



Allergen Residues in Spices: Discovery, Detection and Risk Assessment

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Outline

- A very brief primer on food allergies
- Peanut in cumin – the starting point (2014)
 - a tale of two very different situations
- Almond in paprika and cumin
 - a case of mistaken identity
- Peanut in garlic powder
 - the ongoing nightmare
- Gluten in various spices

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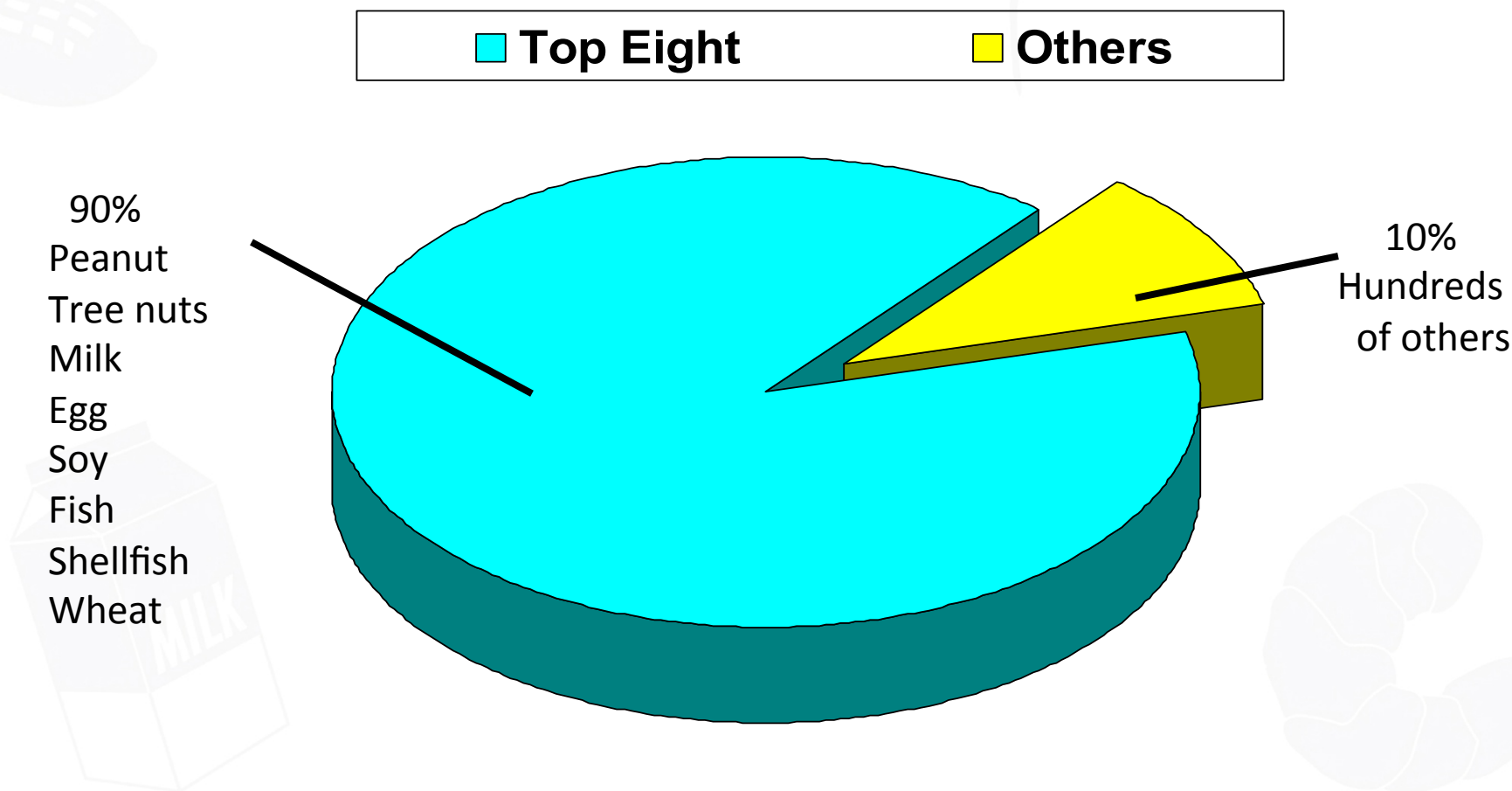


Why Should Spice Industry Be Concerned About Food Allergies?

Why Should Food Allergies & Sensitivities be a Priority Concern for Seasoning Manufacturers?

- Allergic consumers expect it (very aware; high expectations)
- Estimated 10-14 million U.S. consumers have food allergy
- Reactions can occasionally be quite severe, even fatal
- Reactions happen immediately after ingestion
- Threshold dose for provoking a reaction is quite low
- Avoidance is the only strategy for reaction prevention
- Allergic consumers are diligent label readers

