

A GLOBAL PERSPECTIVE ON FOOD ALLERGENS

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Perspectives Require Historical Context

- For the worldwide food industry, the allergen issue emerged slowly at first beginning in late 1980s in several countries but had become a major public health focus in several countries by the late 1990s
- Awareness in the public health agencies began to emerge also in the late 1980s

U.S. – Chaos (1988-late 1990s)

- 8 deaths reported from food allergies by Mayo Clinic group in 1988 – JAMA
- FAAN formed in 1991
- 12 deaths and near-deaths reported by Johns Hopkins group in 1992 – NEJM
- FDA recalls for undeclared allergens begin in earnest in 1992
- FDA Notice to Manufacturers in 1996
- FDA Compliance Policy Guide in 2001
- FDA Guide to Inspections in 2001

International Chaos

- Canada leads the way from late 1980's
 - Severe reaction at food industry party
 - Industry group produces Allergy Beware video
 - Several highly publicized deaths from peanuts
 - Sabrina's Law in Ontario
 - CFIA begins to initiate recalls
 - CFIA institutes “may contain” labeling
 - Schools ban peanuts

International Chaos

- Several deaths occur in U. K.
 - D. Reading's daughter leading to Anaphylaxis Campaign
- Sweden develops allergy death reporting system
- FAO initiates development of Big 8
- Codex Alimentarius Commission adopts Big 8 in 1999
- New regulatory approaches pending in Japan, EU, and Canada

Why Are Allergens Now a Key Issue?

- Increased Awareness
 - Medical Journal Publications 1988, 1992
 - Advocacy Support Groups
- Trend toward “Value Added” Products
- Improvements in Detection
- Company Liability
 - Negative Publicity/Financial Impact

