



# Government Chemist Dissemination Event 2008

## Referee Case Update

**Michael Walker**



## Intend to cover

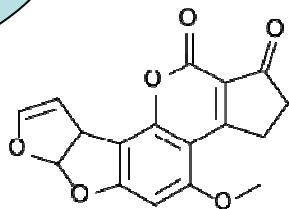
- Objectives
- Process
- Critical issues
- Examples
- Conclusions



# Objectives of Food Law

Consumer Safety  
e.g. Toxic chemicals

Consumer Choice  
VFM for consumers  
e.g. meat speciation,  
irradiation



Health Improvement  
Dietary goals  
e.g. Na

Source  
Dr David Jukes Reading University



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# Food Standards and Safety

Government  
Chemist

Local Authorities  
Public Analysts  
Defra, HPA

Codex  
Europe  
FSA

Enforcement

Policy

Consumers

Food Industry



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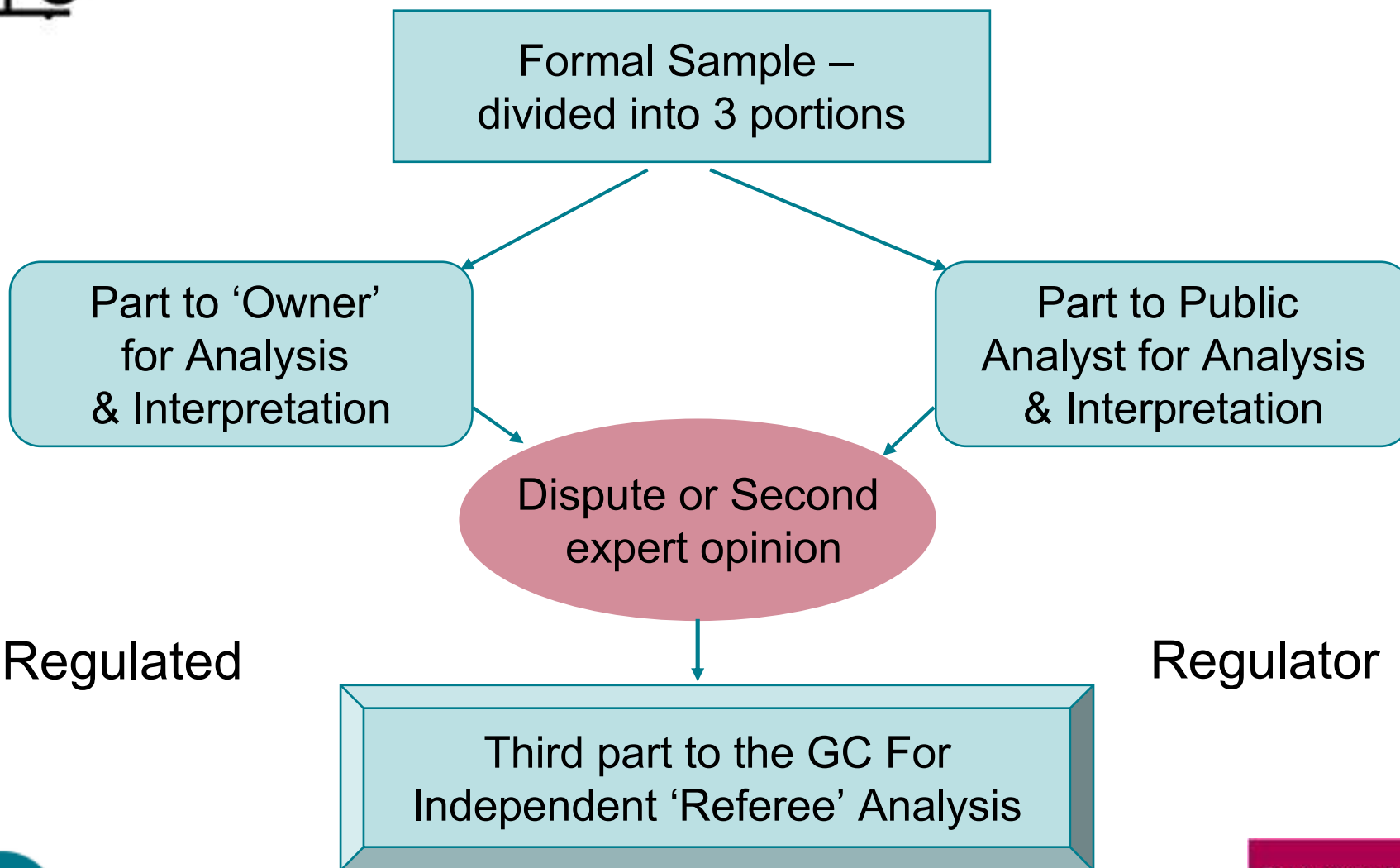
# Objectives of Referee Function

Prevent Disputes  
Method development  
Current awareness  
Dissemination

Assist the court in cases of dispute  
Maintenance of sample integrity  
Accurate and precise results  
Valid, informed interpretation