Food Allergies Prevalence



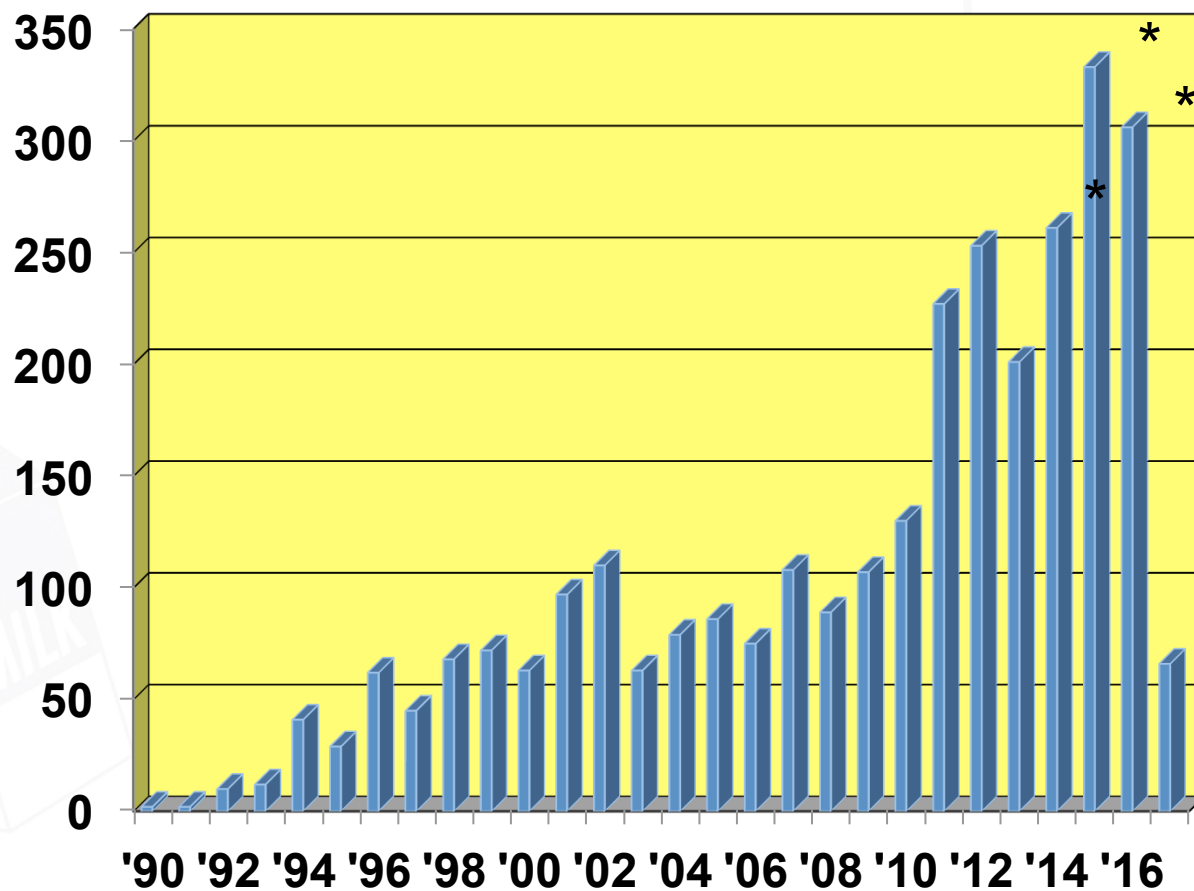
The Risks of Uncontrolled Allergens

- Regulatory risk – undeclared allergens can lead to product recalls, FDA audits, etc.
- Business risk - loss of customers, law suits, failed audits (SQF, etc.), cost of product recalls, loss of consumer confidence, loss of retail space for products with your ingredients, allergen control/sanitation, down time, etc.
- Health risk – undeclared allergens can cause consumers to have reactions (some of which can be severe and even fatal!)