From Early Chaos Came Concern Followed by Commitment and Control

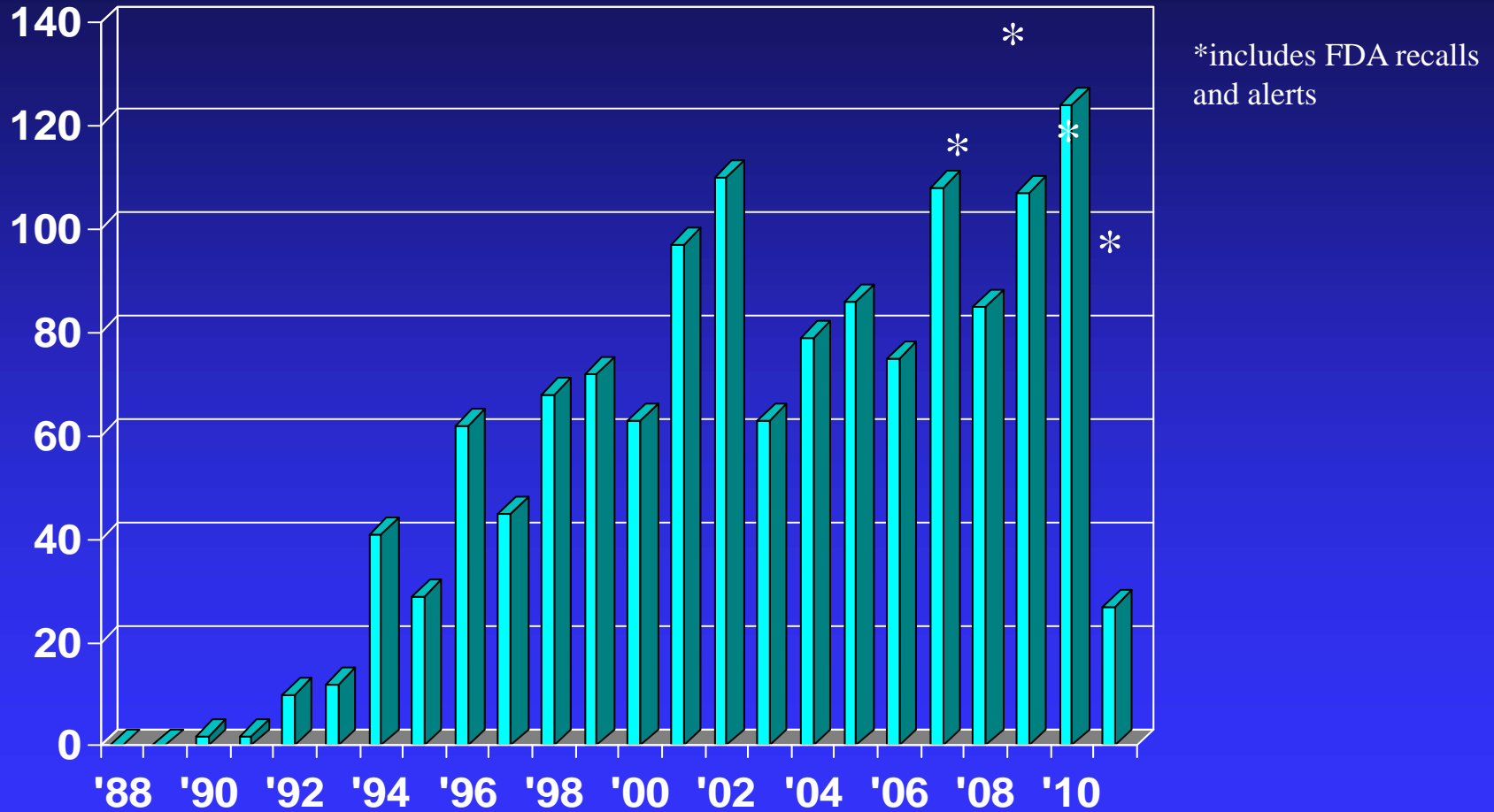
1990 Industry Status on Food Allergens

- Lack of knowledge and awareness
- Lessons from the sulfite issue of 1980's
- Resistance to change
- Complex web
- Lack of recognition of vulnerability
- Focus on the minutiae

Key Food Industry Lessons

- Major company recalls
 - Rework
 - Inadequate cleaning of shared equipment
 - Line cross-overs
 - Packaging errors
 - Ingredient suppliers
 - Custom processors

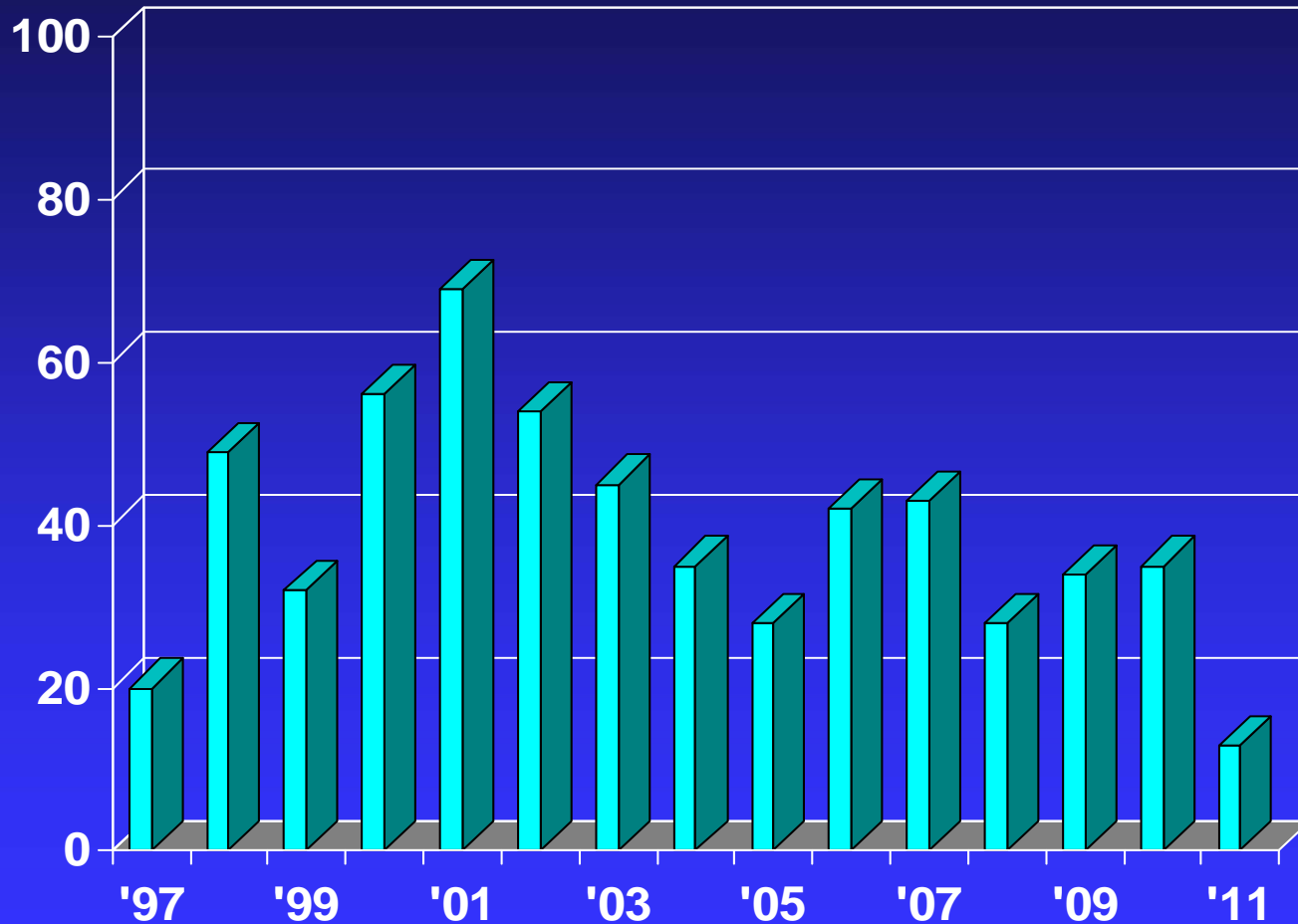
U.S. FDA Food Allergen Recall Incidents 1988-2011