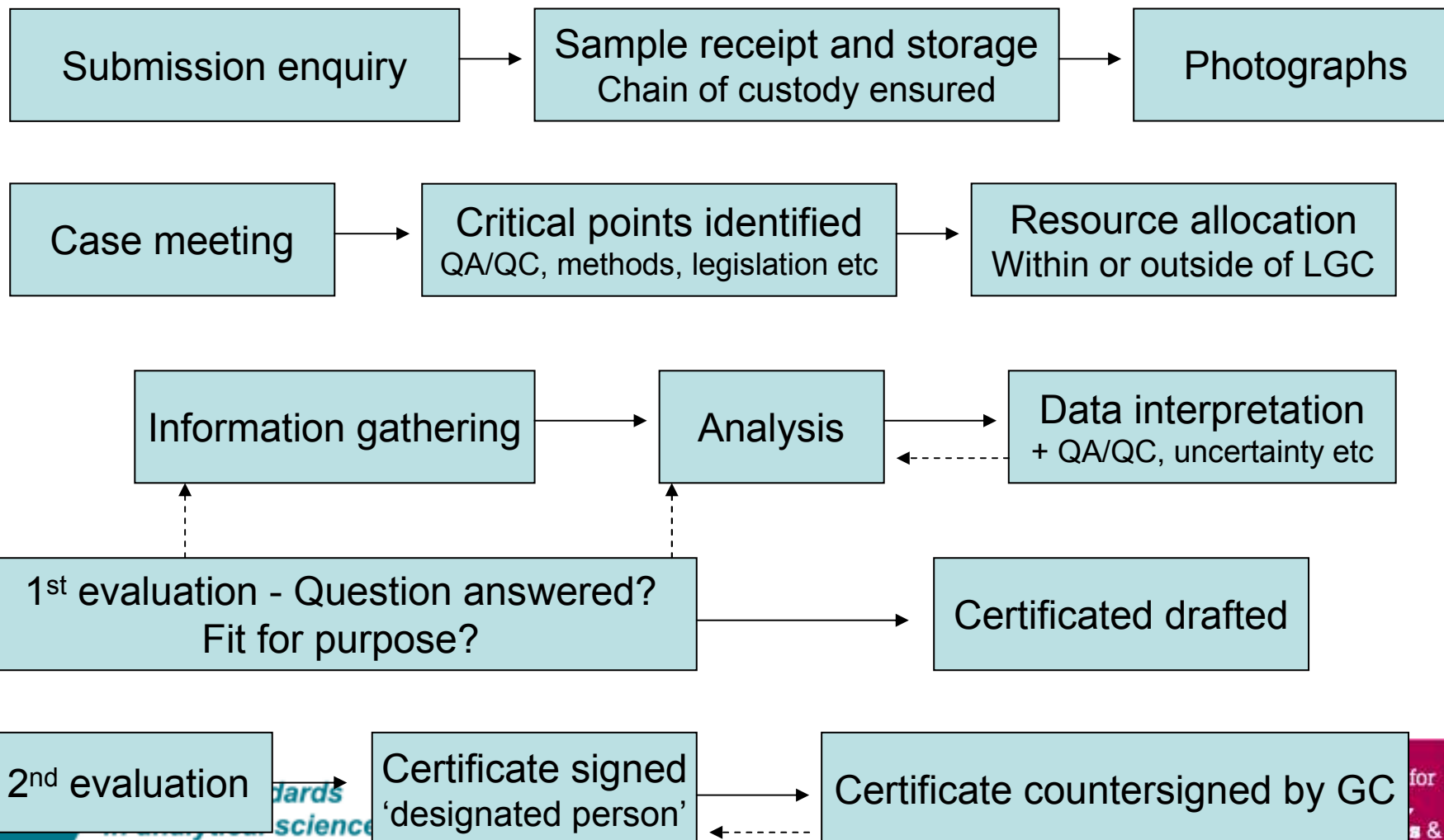


# Formal Sample





# Major events in a 'referee' case



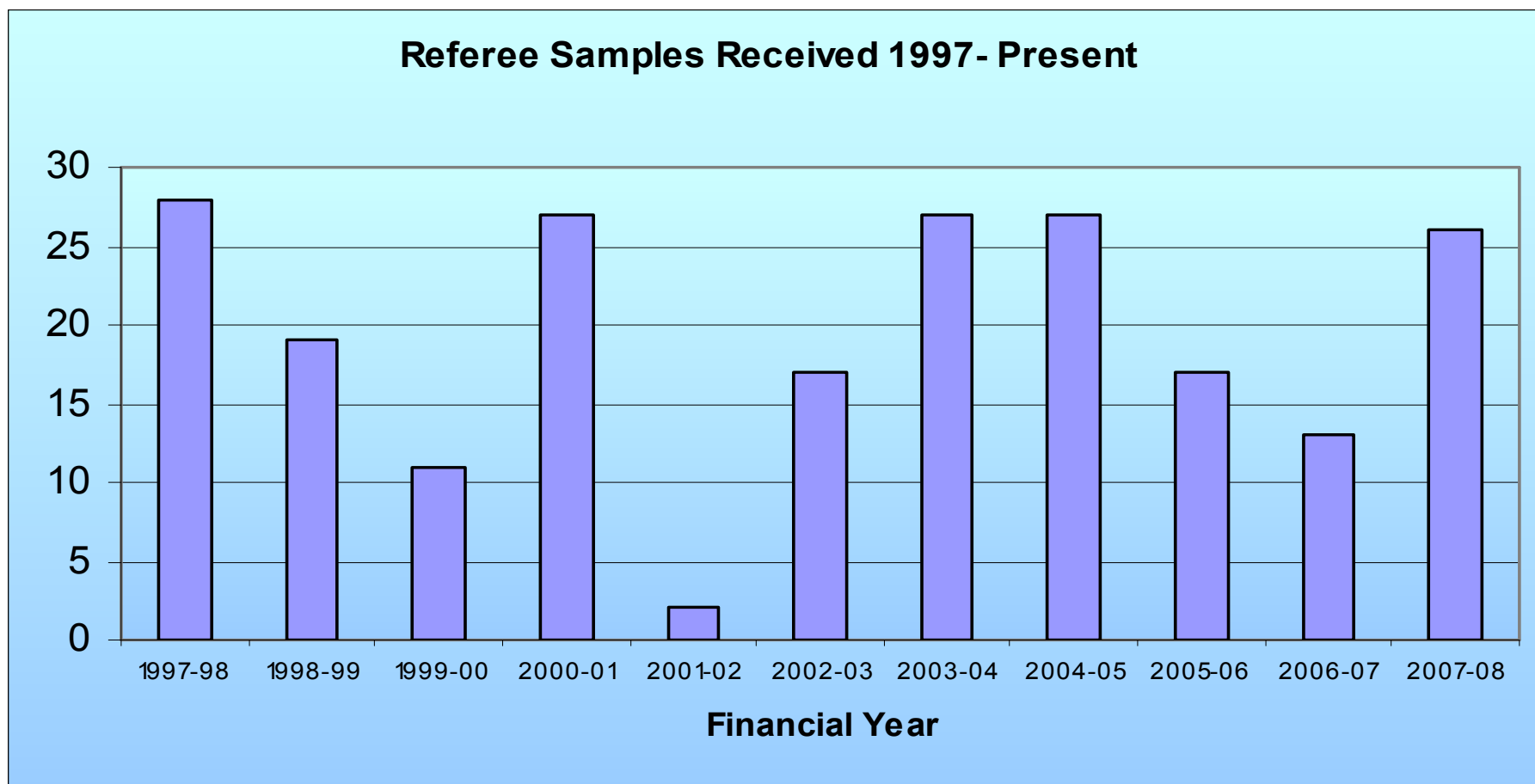


# Critical Quality Criteria

- Sampling and sub-sampling
- Statistically valid experimental plan
- Choice of analytical approach
- Suitability for the sample in question
- Staff training and experience
- Interpretation - background work required
- Uncertainty
  - of test result
  - of information on the certificate