Regulatory Risk

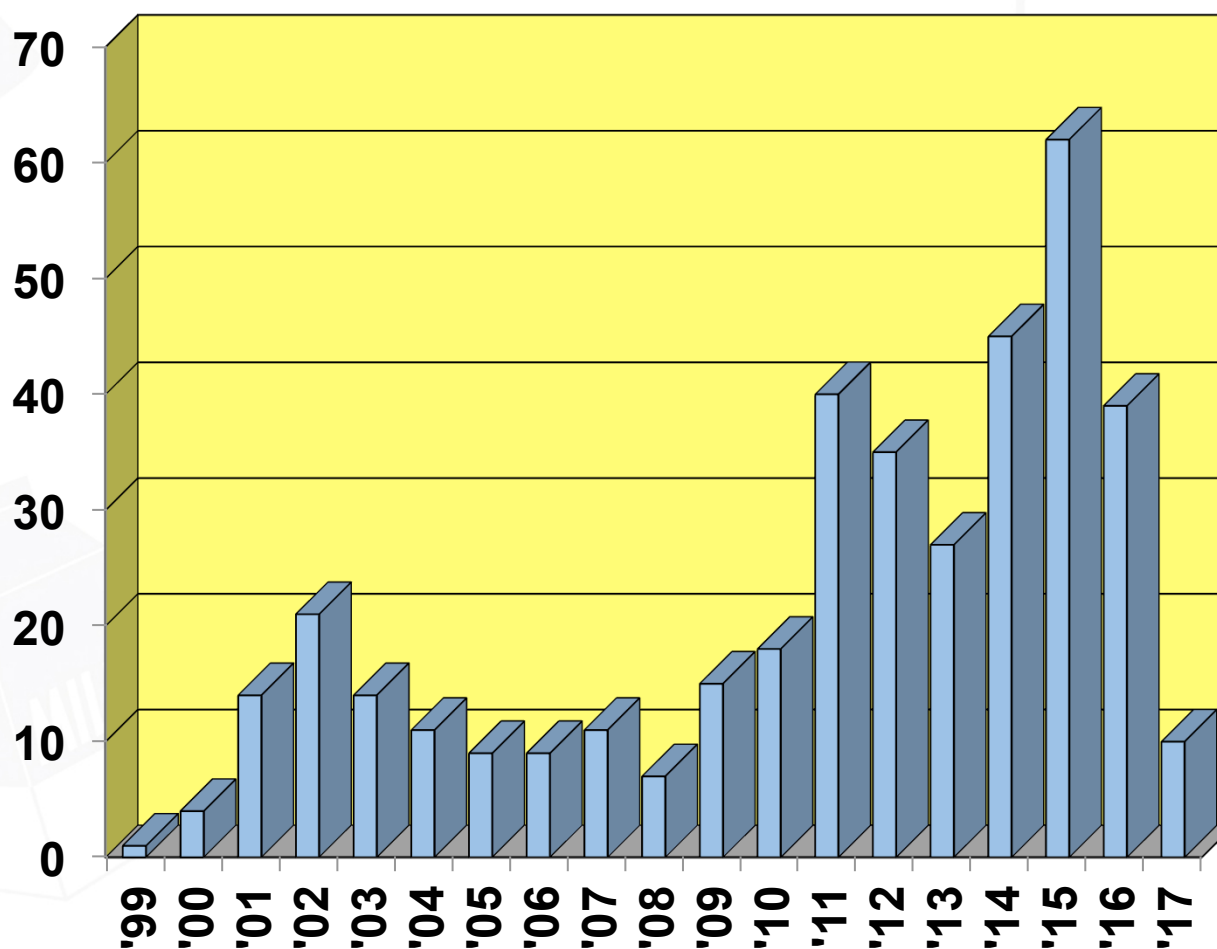
- FDA/USDA put a major emphasis on undeclared peanut residues but consider all of Big 8 as basis for Class I recalls (except wheat and gluten)
- FDA/USDA place a heavy focus on consumer complaints
- FDA/USDA have not established threshold doses so detectable allergen residues can be basis for recalls
- In FARRP experience, FDA does pay some attention on thresholds and risk assessment but only in absence of consumer complaints
- In FARRP experience, USDA pays no attention to thresholds
- FDA/USDA have little understanding of agricultural practice

U.S. FDA Food Allergen Recall Incidents 1988-2017



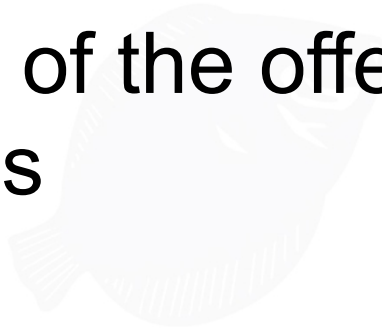
* Includes FDA recalls & alerts

FSIS/ USDA Food Allergen Recalls Calendar Years 1999-2017



Exquisite Sensitivity of Some Food-Allergic Individuals

- Trace amounts of the offending food will trigger reactions



How Much is Too Much?

Milligram amounts!
(ppm concentrations)



Peanut Allergic Patients Present with Different Levels of Sensitivity



Ballmer-Weber and Hourihane

0.2 mg
(0.05 mg)

0.4 mg
(0.1 mg)

1.0 mg
(0.25 mg)

5.0 mg
(1.25 mg)

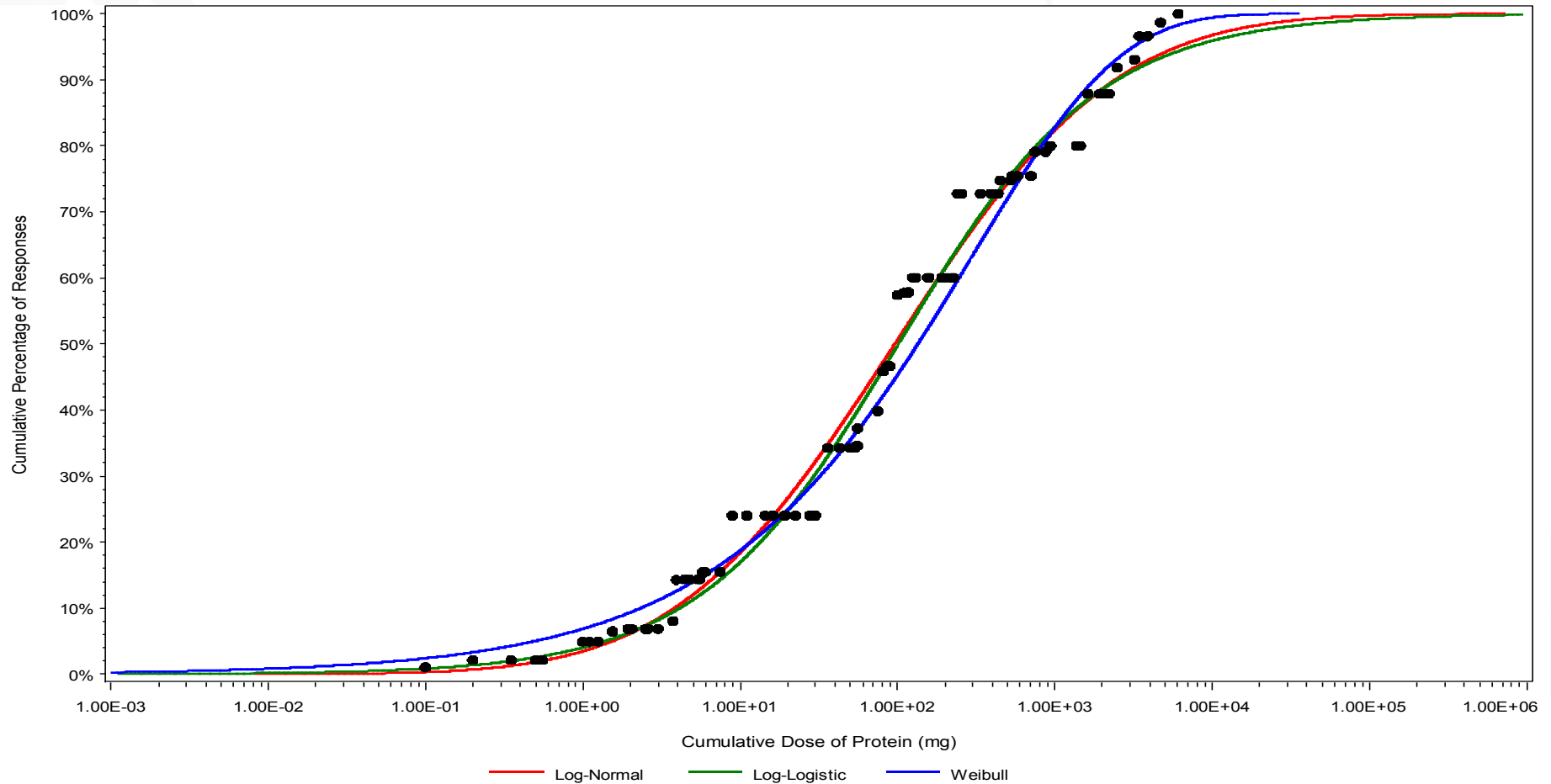
25 mg
(6.25 mg)