Canadian Food Inspection Agency

Food Allergen Recalls

Calendar Years 1997-2011



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Food Industry Response

Commitment

- Institution of improved GMP's
- Institution of improved sanitation practices
- Changes in facility and equipment design
- Employee and management training
- Food Allergy Issues Alliance
- Industry support for FAAN
- Creation of Food Allergy Research & Resource Program

FARRP

- Food Allergy Research and Resource Program
- University of Nebraska
- Food industry consortium created to address/support research and methods development for food allergen issues
- Formed in 1996 (now has 57 member companies from 11 countries)

FARRP Research

- Develop the tools for industry to use to assess and control allergen risks – analytical detection methods, sanitation strategies, etc.
- Develop risk assessment approaches that allow appropriate management of the allergen issue for balanced protection of allergic consumers and maintenance of quality of life

Detection of Allergenic Food Residues

Detection of Allergenic Food Residues

- First method (Skerritt ELISA for gluten) was published in 1990; commercialized soon after
- First peanut ELISA (Neogen) marketed in 1996
- Now – many different methods and formats from numerous companies from around the world
- The food industry now has the analytical tools needed to detect allergen residues

Detection of Allergenic Food Residues

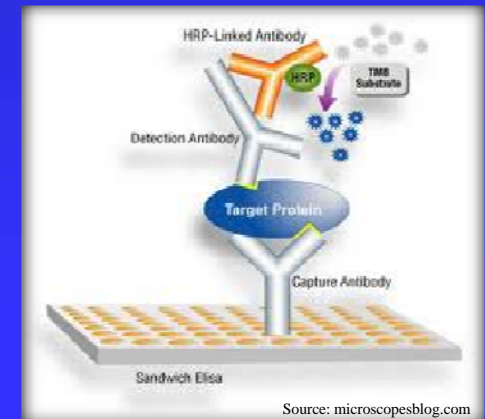
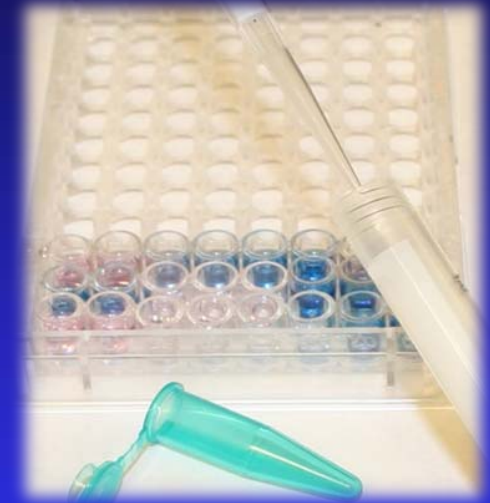
- But be careful!!
- All allergen detection methods are not created equal!!
- The right choice for one application may be wrong for another!
- Results can differ qualitatively and quantitatively
- Food industry ability to select the best method and interpret results still well short of ideal