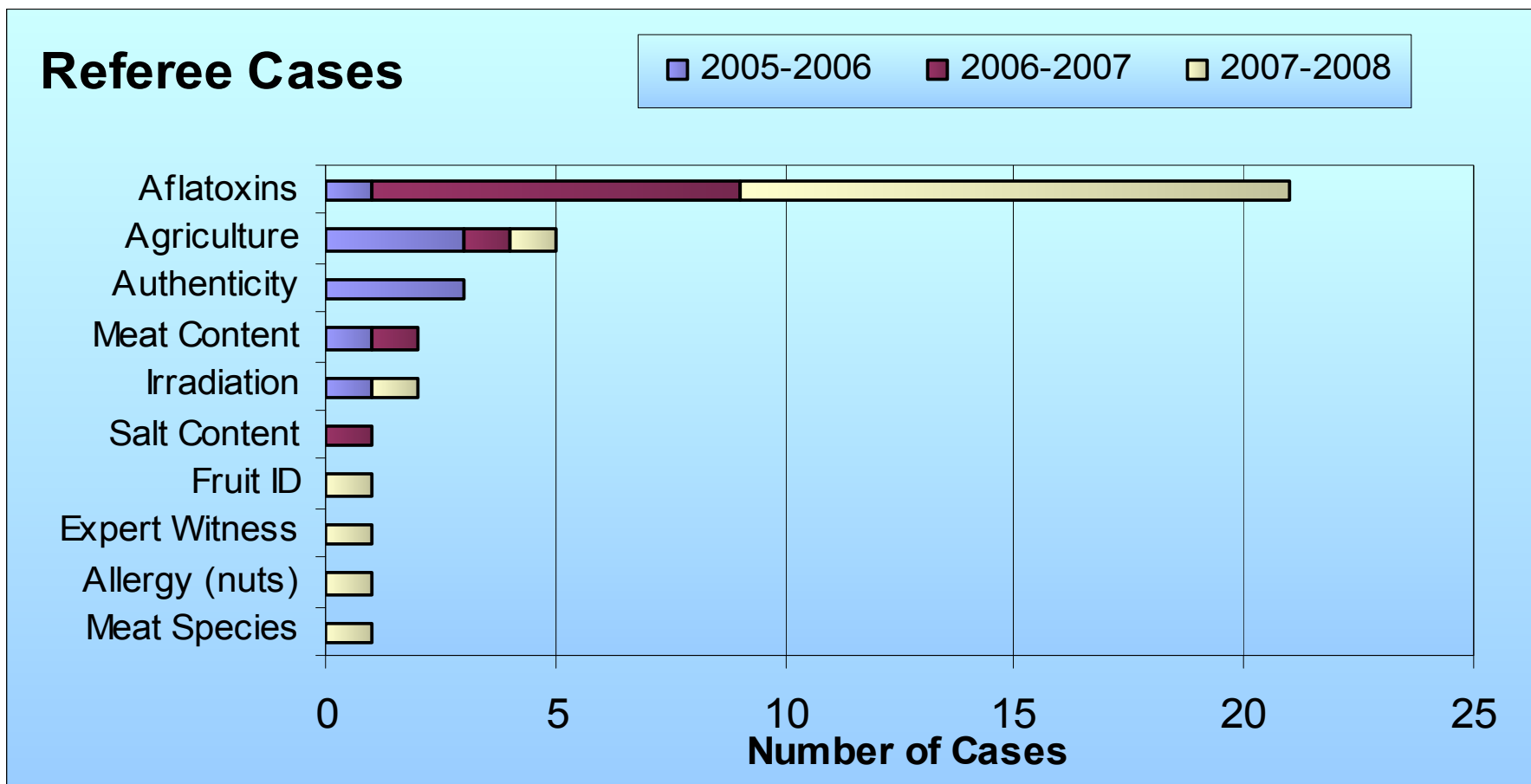


# Referee Samples Received



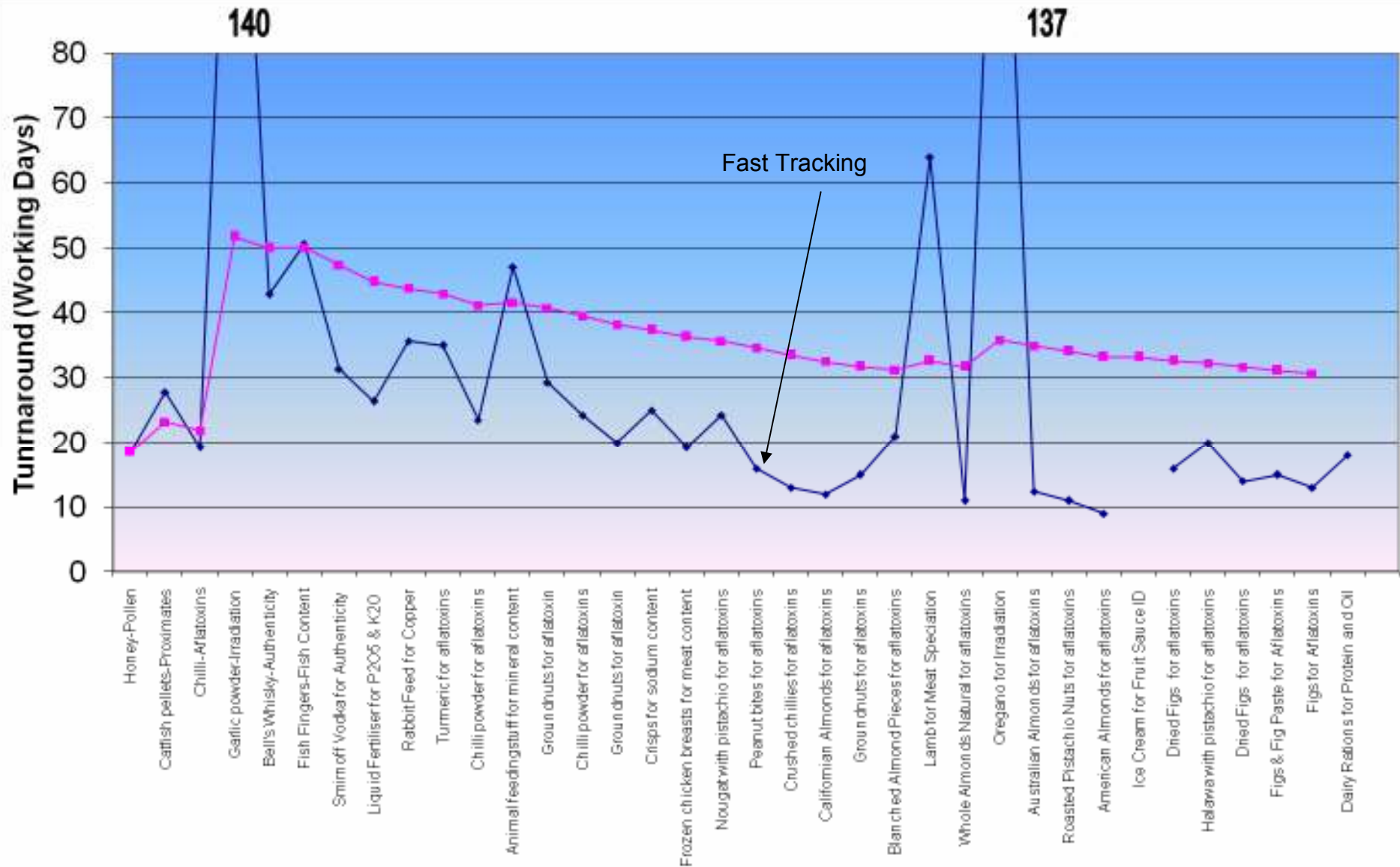


# Referee Cases Summary



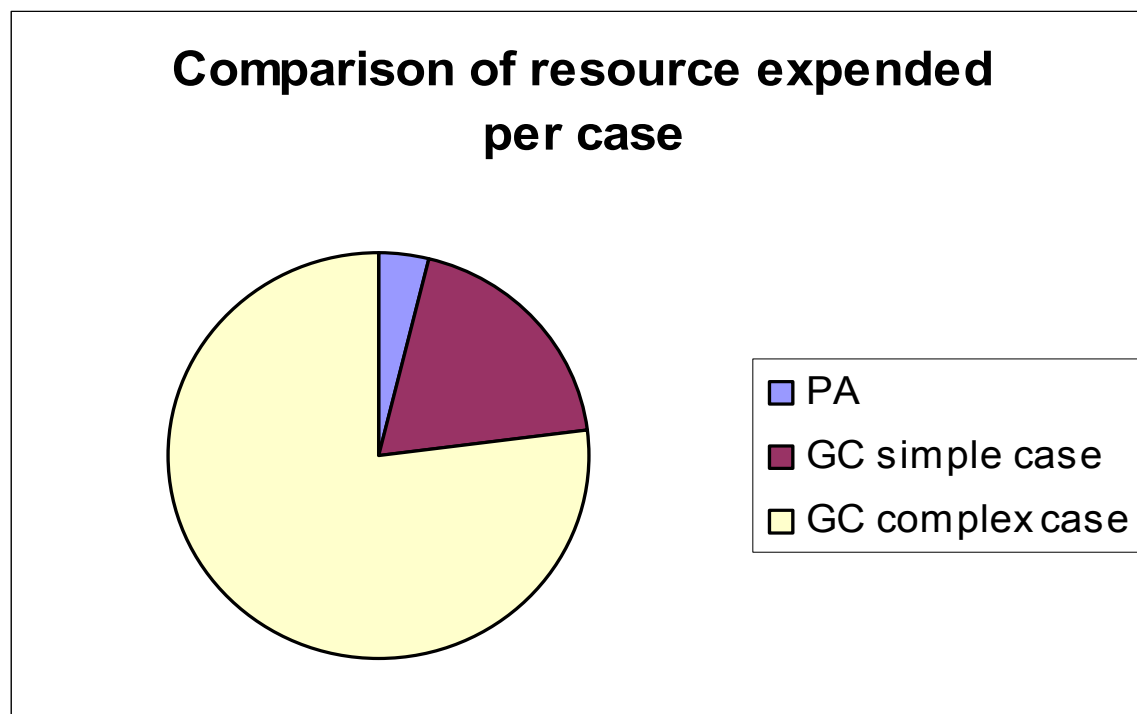


# Referee case turnaround times





## Before we move on to cases



For public analyst and trade laboratories expenditure of resource on such a scale would not be fit for purpose but is required for a referee analysis since the result must be definitive



# Expert Witness – Para Red in Chilli Powder

- Dispute between supplier and downstream food manufacturer regarding Chilli powder supplied in 2005
- Manufacturer incorporated the chilli into products and in May 2005 – presence of para red confirmed (200ppb)
- FSA advised product recall & destruction (>100,000 products affected)
- LC-MSMS method with lower LOD than industry standard HPLC method (LOD 1000ppb)
- Questions
  - Was para red present in the chilli powder?
  - Was LCMSMS a reliable method to use for the determination of para red in chilli powder?



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# Expert Witness – Para Red in Chilli Powder

- Peter Farnell acted as Expert Witness called by manufacturer's lawyers:
- An Expert Witness report was produced
  - Para red was present in chilli powder
  - LCMSMS method is a reliable method for the determination of para red in chilli powder
- Attended 4 day civil hearing and gave evidence
- Ruling given 26<sup>th</sup> July 2007
  - Court accepted Dr Farnell's opinion and based on this and other evidence found against the supplier of the chilli powder



