, nausea, fatigue, vomiting, abdominal pain, and diarrhea. These analytes are

of interest, because many consumers report gastrointestinal symptoms in their pets (vomiting, diarrhea) after feeding sweet potato jerky treats. Since solanine was previously added to the screen, method expansion was done only for chaconine. Glycoalkaloids are stable during cooking and frying but significantly degrade starting around 340 °F (Friedman, 2006). During JPT manufacturing (including the sweet potato type of treats), the dehydration temperature usually does not exceed 160 °F. Glycoalkaloids could still be present in the JPT after manufacturing.

Results

Method expansion for chaconine was successfully completed. We tested 50 samples. None tested positive for either solanine or chaconine.

Method development projects in progress

3.3.8. Amikacin

Rationale

As we described in section 3.3.5 of this document, we expanded our testing list of antibiotics and antivirals. The planned list of analytes included azithromycin, erythromycin, streptozotocin, doxycycline, florfenicol, and amikacin (antibiotics), and ritanovir and acyclovir (antivirals).

Results

The following analytes could not be incorporated into existing screens: erythromycin, streptozotocin, doxycycline, florfenicol and amikacin (antibiotics), and acyclovir (antiviral). The method for florfenicol requires running the LC-MS method in negative ion mode (see section 3.3.10). Work on amikacin method development is still in the planning phase. Work on method development for remaining analytes from the list is currently on hold.

3.3.9. 3-MCPD, and 3-MCPD esters

Rationale

As we previously mentioned in [section 3.3.4](#) of this document, water causes the hydrolysis of ECH to 3-MCPD (Gaca et al, 2011). In addition, studies show that in some foods, 3-MCPD is often found as an ester linked with fatty acids (e.g., mono- or di-esters; Svejková et al., 2004; Weisshaar, 2011; Crews et al., 2013). High levels of 3-MCPD esters are found in edible refined plant oils and fats, and composite food containing them. Lipases can release 3-MCPD from the esters *in vivo*, and a recent study (Abraham et al., 2013) supports equal oral bioavailability of 3-MCPD in the free form and in the ester form.

Results

Work on these methods is still in the planning phase.

3.3.10. Negative Ion liquid chromatography- mass spectrometry (LC-MS) method

Rationale

Briefly, liquid chromatography (LC) separates the components of a sample based on differences in their affinity (or retention strength) for the stationary phase or mobile phase. The machine detects the separated components using ultraviolet (UV) light, fluorescence, or electrical conductivity based on the components' properties. Chromatography offers great resolution. However, accurately qualifying and quantitating substances can be difficult if multiple components elute at approximately the same time, such as during simultaneous multi-analyte analysis. In contrast, mass spectrometry (MS) offers a highly sensitive detection technique that ionizes the sample components using various methods. The machine separates the resulting ions in a vacuum based on their mass-to-charge ratios and measures the intensity of each ion. Since the mass spectra provided by MS can indicate the concentration level of ions at any given mass, it is extremely helpful for qualitative analysis. In other words, the mass information is particular for a specific molecule, and MS enables direct identification of these molecules. Unfortunately, this only applies when measuring a single component. If multiple components are injected simultaneously, it becomes extremely difficult to analyze the spectra. Therefore, LC-MS systems combine the outstanding separation resolution of liquid chromatography with the outstanding qualitative capabilities of mass spectrometry. LC-MS systems can operate in positive or negative ion mode depending of the analytes of interest. Our previous testing was done in positive ion LC-MS mode. However, we have identified several analytes of interest during our investigation (e.g., mycotoxins: deoxynivalenol (DON) and fumonisin, or antibiotic-florfenicol) that require running LC-MS in negative ion mode. DON is one of several mycotoxins produced by certain *Fusarium* species that frequently grow on corn, wheat, oats, barley, rice, and other grains in the field or during storage. DON affects animal and human health causing acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever (Sobrova et al., 2010). Fumonisin and other mycotoxins may have chronic effects on companion animals. A number of surveys have shown that they are present in significant amounts in commercial pet food. Zearalenone and Fumonisin B1 for instance, were found in 84 and 100 percent of samples, respectively (Leung et al., 2006). We explained rationale for florfenicol testing in "Selected Antibiotics/Antivirals" section. Please see that section for more details.

Results

Work on this method is still in the planning phase. We are seeking additional analytes of interest, in addition to those already planned (DON, fumonisin, florfenicol).