Detection Methods

- Enzyme Linked Immunosorbent Assay (ELISA)
- General Protein Tests
- ATP/Bioluminescence Tests
- Polymerase Chain Reaction (PCR)

ELISA and ELISA-Based Technologies

- Include ELISA kits, lateral flow devices (dipsticks), swabs
- Current state-of-the-art
- Specific
- Sensitive
- 5 min-6 hr analytical process



ELISA

- Specific – detects protein(s) from source; not always specific for an allergenic protein but that is rarely an important concern
- Sensitive (low ppm and could be less)
 - FARRP/Neogen methods – Limit of Quantitation (LOQ) of 1-2.5 ppm
 - No clinical reason to “chase molecules”
- Quantitative (96 well) and Qualitative (lateral flow and swab) formats

Commercial ELISAs

- Peanut
- Milk
- Egg
- Gluten
- Almond
- Hazelnut
- Walnut
- Soybean
- Crustacea
- Mustard
- Lupine
- Sesame seed
- Buckwheat

FARRP Confidential Analytical Testing: ELISAs

Fully Developed

- Peanut
- Milk
- Egg
- Processed Soy
- Soy Flour
- Almond
- Hazelnut
- Shrimp Tropomyosin
- Lupine
- Sesame
- Gluten/Gliadin (wheat, barley, rye)
- Buckwheat*
- Walnut*
- Mustard
- Clam*
- Pecan*
- Cashew*

*In-house ELISAs

In Development

- Pistachio**

**in use for analysis

All ELISAs Are Not Created Equal

- Specificity
- Sensitivity
- Format
- Quantitative vs. Qualitative

ELISA Points of Difference

- Antibody Specificity – total protein vs. allergen
- Polyclonal vs. Monoclonal
- Calibrators
- Effects of Processing on Detection
- Extraction Methods
- Sensitivity Limits

Key ELISA Decisions

- What do you want to measure?
 - Select appropriate detection system according to major components in the product
 - Example: Milk
 - Neogen: Total Milk
 - r-Biopharm: β -lactoglobulin
 - ELISA Systems: Casein
- What protein source is used as the standard in the method?
- What units are the results reported in?
 - Example: ppm casein or ppm NFDM

ELISA Specificity

- Total Peanut vs. Ara h 1
- Total Milk vs. Casein vs. β -Lactoglobulin
- Soy Flour vs. Processed Soy

FARRP/Neogen Corp. Collaboration

- Allergen ELISA test kits (Quantitative - Veratox®)
 - Almond, Casein, Egg, Gliadin, Gliadin R5, Hazelnut, Lupine, Total Milk, Mustard, Peanut, Soy Allergen, Soy Flour)
 - Limit of Quantitation: 2.5 ppm
 - Extensively validated by Neogen and FARRP in a variety of food matrices using standards developed by the food industry
 - 15 min sample prep; 30 minute assay time



FARRP/Neogen Corp. Collaboration

- Allergen ELISA test kits (Qualitative - Alert ®)
 - Almond, Egg, Gluten/Gliadin, Total Milk, Peanut, Soy Allergen, Soy Flour
 - 2-15 min sample extraction time; 30 minute assay time
 - Color compared to 5 or 10 ppm standard (+/- assessment)
 - Used primarily for sanitation assessment