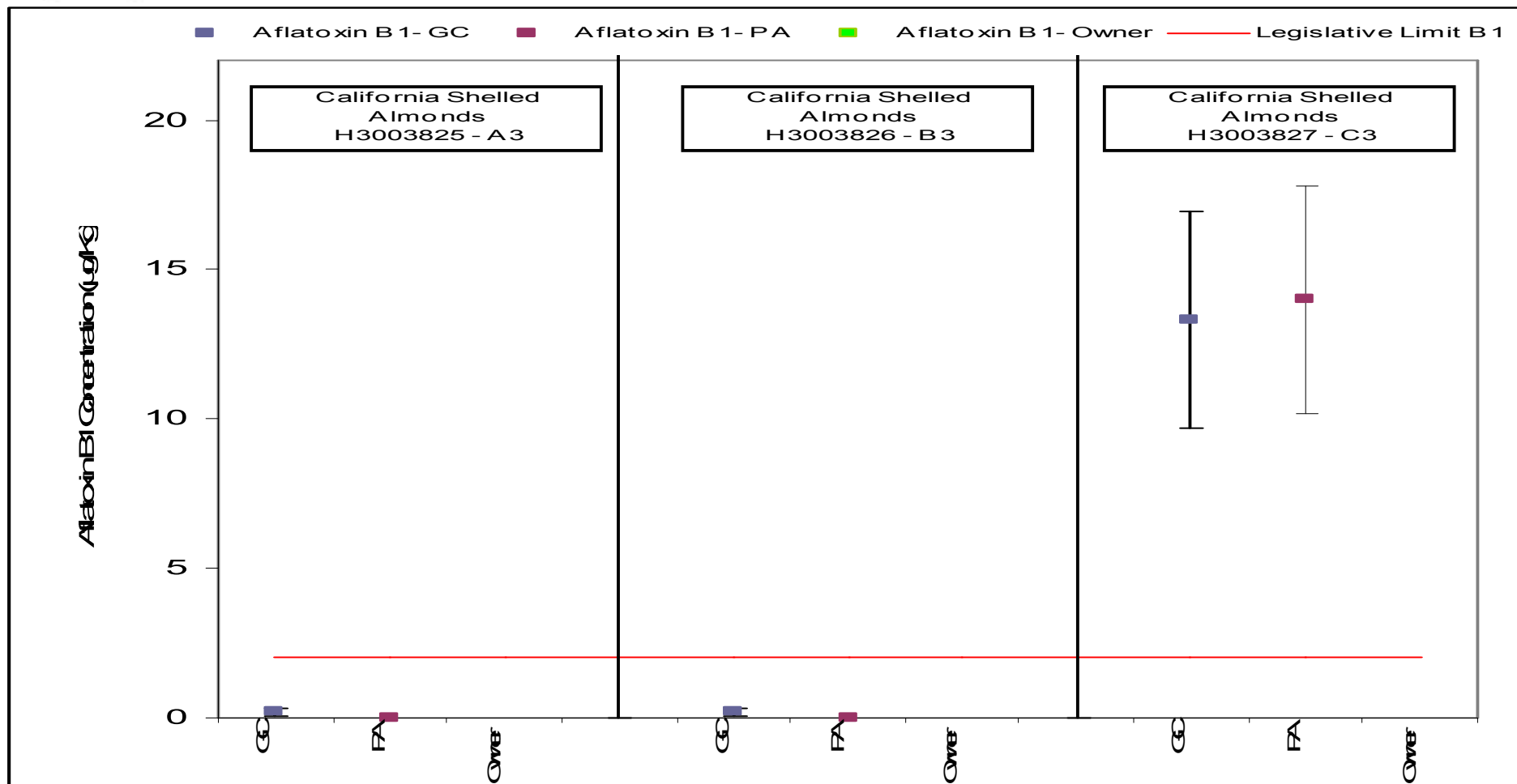
# Californian Almonds – Aflatoxins

- PA analysed sample and failed consignment
- Owner requested 2<sup>nd</sup> expert opinion under 88
- The GC results confirmed those of the PA
- The consignment was re-exported to the country of origin (California)





# California Shelled Almonds





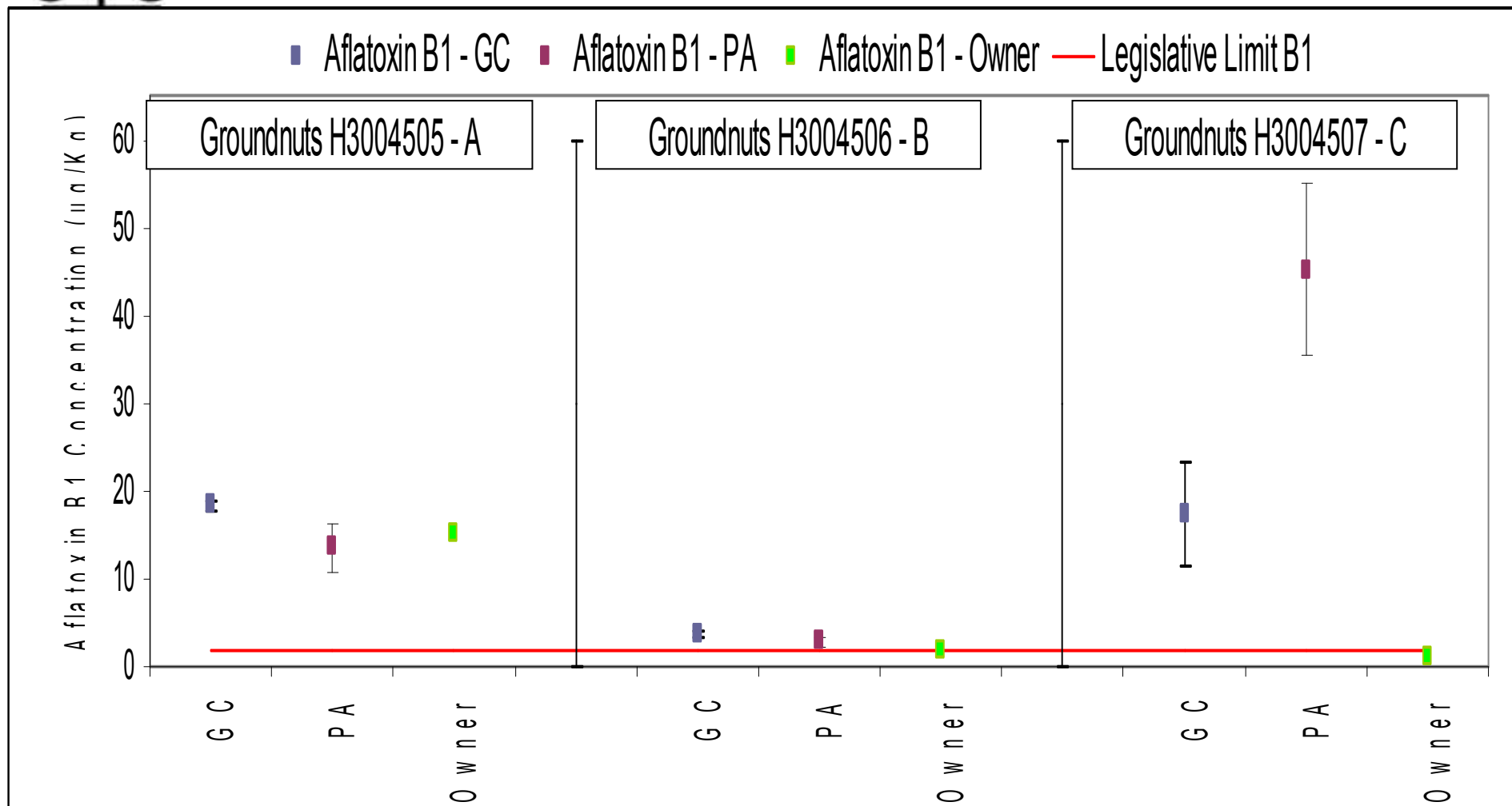
# Groundnuts – Aflatoxins



- PA analysed sample and failed consignment
- Owner had sample analysed in UK (not an OFCL) – also failed consignment
- Owner requested 2<sup>nd</sup> expert opinion under 882/2004/EC
- The GC results confirmed those of the PA and the owner
- The importer re-exported the consignment to Rotterdam for oil extraction