100 mg
(25 mg)

400 mg
(100 mg peanut protein)

*0.4 mg peanut (0.1 mg peanut protein) is the eliciting dose of the most sensitive peanut-allergic patient reported in the published clinical literature

Peanut Threshold Population Distribution (expressed as mg peanut protein)



Detection Methods



Detection Methods

- Detect proteins from the allergenic source
- Most common approach is immunoassay (ELISA)
- Specific, sensitive, rugged in some formats
- But not all ELISAs are created equal
- Matrix effects can also be challenging especially when you have a product with reactive chemicals (e.g. spices)
- Matrix effects can cause false negatives or weak false positives

Detection Methods

- Alternative methods include:
 - PCR (detects DNA from source)
 - Mass spectrometry (also detects protein)
 - Total protein from all sources
 - ATP
 - Visual inspection

ELISA and ELISA-Based Technologies Used for Analysis

- Include ELISA kits, lateral flow tests, swabs
- Have become the standard method for allergen validation
- Specific and sensitive
- Rapid analytical assessment
 - 10 min-1 hr analytical process

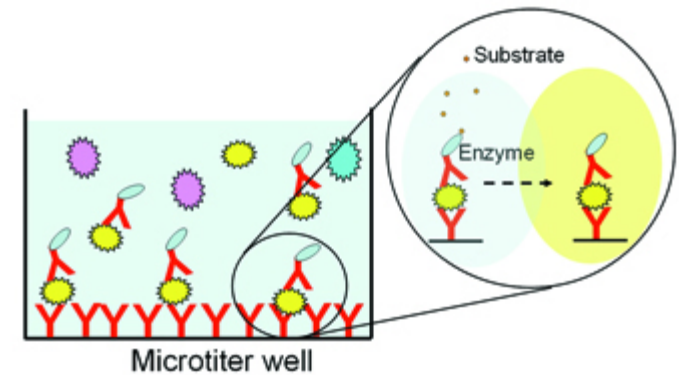
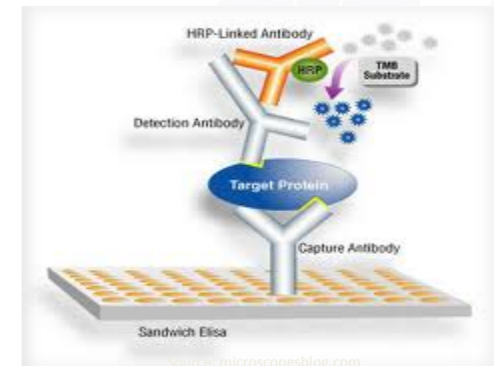


Figure 2: Principle of an ELISA: Allergens are detected by a specific enzyme-labeled antibody and a specific capture antibody on the wall of a microtiter well. After conversion of a substrate by the enzyme, a colored product is formed. The color is read in a microplate-compatible spectrophotometer.



