



Canadian Food
Inspection Agency

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Canadian Food Inspection Agency



Our vision:

To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:

Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Recent Feed Contaminant Incidents: Aflatoxin in Corn and Dioxins in Hydrogenated Palm Oil

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AAFCO Laboratory Methods and Service Committee meeting

2015-08-04

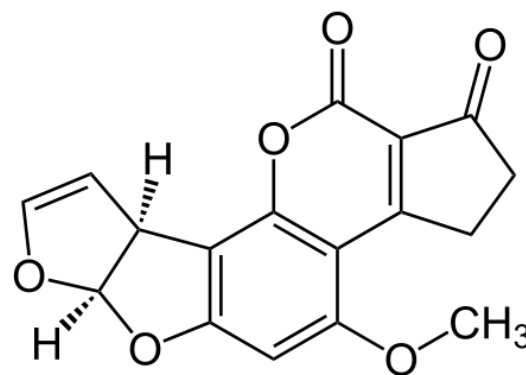
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Case 1: Aflatoxins in organic Indian corn

- Aflatoxins produced by fungi (*A. flavus* and *A. parasiticus*)
- Significant health concern in animals and humans: aflatoxicosis (liver), carcinogenic, immune suppression
- Can end up in meat, eggs and milk products
- An AFLA outbreak in 2003 led to 120 deaths in Kenya
- Canadian limit in feed 20 ppb (total of AFLA B₁, B₂, G₁, G₂)
- Aflatoxin B₁ most toxic and usually most abundant



Aflatoxin B₁

CFIA Monitoring Plan – AFLA in Feed

- CFIA monitors imported feed and feed ingredients for aflatoxins (multi-analyte LC-MS/MS method)
- Small sampling plan: ~30 samples per year, can be grains and single ingredients (plant origin) and mixed feeds
- Aflatoxin contamination can occur in crops, such as corn, from countries with warmer climates (eg. India, China, U.S.)
- No aflatoxin monitoring for samples originating in Canada



Too COLD!!!



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Methodology and Capabilities

- LC-MS/MS multi-analyte method (AB Sciex API 5000):
 - Aflatoxins (AFLA B1, B2, G1, G2)
 - Ochratoxin A (OTA)
 - Zearalenone (ZEA),
 - T2, HT-2, Neosolaniol (NEO)*, Diacetoxyscirpenol (DAS)*

*Used for information only and not reporting

- 50 g sample shaken for 2 hrs in acetonitrile – water 84+16 (v/v)
- Extract diluted 1/10 in 10% methanol in water with 0.1% formic acid and 5 mM ammonium formate
- Isotope labelled internal standards used to compensate for ion suppression/enhancement



Methodology and Capabilities

- Validation results in many feed types and ingredients, including DDGs and corn gluten

Toxin	LOQ (ppb)	% Rec (spike)	CV
Aflatoxin B ₁	1.0	110.2 (4.29 ng/g)	2.1
Aflatoxin B ₂	1.0	105.5 (4.87 ng/g)	3.3
Aflatoxin G ₁	1.0	112.4 (5.41 ng/g)	2.6
Aflatoxin G ₂	1.0	107.7 (3.81 ng/g)	9.4
Ochratoxin A	10	92.7 (33.8 ng/g)	12.6
T2	10	111.9 (59.5 ng/g)	3.5
HT-2	15	105.5 (53.3 ng/g)	6.1
Zearalenone	10	104.9 (100 ng/g)	5.4



Results

- All samples corn from India, most labelled organic

Lab #	AFLA B ₁	AFLA B ₂	Total AFLA	Other Mycotoxins	Area Sampled	Date Sampled
2014-0553	164.4	15	179.4	No	British Columbia	2014-11-03
2014-0880	229.4	18.2	247.6	FUM (0.17 ppm)*	British Columbia	2015-02-03
2014-0881	243.6	17.5	261.1	No*	British Columbia	2015-02-03
2014-0882	379.1	32.1	411.2	No*	British Columbia	2015-02-03
2014-0920	92	7.1	99.1	No*	British Columbia	2015-02-10
2014-0959	26.8	2.1	28.9	No	Quebec	2015-02-20
2014-0985	20	1.4	21.4	OTA (24 ppb)	British Columbia	2015-02-27
2014-1001	8.9		8.9	No	Quebec	2015-03-05
2014-1003	20.3	1.7	22	No	Quebec	2015-03-05
2014-1006	39.5	3.4	42.9	OTA (35 ppb)	Quebec	2015-03-05
2014-1008	29.2	2.5	31.7	No	Quebec	2015-03-05
2014-1119	10.9		10.9	No	Quebec	2015-03-17
2014-1116	9.7	1	10.7	No	Quebec	2015-03-23
2014-1130	10.2		10.2	No*	British Columbia	2015-03-26
2015-0012	49.3	5.7	55	OTA (27 ppb), FUM (0.45 ppm)*	Quebec	2015-03-31
2015-R-0007	8.7		8.7	OTA (45 ppb)	Quebec	2015-05-06