# Groundnuts





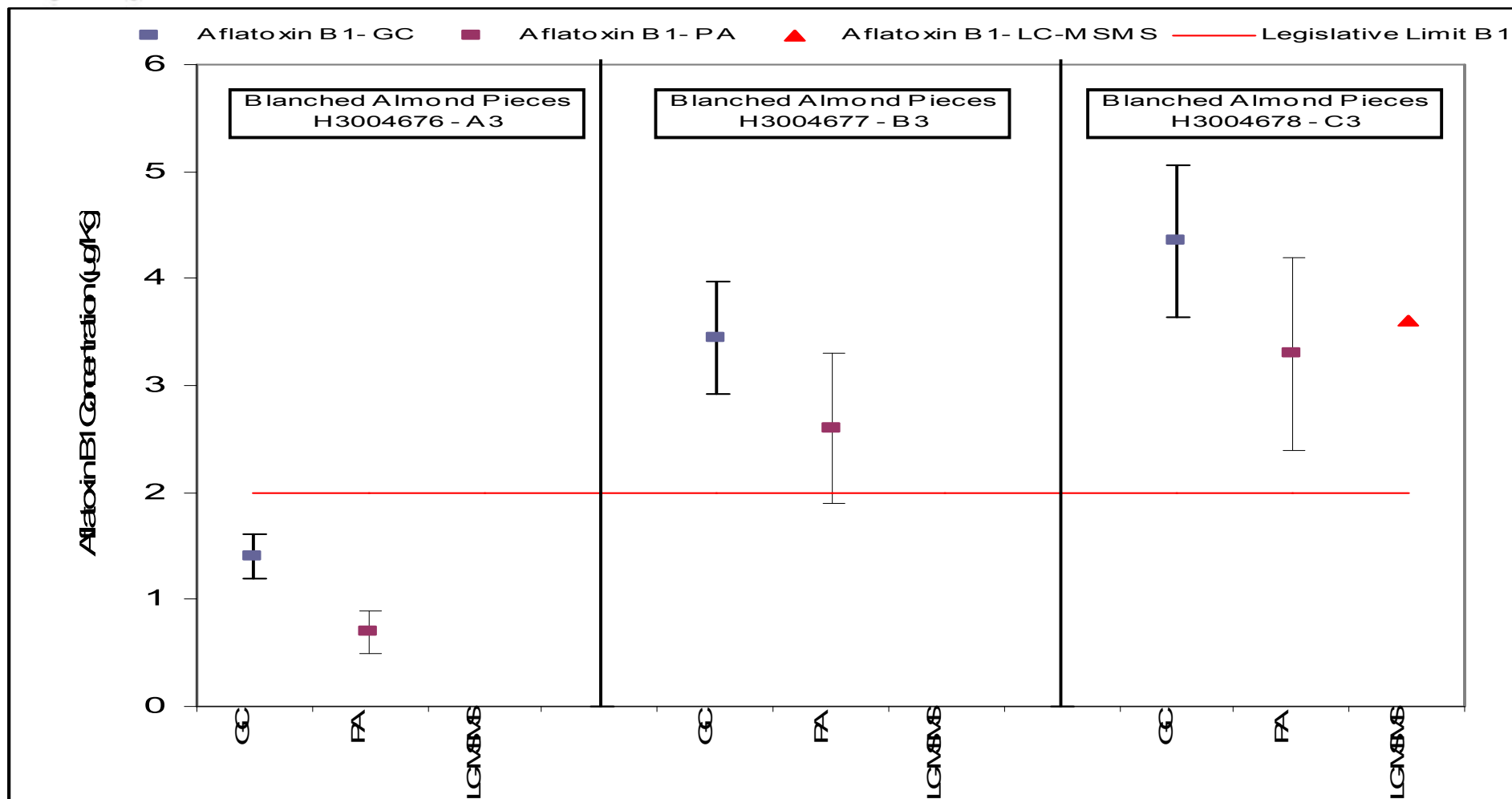
# Blanched Almond Pieces – Aflatoxins



- PA analysed sample and failed consignment
- Owner requested 2nd expert opinion under 882/2004/EC
- The GC results confirmed those of the PA



# Blanched Almond Pieces





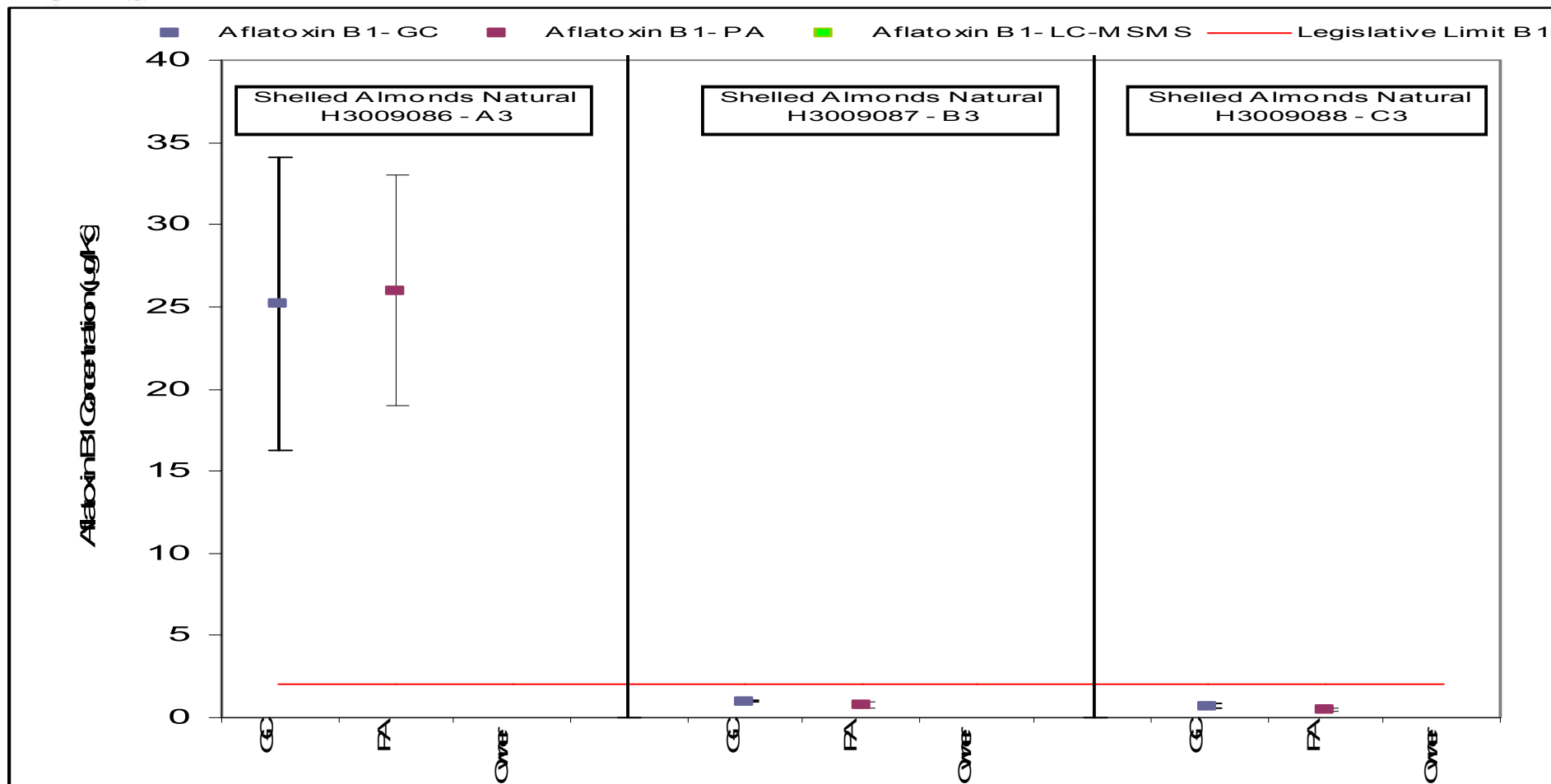
# Shelled Almonds – Aflatoxins



- PA analysed sample and failed consignment
- Owner requested 2nd expert opinion under 882/2004/EC
- The GC results confirmed those of the PA
- Consignment re-exported back to USA