Lateral Flow ELISAs

- Lateral flow device (strip test/ dipstick)
 - Qualitative ELISAs
 - Used primarily for sanitation assessment, but can be used for food product testing
 - 10 minute assay time
 - 5 ppm limit of detection depending matrix

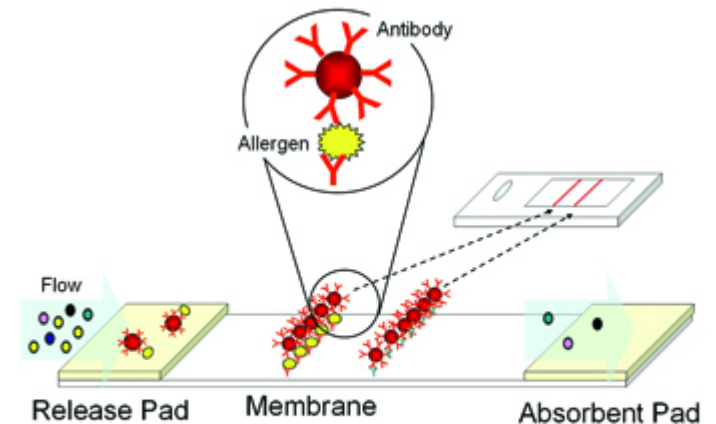


Figure 1: Principle of a strip test: Anti-allergen antibody-coated colored beads form a complex with allergens in the sample and anti-allergen antibodies on the strip. This leads to a colored test line indicating a positive (i.e., allergen-containing) sample. A colored control band indicates correct performance of the test.

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- Gluten in various spices



The Peanut in Cumin Story in North America

Initial Peanut in Cumin Situation

- November 2014 – CFIA conducted a random retail analysis of a taco seasoning product
 - Taco seasoning was positive for peanut (and almond)
- FARRP assisted the company with analysis of retain samples of taco seasoning
 - Concentrations of peanut ranged from 1000 to >5000 ppm peanut using several ELISA kits
 - Individuals ingredients were then analyzed
 - Cumin was found to be positive for peanut (>5000 ppm peanut)
- A recall of taco seasoning and sauce was initiated

Initial Peanut in Cumin Situation

- Late December 2014 – a second series of FDA and USDA-FSIS recalls initiated involving well over 500 products and 30+ companies
 - Concentrations of peanut ranged from 100 to >5000 ppm peanut (FARRP lab and several other contract labs)
 - Back calculation of positive results in some finished products would lead to levels of 50,000 to 105,000 ppm peanut (5-10% peanut in the cumin!!!)
- Ground cumin from sourced from Turkey was implicated in both instances
- FDA did receive consumer reports of alleged allergic reactions from peanut-allergic individuals

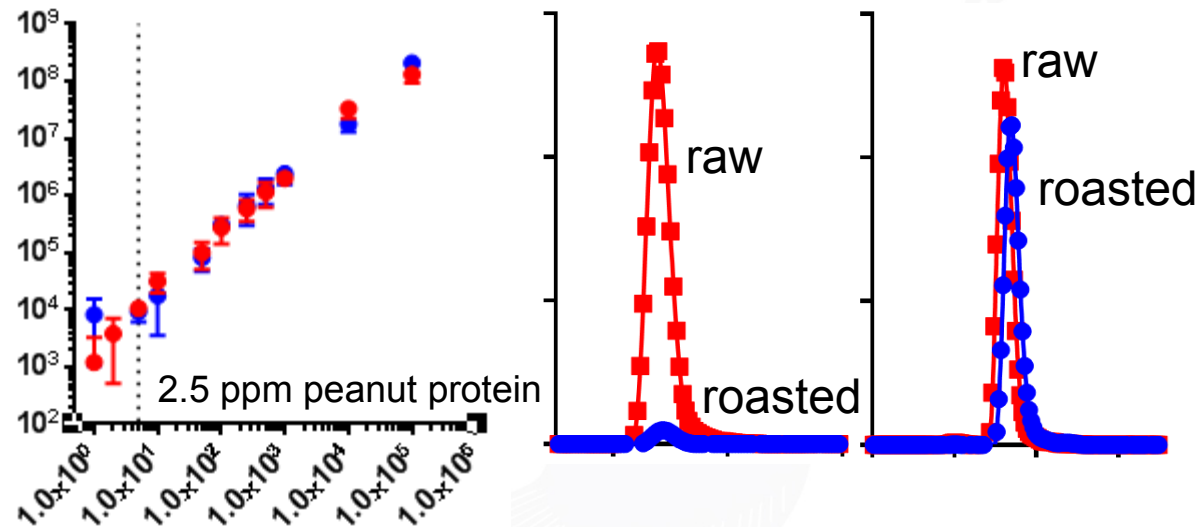
Analytical Assessment

- Quantitative peanut ELISAs were uniformly positive in FARRP Lab and other labs; LFDs also positive
- Some cumin samples contained no detectable peanut so no indication of false positive
- Also conducted an IgE-immunoblot analysis of select cumin samples
 - Binding of IgE from a peanut-allergic individual observed at MW bands of Ara h 1, 2 and 3

Analytical Puzzle Solved

- Commercial peanut ELISAs are excellent way to test cumin samples for peanut; improved extraction may be desirable
- PCR gave variable results but happened because one commercial PCR method does not detect raw peanut
- Mass spectrometry results have confirmed the presence of peanut residues in cumin but it required the development of appropriate method

Finalized method (cumin)



- Uses 4 peptides
- LoD 1 ppm peanut protein.kg cumin-1
- 20 points across peak / 3 or more transitions
- Linear quantitation >4 orders of magnitude (no dilution)
- High sample weight (1g), rapid, inexpensive, simple extraction
- Equivalent detection of raw and processed peanut
- Can distinguish between raw and roasted peanut in cumin

Initial Peanut in Cumin Situation

- Risk associated with 50,000 ppm peanut in cumin
- 50,000 ppm peanut ($\mu\text{g/g}$) x 2% cumin in finished product