3.3.11. Glyceraldehyde and other glycerol breakdown products

Rationale

Since the beginning of our investigation, we focused JPT testing on possible contaminants/toxicants in the main ingredients JPT (e.g., chicken breast, duck breast, sweet potatoes) and some secondary ingredients (e.g., glycerol, food additives/preservatives, food-

flavoring agents, food dyes, and others). This method development focuses on glycerol breakdown products, because glycerol is present in the majority of JPT products, often in quite large amounts (10-20 percent). Information about these breakdown products could provide insight into potential causes of JPT related illness. Figure 1 shows several glycerol breakdown products (Gil et al., 2011). These compounds may form due to glycerol oxidation during storage, during JPT manufacturing, during irradiation, or in combination with other factors (e.g., use of low quality, nonfood-grade glycerol). So far, we have requested method development for GA and DGA (described in separate section of this document), tartronic acid, glyoxylic acid, and glyceraldehyde. As shown in Figure 1, glycerol is converted to glyceraldehyde by simple oxidation. Glyceraldehyde is isomeric with dihydroxyacetone (DHA), which can also be formed by oxidation. We tested treats for DHA in the past, and so far, only 1 sample tested positive in very small amounts (trace).

Results

Method development for glyceraldehyde is underway. Method development was completed for tartronic and glyoxylic acid, with a reporting limit of 500 ppm. Other glycerol breakdown products may also be added to this method development request in the near future.

3.3.12. Compound Discoverer Software

Rationale

Compound Discoverer is new software available from Thermo Fisher Scientific Inc., used for detection and identification of small molecules. The software analyzes analog and mass spectrometer data, detects components with targeted and untargeted mechanisms, and uses very high resolution to perform fine isotope searches.

Results

The method development is underway.

4. Evaluation of Jerky Pet Treat Irradiation

Food irradiation is acknowledged as a safe process to improve food quality by reducing microbial contamination. As we previously discussed in our 2013 rationale document, the literature indicates irradiation of food does not negatively affect dogs. Blood et al. (1966) reported, however, that dogs fed a diet of high-dose irradiated pineapple jam developed glucose in the urine. Current FDA regulations allow a maximum absorbed dose of 50 kGy in animal feed, pet food, and treats.

Currently there are no validated methods to determine the dose of radiation that was used to ensure the product was properly irradiated, not too low and not too high. Until a validated method is available, this aspect of the investigation is pending.

5. Formaldehyde Testing

FDA received many testing suggestions from pet owners and other groups. This testing was done as a response to some of those suggestions.

Rationale

Formaldehyde is used illegally as a food preservative in some countries. In the past 10 years, there were several scandals involving the addition of formaldehyde to food to help extend shelf life. One incident in Thailand identified 11 slaughterhouses that treated (soaked) rotten chicken in formalin (The Nation, 2011, link:

<http://www.nationmultimedia.com/2011/06/16/national/Illegal-business-being-run-by-a-gang-30157928.html>). Ingesting large amounts of formaldehyde causes vomiting, abdominal pain, dizziness, renal injury, and sometimes death. Exposure to formaldehyde is also associated with hepatotoxicity in many species (Beall et al., 1984).

Results

During FY 2014, we tested 6 consumer complaint-related samples. All samples tested negative.

6. Radioactivity Testing

As with formaldehyde testing, this testing was done as a response to test suggestions we received from pet owners and other groups.

Rationale

One of the main presentations of the acute radiation syndrome is gastrointestinal signs (CDC, 2005, link: <http://emergency.cdc.gov/radiation/arsphysicianfactsheet.asp>), including nausea, vomiting, diarrhea, loss of appetite, and abdominal pain. Since approximately 60 percent of the case reports include gastrointestinal signs, we evaluated a number of JPT for radiation.

Results

During FY 2015, we evaluated 15 consumer case-related samples for gross alpha, beta, and gamma radioactivity. Gross alpha analysis of samples did not detect any alpha activity. Gamma-ray analysis results showed existence of potassium (K)-40, which is a naturally occurring radioisotope emitting both gamma ray and beta particles. Gross beta analysis detected beta activity that was consistent with the gamma analysis finding for K-40. These findings indicate that jerky samples are negative for harmful radioactive compounds.

7. Viral Testing

In September of 2014, USDA requested testing of jerky pet treats for porcine epidemic diarrhea viruses (PEDv) responsible for the 2014 USA national outbreak. USDA's epidemiology investigations

and studies suggested one possible scenario for introducing PEDv into the U.S. was through virus-contaminated imported JPT. In 2015, we tested 43 samples (34 case-related, 6 store-bought, and 3 control samples). All were negative for PEDv.

8. References

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