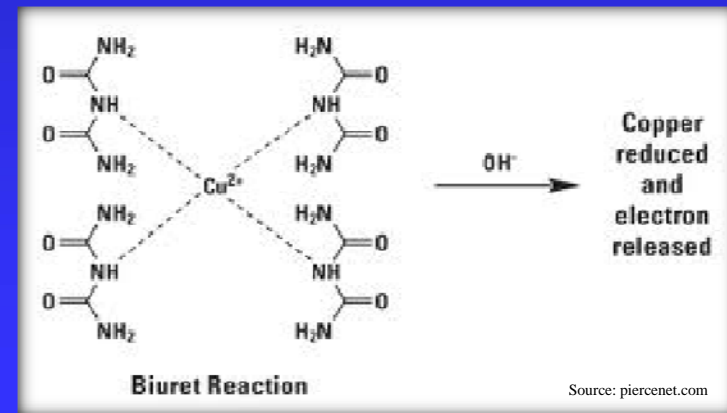
FARRP/Neogen Corp. Collaboration

- Allergen ELISA test kits (Qualitative – Reveal® 3D)
 - Almond, Casein, Egg, Gluten, Hazelnut, Peanut, Shellfish, Soya, Total Milk
 - Lateral flow device (strip test/ dipstick)
 - 10 minute assay time
 - 5 ppm limit of detection depending on food matrix
 - Used primarily for sanitation assessment, but can be used for food product testing



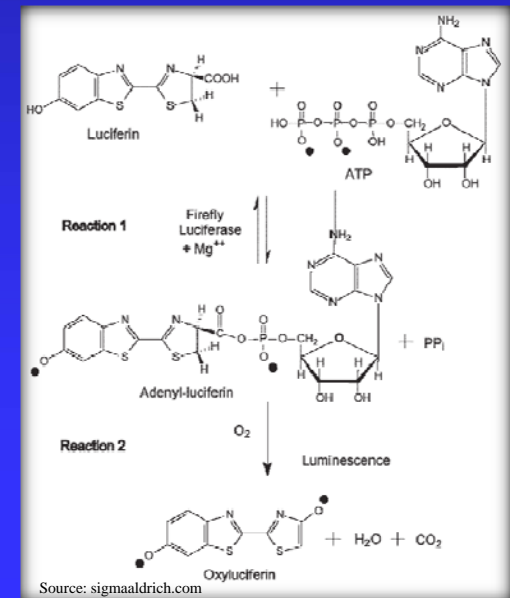
General Protein Tests

- 3M™ Clean-Trace™ Surface Protein (Allergen)
 - Swab method for detection of protein
 - Based on biuret/BCA reaction
- Detects protein regardless of source but not specific for allergenic source of protein
- Detection limits not low enough for allergen detection
 - limit of detection: 3-20 μg protein
- May not correlate to allergen ELISAs



ATP/Bioluminescence Tests

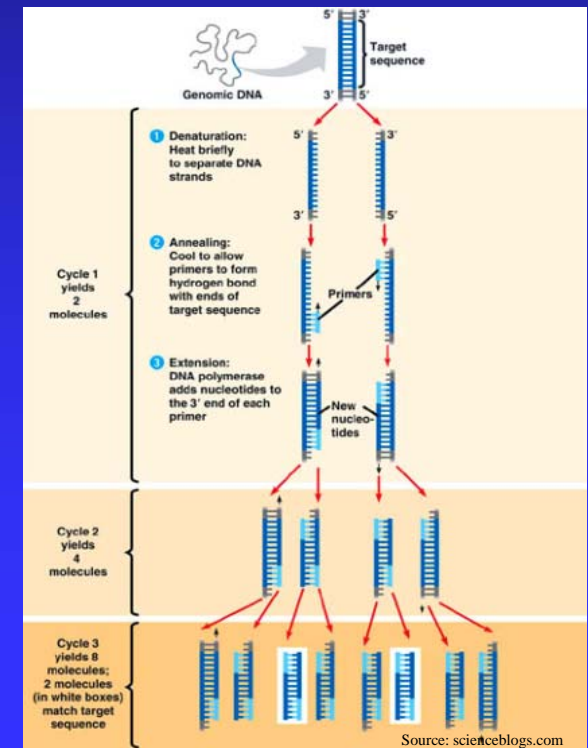
- None are specific for allergens
- ATP levels vary between foods
- Does not prove presence of protein
 - Protein makes the problem with allergens
- Have not been shown yet to correlate with specific ELISA tests in research so far



PCR

- Specific – to the source but not to the allergenic proteins
- Sensitive (very)
- Semi-quantitative
- Depends on specific DNA primers
- Available for many allergenic food sources
- Rapid detection and can be adapted for multiple screens (e.g. detection of several tree nuts)

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PCR Methodology

- PCR (DNA) tests available for many allergenic foods but must send out samples
 - Not practical for in-plant use
 - expensive equipment required (>\$30,000 USD)
 - isolated lab required to avoid contamination
 - Does not prove presence or absence of protein/allergen

Issues with PCR

- These cannot be differentiated by PCR
 - Beef/milk
 - Egg/chicken

Things You Can Test

- CIP rinse water
- Equipment surfaces
- Environmental surfaces
- In-process product (“throwaway”)
- “Push-through” – product, ingredient, etc.
 - Ice, salt, flour, other things used to “scour” equipment

Status of Allergen Testing in U.S.