*TRICO7 and FUM methods performed as well



Results

- Some samples 10 to 20 times greater than allowed AFLA levels in feed
- Small amount of other mycotoxins present
- Risk assessment based on levels: foods of animal origin (milk, eggs, meat) for human consumption, determined no risk at levels found
- Animal Feed Division (AFD) wanted a Border Lookout (detain and test)

Testing Challenges

- CFIA AFD wanted to detain all feed corn imports from India to have them tested: CFIA vs private labs
- Proper sampling methods?
- AFD and LCD wanted quick turn-around: 5 days
- Current service standard is 45 days
- Grinding and sample processing was rate-limiting step for the lab (4-6 samples per day/person)
- Possible carryover with these levels? Grind rice to avoid carryover?
- 2 dilutions required to fit B_1 and B_2 onto cal curves

Response and Conclusions

- Border alert published: all corn shipments from India detained and examined (mould, pests, etc)
- Regulated party must take a representative sample (sampling procedure must be provided to CFIA)
- Regulated party must use a laboratory accredited for aflatoxin testing in feed matrices
- Results sent to CFIA (<20 ppb AFLA release)
- Every tenth shipment sampled by a CFIA inspector and sent to the CFIA lab for testing (5-7 day TAT)
- Non-compliant product can't be sold in Canada, nor used to make single ingredient or mixed feeds

Response and Conclusions

- Many questions:
 - What about food? Regulations, food safety concerns
 - What are we seeing now? Not much...
 - Is this still a concern? Don't know...
- Why organic corn from India?

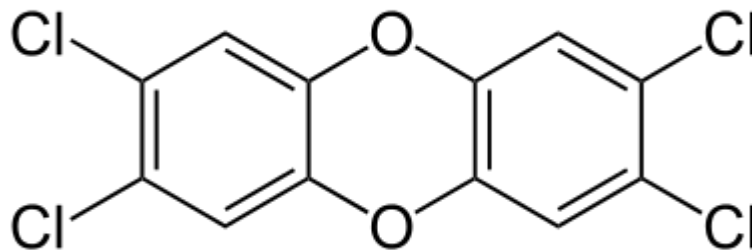
Cheaper to import than produce organic corn in Canada
- CFIA action cited in Forbes online:

Organic Offshoring: As Demand Rises, Increase In Imports Poses Safety Risks

<http://www.forbes.com/sites/stevensavage/2015/06/03/is-the-organic-brand-at-risk>

Case 2: Dioxins in Malaysian Palm Oil

- Dioxins, furans, and PCBs classified as persistent organic pollutants (POPs)
- Highly toxic - cause cancer, damage immune system, cause reproductive and developmental problems
- Bio-accumulate in the food chain, fatty tissue in animals
- By-products of industrial and natural processes (burning)
- 1999 high levels of dioxins in eggs and poultry from several European countries, traced to feed (PCB waste added)
- Estimate that 80% of dioxins in food originate from feed



Dioxin: 2,3,7,8-tetrachlorodibenzo para dioxin (TCDD)

CFIA Monitoring Plan – Dioxins in Feed

- CFIA monitors livestock feed, targeting feed and feed ingredients that have higher dioxin potential
- Sampling plan: ~50 samples per year

fish meal, fish oil, mineral sources, zinc oxide and copper sulfate, mineral complexes (chelates, amino acid complexes, proteinates, etc.) and hydrogenated vegetable (palm) oils, vegetable (palm) oils, palm fatty acid distillates, and palm palmitic acid

- Canadian limits (values in ng WHO-TEQ/kg)

Fish Meal	3	Minerals, complexes etc.	1.5
Fish Oil	16	Vegetable Oils (palm)	0.75 (D&F)
Fish Feed	6	Vegetable Oil By-Products	1.5