# Shelled Almonds





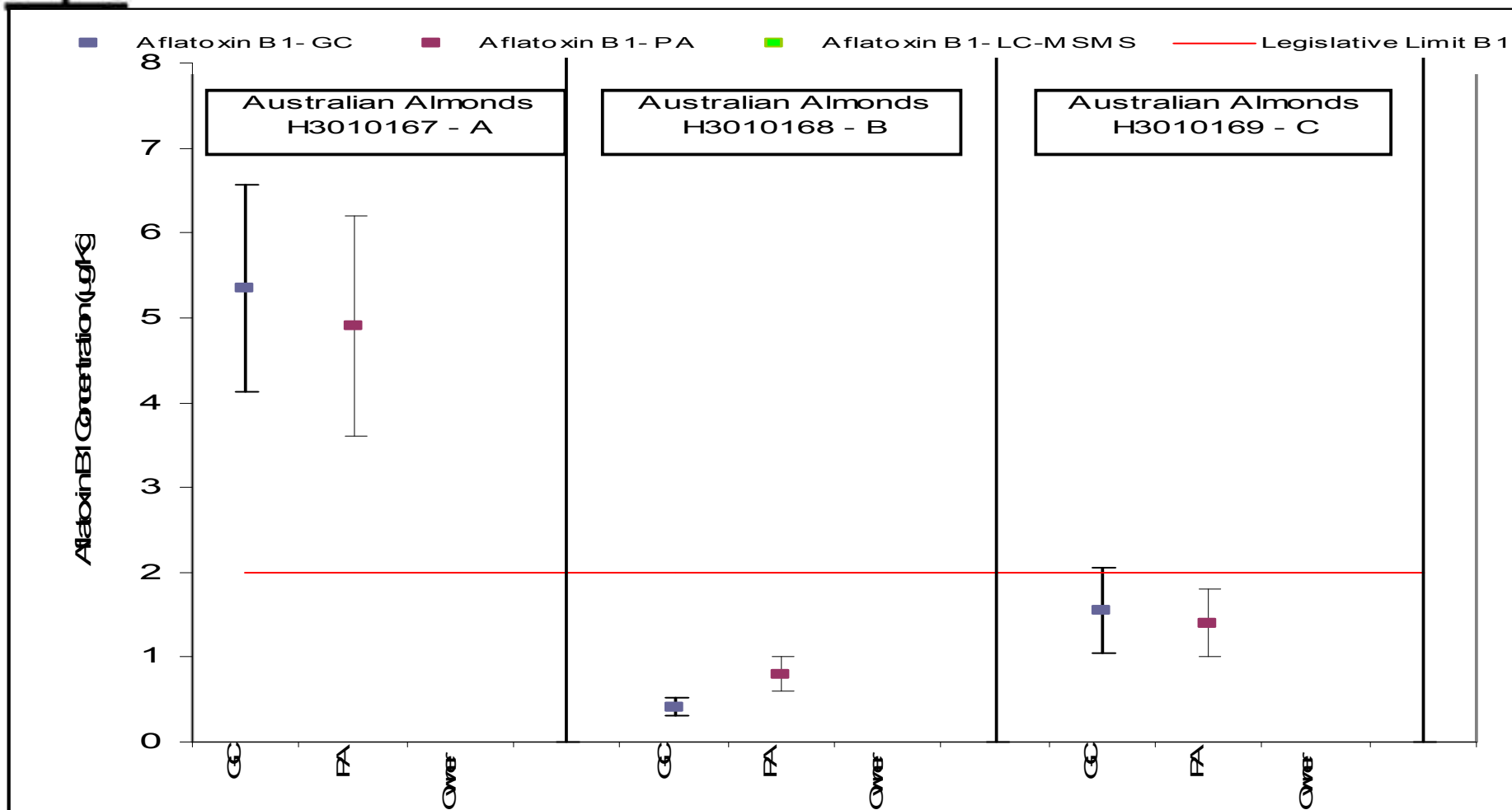
# Australian Almonds – Aflatoxins

- PA analysed sample and failed consignment
- Owner requested 2<sup>nd</sup> expert opinion under 88
- The GC results confirmed those of the PA





# Australian Almonds





# Homogenisation



- Grinding shells separate to kernels
- Only possible for the blank

- Wet blending with Waring blender
- Suitable for further homogenisation of the slurried sample