- Many companies are testing for allergen residues
- ELISA or lateral flow-type is the preferred method
- Some do in-house testing, others use contract labs
- Most companies are not testing finished product
 - Are testing to validate sanitation methods
 - environmental swabbing
 - push-through materials
 - Some testing of finished product advised after sanitation methods are validated

Thresholds and Risk Assessment

How Much is Too Much?

How Clean is Clean Enough?

When is it Appropriate to Apply an
Advisory Label?

Today's Situation

- We live in a world without thresholds where uncertainty abounds regarding the safety (or lack of safety) of various products for food-allergic consumers
- In some countries (e.g. USA), that world is reasonably safe (at least for packaged foods) but loaded with restricted choices

The Ideal Future

- We establish finite thresholds based upon scientifically defensible clinical data
- We then become much more certain about the level of risk posed by any given product for food-allergic consumers
- The world remains reasonably safe (at least for packaged foods) with many fewer restrictions
- All countries are equally safe with respect to food allergies

Why Are We Interested in Thresholds? Circa 2000

- ❖ Very small amounts of specific allergens can provoke reactions in some individuals, but
 - we don't know in how many
 - we don't know how small the amounts are
 - we don't know how severity of reaction relates to an individual's sensitivity
 - allergic people are known to react differently on different occasions

So it is difficult to assess how much needs to be done to achieve the desired level of safety with respect to allergens.

Terminology

- NOAEL = no observed adverse effect level
the highest amount that an individual can tolerate before experiencing symptoms
- LOAEL = lowest observed adverse effect level
the lowest dose that would provoke an allergic response in an individual
- Objective NOAEL/LOAEL – based on observable symptoms
- Subjective NOAEL/LOAE – based on non-confirmable response

Terminology

- Individual Threshold – LOAEL or NOAEL for an individual patient
- Population Threshold – LOAEL or NOAEL for a group of food-allergic individuals
 - all peanut-allergic individuals
 - peanut-allergic individuals in a particular clinic or group/sub-group

Terminology

- Regulatory Threshold – an allowed amount or concentration that would be safe for the vast majority of individuals in a group e.g. peanut-allergic consumers
 - based upon population NOAEL/LOAEL and risk assessment modeling
- Food Industry Threshold – an amount or concentration that triggers labeling to protect the allergic consumer; predicated upon regulatory threshold where such thresholds exist

Historical Approach to Dose/Response

- Physicians recommended complete avoidance (ZERO threshold)
- Ingestion of small amounts (not well defined) could elicit allergic reactions
- DBPCFC was the gold standard for diagnosis but challenges often started at 400 – 500 mg
- 20%+ of patients reacted to first challenge dose – some severe rxns

Historical Approach to Dose/Response

- Peanut-allergic consumers have practiced complete avoidance (zero threshold)
- Peanut-allergic consumers still experienced occasional allergic reactions (hidden ingredients, cross contact, FOOD SERVICE)
- Unexpected allergic reactions to peanuts were occasionally severe leading to widespread belief that low doses elicited severe reactions

Status of Dose/Response Knowledge circa 2005

- Trace amounts (low mg) can elicit allergic reactions; individual thresholds variable
- A few clinics started doing very low dose DBPCFC and proved that safe doses exist for every subject and that severe reactions did not occur at very low doses (low mg)

Current Situation

- Public health authorities have not established regulatory thresholds for peanut or other allergenic foods
- Labeling regulations in some countries based on de facto zero threshold
- Industry acutely aware of allergens, no guidance on thresholds so rampant use of precautionary/advisory labeling