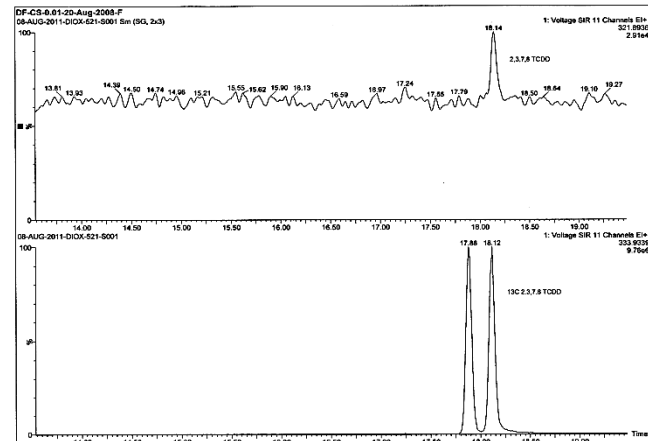
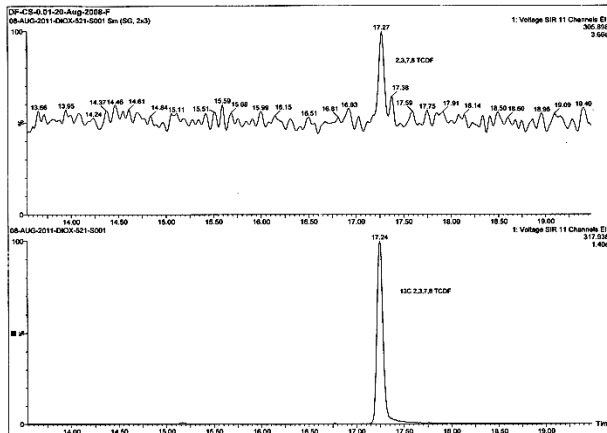
Methodology and Capabilities

- CFIA Calgary laboratory method analyzes for:
 - 7 Polychlorinated dibenzo-dioxins (PCDD)
 - 10 Polychlorinated dibenzo-furans (PCDF)
 - 12 Polychlorinated Biphenyls (PCB's)
- Method is based on US EPA 1613b and MOE 1/RM/19
- 5 g of feed soxhlet extracted in ethanol/toluene (70:30), isotope labelled internal standards added to sample
- Extracts cleaned using a series of chromatographic columns (multi-layer silica, alumina and carbon)
- Cleaned extracts are concentrated and analyzed via GC/HRMS (Waters Autospec-Ultima)



Analytical Challenges

- After hot extraction, samples cooled to gelatinous mass
- Needed to reheat to pass through clean-up column
- Sample would solidify in filter flask when still warm
- Acid treatment (sulfuric) worked but recoveries suffered
- Final solution: smaller sample size; this led to some solidification, but able to clean-up with acid silica column



Results

- Hydrogenated palm oil sampled in May 2014 from a farm in Ontario (farm inventory 17 x 25 kg bags)
- Results in July 2014: 1.793 ng WHO-TEQ/kg (D & F)
- Exceeds 0.75 ng WHO-TEQ/kg (D & F), non-compliant
- AFD took 3 more samples from 3 different lots

Total TEQ (ng WHO TEQ/kg)	Sample 1	Sample 2	Sample 3
Dioxins	1.023	1.567	1.001
Furans	0.139	0.000	0.120
PCBs	0.001	0.001	0.037
Total (Dioxins & Furans)*	1.942	1.727	1.770
Total*	1.994	1.785	1.823

*totals expressed as upper bound limits (DL's included)



Response and Conclusions

- Risk assessment: product intended for lactating livestock, milk transfer model used, no risk found
- After first result, initiated recall (voluntary) at the farm level for that lot of product (early August)
- After 3 more lots found non-compliant, decision to detain and/or recall all production lots in Canada (Sept 2014)
- Product was all over the country (Ont, Que, Sask)
- Tens of thousands of 25 kg bags either detained or recalled (all lots imported since Feb 2014)
- Notification sent to Malaysian authorities that non-compliant product was being returned

Acknowledgements

I'd like to thank the following CFIA personnel for providing the information used in this presentation

- Mélanie Titley, Special Project Chemist, Feed & Fertilizer Chemistry Section – CFIA Ottawa Laboratory (Carling)
- Paul Houle, Supervising Chemist, Environmental Contaminants Group – CFIA Calgary Laboratory
- Jennifer Kormos, Senior Feed Toxicologist, Risk Analysis and Toxicology Section – Animal Feed Division



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