= 1000 ppm x 100 g serving of a product

Exposure dose = 100 mg of peanut

(25 mg peanut protein)

Dose of Peanuts Causing Reactions in Peanut-Allergic Individuals



0.2mg

(0.05 mg)

0.4mg

(0.1 mg)

1.0mg

(0.25 mg)

5.0mg

(1.25 mg)

25mg

(6.25 mg)

100mg

(25 mg)

400mg

(100 mg)

Lowest Eliciting Dose in mg whole peanut (mg peanut protein)

Percent of Peanut-Allergic Population That Would React To Dose

0.3%

1%

4.25%

14%

30%

50%

Ballmer-Weber and Hourihane

The Ongoing Cumin Situation

- After the initial series of recalls involving cumin, many companies began testing for peanut residue in cumin and other spices
- Random low level positives were found in whole cumin seed with no visible signs of whole or parts of peanut
 - Generally ranging between 5 and 25 ppm peanut
- Likely due to incidental cross-contact due to agricultural comingling

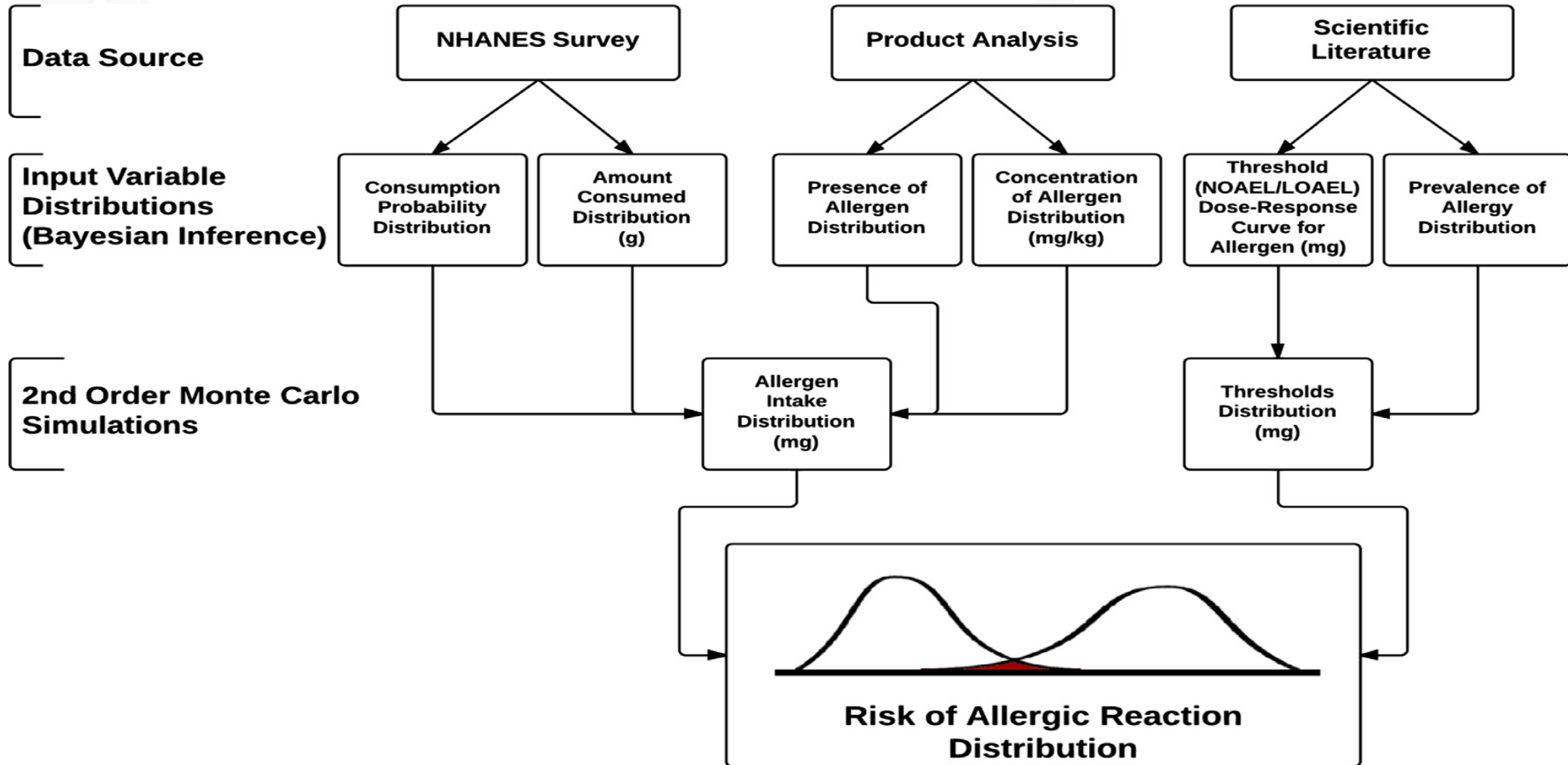
The Ongoing Cumin Situation

- In India, peanuts and cumin grown on same farms
- Opportunities for comingling on farms, in transportation, at the local markets, later
- Shared burlap bags were one focus for comingling
- Some spice companies offered new burlap bags and levels of peanut in cumin dropped
- Occasional positives now (<10 ppm) but most samples have no detectable peanut