Current Situation

- Quality of life for food-allergic consumers suffers partially as a result of seriously restricted food choices
- Some food-allergic consumers ignore products with precautionary labels
- Some physicians advise food-allergic patients to ignore precautionary labels
- Allergic reactions continue to occur but rarely with packaged foods (USA)

US FDA Allergen Thresholds

- Threshold Working Group Report
- “Approaches to Establish Thresholds for Major Food Allergens and for Gluten in Food” (March, 2006)
(Journal of Food Protection, Vol. 71, No. 5, 2008, Pages 1043–1088)

Current Focus

- The FDA Threshold Working Group Report of 2006 favored use of the quantitative risk assessment-based approach
- QRA based on knowledge of individual threshold doses within the overall population of individuals with a particular food allergy and then uses statistical dose distribution modeling
- Very data intensive!!

FDA Conclusion

- Conclusion Finding 4 – ‘the quantitative risk assessment-based approach provides the strongest, most transparent scientific analyses to establish thresholds for the major food allergens. However, . . . the currently available data are not sufficient to meet the requirements of this approach. A research program should be initiated to develop applicable risk assessment tools and to acquire and evaluate the clinical and epidemiological data needed to support the approach.’”
- Do we have or can we create enough data to use this approach?

The FARRP Approach

- Acknowledge help from Unilever (Rene Crevel and David Sheffield), FARRP colleagues (Joe Baumert, Jamie Kabourek, and Ben Remington) and ILSI-North America
- First attempt – peanut
- Peanut - prevalence, severity, and likely availability of data
- Use of this approach outlined in recent publication: Crevel et al. Food Chem. Toxicol. 45:691-701 (2007)
- Can we find enough data points in the literature to use this model? Uncertainties? Data gaps?

FARRP Peanut Threshold Study

Task #1

- Mined individual NOAEL and LOAEL data for peanut from existing published literature
- Focused on objective NOAELs and LOAELs
- Taylor SL, Crevel RWR, Sheffield D, Kabourek J, Baumert J. 2009. Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals. *Food Chem. Toxicol.* 47:1198-1204.

ILSI- FARRP

Peanut Threshold Study

Task #2

- We mined additional existing but unpublished clinical data on individual threshold doses
- In examining clinical literature, determined that clinical group in Nancy France might have data on low dose challenges of large numbers of peanut-allergic subject that have not been published
- We had only gleaned 21 individual thresholds for peanut from 3 publications from this group in Task #1
- Obtained data on 286 peanut-allergic subjects!!
- Limited selection bias because everyone challenged

Log-Normal (expressed as peanut)