The Current Cumin Situation

- Are these low level positive results found in whole cumin seed a public health risk?
- Quantitative (Probabilistic) Risk Assessment can provide a thorough, transparent analysis of the potential risk

Quantitative Risk Assessment



Concentration of Peanut in Tacos

Seasoning Product	ppm Peanut in Cumin	% Cumin in Seasoning Blend	ppm Peanut in Seasoning Blend	% Seasoning Blend in Taco Meat (including water)	ppm Peanut in Taco Meat (including water)	Proportion of Meal Component to Total (highlighted item indicates component that includes seasoning in question)	ppm Peanut in Prepared Taco Product
Taco Seasoning	10	8.4	0.84	6.241	0.052	Taco Meat: 35% Tortilla: 45% Lettuce/tomato: 5% Cheese: 10%	0.018
	25	8.4	2.1	6.241	0.131		0.046

Consumption of Tacos Using the NHANES Dietary Survey

			Daily Consumption Estimates (g)			
Prepared Food Product Category	# of Individuals Who Reported Consuming the Product	Estimated % of U.S. Population that Consume the Product	Average	90th Percentile	95th Percentile	99th Percentile
Tacos	1526	4.63	208	396	489	724

Quantitative Risk Assessment Results - Tacos

Seasoning Product	ppm Peanut in Cumin	Calculated ppm Peanut in Prepared Product	Prepared Product Category	User Risk	Peanut-Allergic Population Risk	Overall U.S. Population Risk
Taco Seasoning	10	0.018	Tacos	2.8 reactions per 1 million peanut-allergic individuals (0.00028%)	1.3 reactions per 10 million peanut-allergic individuals (0.000013%)	1.0 reaction per 1 billion individuals (0.0000001%)
	25	0.046		1.6 reactions per 100,000 peanut-allergic individuals (0.0016%)	7.6 reactions per 10 million peanut-allergic individuals (0.000076%)	6.1 reactions per 1 billion individuals (0.00000061%)

Concentration of Peanut in Chili

Seasoning Product	ppm Peanut in Cumin	% Cumin in Chili Powder	% Cumin in Prepared Chili (includes added cumin as an ingredient + cumin in the chili powder)	ppm Peanut in the Prepared Chili Product
Chili Powder + Cumin	10	6	0.082	0.0082
	25	6	0.082	0.021

Consumption of Chili Using the NHANES Dietary Survey

			Daily Consumption Estimates (g)			
Prepared Food Product Category	# of Individuals Who Reported Consuming the Product	Estimated % of U.S. Population that Consume the Product	Average	90th Percentile	95th Percentile	99th Percentile
Chili	790	2.39	316	572	841	1508

Quantitative Risk Assessment Results - Chili

Seasoning Product	ppm Peanut in Cumin	Calculated ppm Peanut in Prepared Product	Prepared Product Category	User Risk	Peanut-Allergic Population Risk	Overall U.S. Population Risk
Chili Seasoning	10	0.0082	Chili	1.2 reactions per 1 million peanut-allergic individuals (0.00012%)	2.9 reactions per 100 million peanut-allergic individuals (0.0000029%)	2.3 reactions per 10 billion individuals (0.000000023%)
	25	0.021		1.1 reactions per 100,000 peanut-allergic individuals (0.0011%)	2.5 reactions per 10 million peanut-allergic individuals (0.000025%)	2.0 reactions per 1 billion individuals (0.0000002%)

Quantitative Risk Assessment Conclusions

- Trace levels of peanut (2.5 to 25 ppm) in whole cumin that is used in finished products do not present a public health risk based on the clinical threshold information for peanut-allergic individuals
- Regulatory authorities have NOT established regulatory thresholds/action levels for food allergens
 - Products may be subject to recall despite the low levels in both the cumin and finished products

What Can We Do Moving Forward

- Need to have a better understanding of the incidental peanut residue found in whole cumin seed
 - Where is the cross-contact occurring?
 - Can it be controlled?
 - Is the cross-contact due to agricultural practices (i.e. shared agricultural equipment, storage, shipping, etc.)?
- If agricultural comingling is the cause, can we leverage the fact that Congress exempted raw agricultural commodities for source labeling under FALCPA?
 - Ex. Soy in wheat flour, corn flour, etc.

Remaining Regulatory Challenges

- FDA never found any grossly contaminated cumin in analysis done in their labs; FDA seems convinced that the root cause is poor manufacturing practices that can be corrected
- FDA investigation of root cause in Turkey does not seem to have occurred
- FDA does not admit that they have received any legitimate consumer complaints (Marler law suit)
- FDA never considered agricultural comingling as a root cause until FARRP forced this issue in April 2015

Remaining Regulatory Challenges

- FDA has poor understanding of agricultural comingling and the realities of the food chain
- FDA does not recognize the existence of threshold doses and does not employ quantitative risk assessment but they do look at FARRP QRAs
- USDA-FSIS would recall even if non-detectable peanut in product if detectable peanut at any level found in cumin
- FDA would recall if consumer complaints occurred
- FDA defaults to blaming poor GMPs

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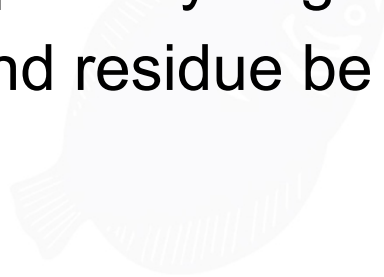
Almond in Paprika and Cumin: A case of mistaken identity?