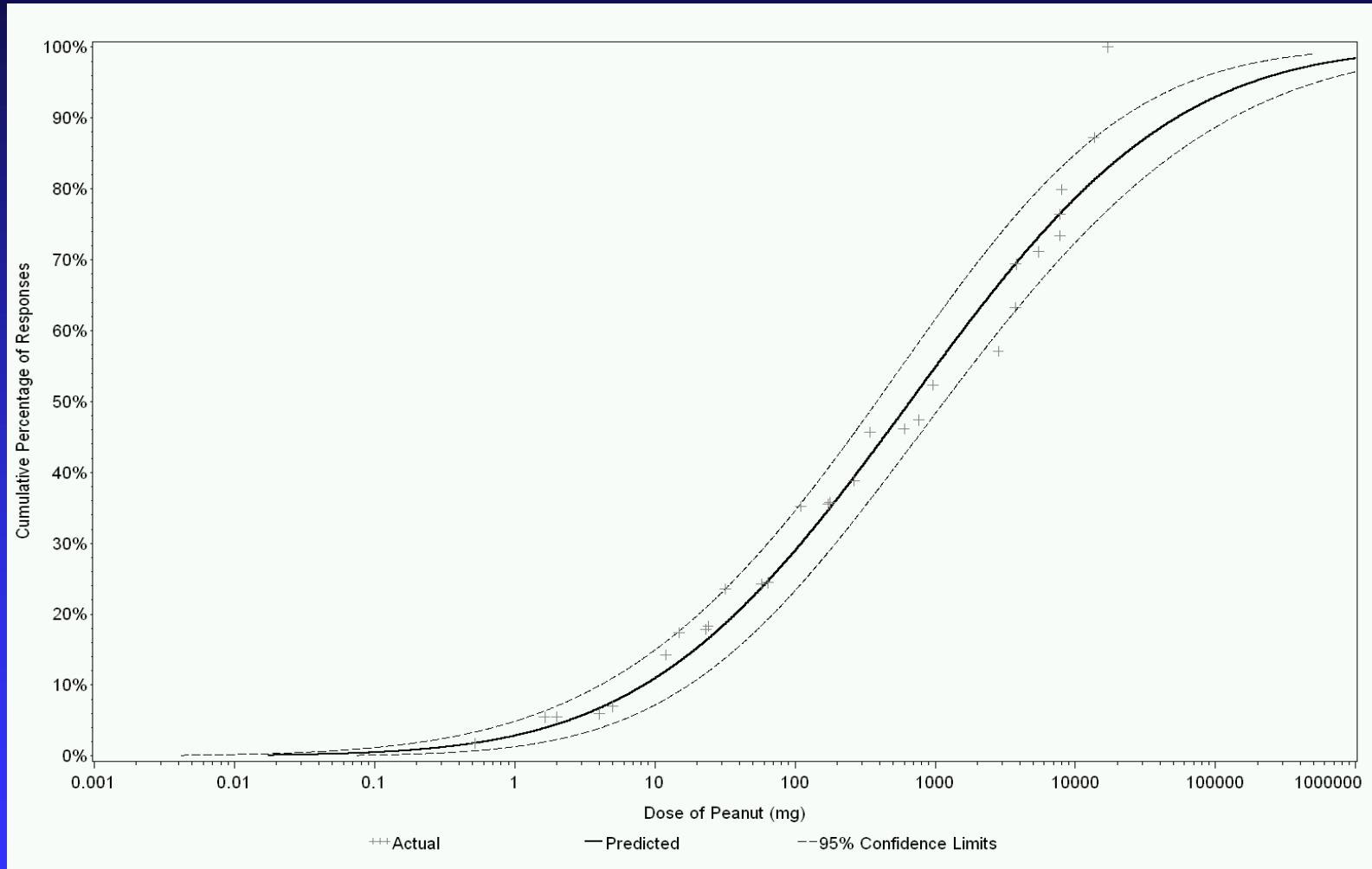


Table 2. ED₁₀ and ED₀₅ Doses for Whole Peanut as Assessed by the Log-Normal Probability Distribution Models

Source	Total No. of Peanut Allergic Individuals	ED₁₀	95% CI	ED₀₅	95% CI
Nancy Data	286	14.4	10.7, 19.6	7.3	5.2, 10.4
Published Papers¹	164	14.1	6.6, 29.9	4.2	1.7, 10.1
Combined	450	12.3	9.0, 16.8	5.2	3.6, 7.4

¹Nine published studies yielded NOAELs and LOAELs for 164 peanut-allergic individuals. Twenty-one individuals from 3 papers (A, B, and D; See Taylor et al., 2009) were excluded from analysis to avoid potential duplication of individuals as these studies included individuals from the Nancy clinic.

All values reported in mg of whole peanut

Table 4. ED₁₀ doses for whole peanut as assessed by the log-normal probability distribution model for severity grade.

Severity Grade	Total No. of Peanut Allergic Individuals	ED₁₀	95% CI
Severe¹	40	10.4	4.8, 22.6
Non-Severe²	123	10.2	6.4, 16.1
No Prior History³	123	27.0	17.4, 42.0

¹Severe reactions include three organ systems, asthma requiring treatment, laryngeal edema, and/or hypotension.

² Non-severe reactions include one or two organ systems, abdominal pain, rhinoconjunctivitis, urticaria, eczema, non-laryngeal angioedema, and/or mild asthma (peak flow rate <80%)

³History of prior allergic reactions and severity of reactions were not available. These individuals were identified as being sensitized to peanut by means of diagnostic tests.

All values reported in mg whole peanut

Task #2

- Taylor SL, Moneret-Vautrin DA, Crevel RWR, Sheffield D, Morisset M, Dumont P, Remington BC, Baumert JL. 2010. Threshold dose for peanut: risk characterization based upon diagnostic oral challenges of a series of 286 peanut-allergic individuals. Food Chem. Toxicol. 48:814-819.