Almond in Paprika & Cumin

- November 2014
 - CFIA found undeclared almond in a taco seasoning together with undeclared peanut but peanut became the focus
- Late December 2014/early January 2015
 - Retail cumin sample in U.K. tested positive for almond
 - Paprika tested positive for almond by several ELISA methods
 - Levels generally in the 50 to 100 ppm range
 - Situation first developed in the U.K. but also observed in the U.S. and Canada

Almond in Paprika & Cumin

- The paprika and cumin were sourced from Turkey
 - Almonds not commonly processed or handled in facilities where the paprika supposedly originated
 - Where could the almond residue be coming from?



Almond in Paprika & Cumin

- Mahlab spice a potential source of positive almond results???
 - Made from ground cherry pit seeds
 - Cherry belongs to the genus – *Prunus*
 - Includes cherry, plums, peaches, nectarines, apricots, and almonds
- Proteins from closely related species could have sufficient protein homology to cross-react in ELISAs



Almond in Paprika & Cumin

- Paprika that tested positive by several ELISA methods was negative for the presence of almond DNA by PCR
 - ELISA cannot distinguish between cherry pit proteins and almond proteins
 - To my knowledge though, a PCR analysis to confirm the presence of cherry DNA has not been conducted
- CFIA retracted 2 recalls associated with positive almond ELISAs in paprika
 - Utilized a LC-MS/MS method to distinguish almond protein from cherry pit proteins in mahlab

Remaining Analytical Challenges

- Regulatory agencies have now backed off and ascribed the positive ELISA results to undeclared cherry pits and not almond
- Analytical uncertainties do exist because almond and cherry pit are indistinguishable by ELISA
- But Health Canada identified that the positive results were due to cherry pits using mass spec

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Outline

- A brief primer on food allergies
- Should we have really been surprised?

Our experience with gluten and food allergens in unexpected places

- Peanut in cumin?!?!?
 - A tale of two situations
- Almond in paprika and cumin
 - A case of mistaken identity???
- Peanut in garlic powder!! (Oh my!)



Peanut in Garlic Powder The ongoing situation

Peanut in Garlic Powder

- **November 2014**

- FARRP Lab found low undeclared levels of peanut in garlic powder when testing ingredients for company involved in the Canadian taco seasoning recall but focus became very high levels of peanut in ground cumin in that product

- **December 2014/March 2015**

- Very infrequent testing of garlic powder
- A few low positive results were obtained

- **May/June 2015**

- Several FARRP companies find recurrent levels of undeclared peanut in garlic powder at levels ranging from 10 ppm up to >200 ppm
- Source – China garlic

Peanut in Garlic Powder

- **Spice company filed RFR with FDA for peanut in garlic**
 - Their supplier was notified and, in turn, notified all of their other customers of possible undeclared peanut in garlic
 - FARRP Lab analyzed >100 products and found low levels of peanut in garlic but many finished products contained no detectable peanut because of low use level of garlic
 - FARRP conducted about a dozen QRAs for various companies and these were presented to FDA
 - Ultimately FDA quietly indicated that the original garlic supplier could market the lot of garlic powder that originally tested positive for peanut; FDA indicated that there was no need to file RFRs for “low levels” of undeclared peanut in garlic
 - Several companies send investigative delegations to China but no root cause has yet been found for peanut in garlic

Peanut in Garlic Powder

- **Spice importer claims that the peanut ELISA results are false positives**
 - But some garlic powder samples do not contain detectable peanut
 - Positive ELISA results only found in garlic powder and granules; never in flakes, minced or cloves
 - Claim made that sprouted garlic was source of false positive but FARRP disproved that assertion
 - FARRP spiked peanut flour into garlic powder and got excellent recovery and detection by several commercial ELISA methods; confirmed the positive results with mass spec
 - The situation began to subside during first 9 months of 2016
 - But Chinese garlic powder continues to test positive for peanut, although not all lots and perhaps not all suppliers

Peanut in Garlic Powder

- **Round 2**

- At a consumer group meeting in Germany in November 2016, a food manufacturer discloses the presence of undeclared peanut in garlic powder to allergic consumers
- Leads to a wave of testing in EU with findings of positive results
- But FSA has begun to do QRA and appears not very excited about these low levels in garlic powder when there are no consumer complaints
- The EU market is disrupted nonetheless

Peanut in Garlic Powder

- Round 3

- Early 2017, one of the 4 major U.S. importers of Chinese garlic sends a letter to their customers revealing the presence of undeclared peanut in garlic; tells concerned customers to seek QRAs from FARRP
- A similar letter appears in Australia and causes widespread concern and analytical testing of garlic for peanut residues
- Will QRAs have any impact on FSANZ?
- The root cause of peanut in Chinese garlic needs to be revealed and corrected

Gluten in Spices

- With the ongoing popularity of gluten-free diets, many food companies are manufacturing gluten-free options; many companies test all incoming ingredients for gluten
- Occasional positive gluten results are found in various spices but especially small seeded spices
- Could be agricultural comingling but this has not been proven
- Gluten-containing grains are grasses; not surprisingly all grass seeds contain gluten residues
- Therefore the gluten found in spices could come from seeds to wild grasses but this is speculation at this point

Thank You for Your Attention

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