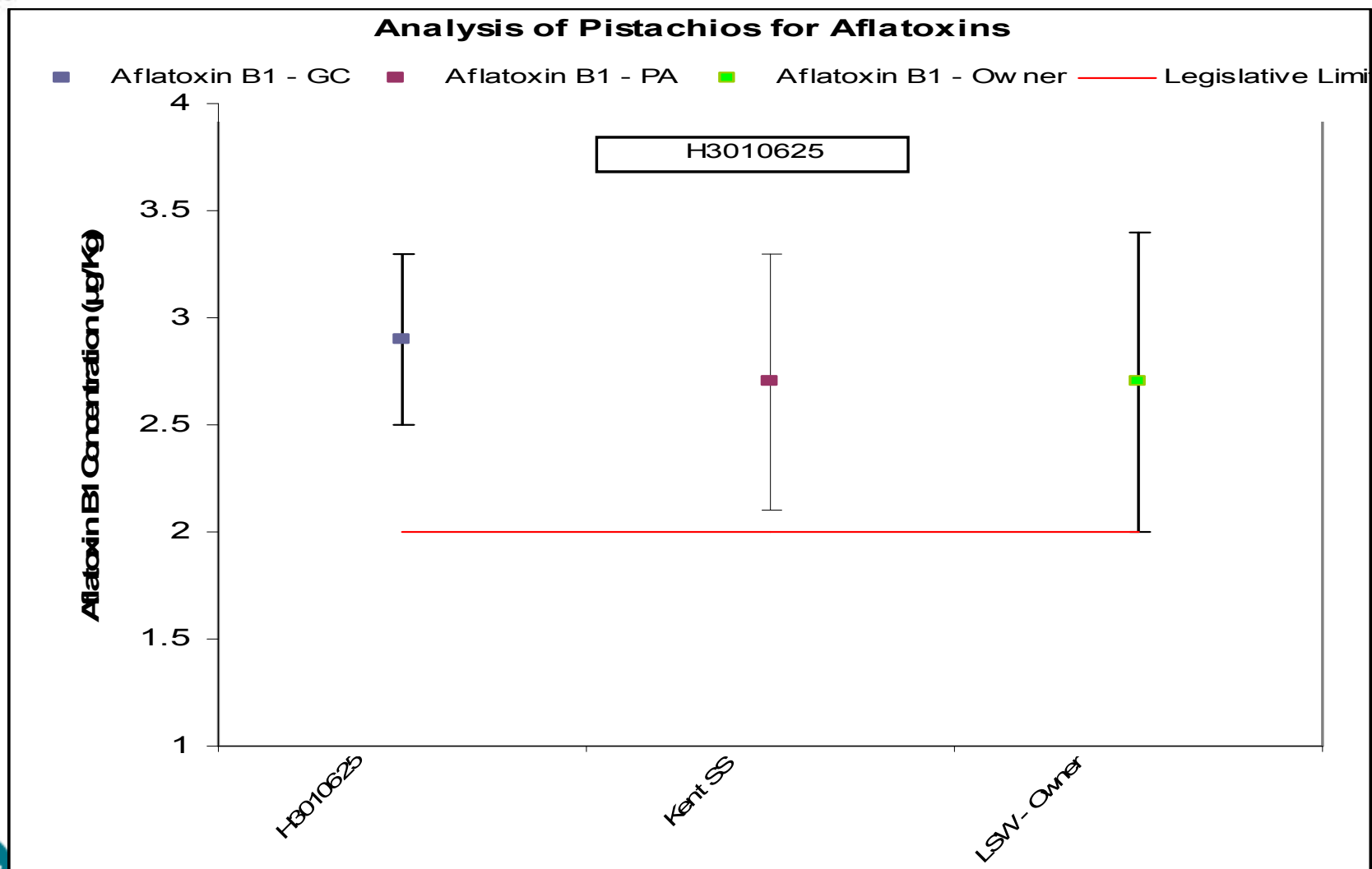
# Roasted Pistachios – Aflatoxins

- PA analysed sample and failed consignment
  - Aflatoxin B1 =  $2.7 \pm 0.6$
- Owner analysed 2<sup>nd</sup> Portion (OFCL)
  - Aflatoxin B1 =  $2.7 \pm 0.7$
- Owner disputing the uncertainty measurement





# Roasted Pistachios – Aflatoxins





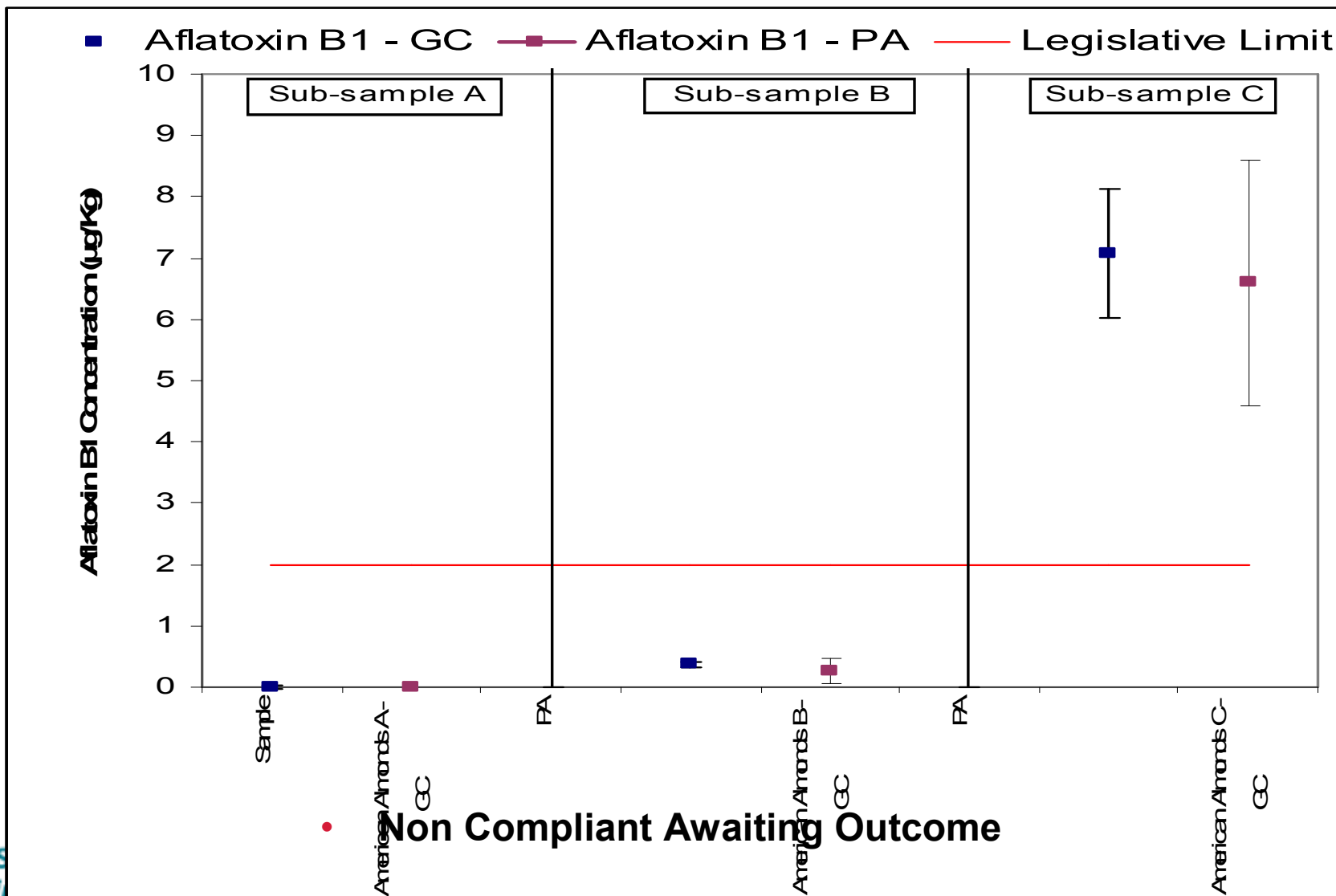
# American Almonds – Aflatoxins



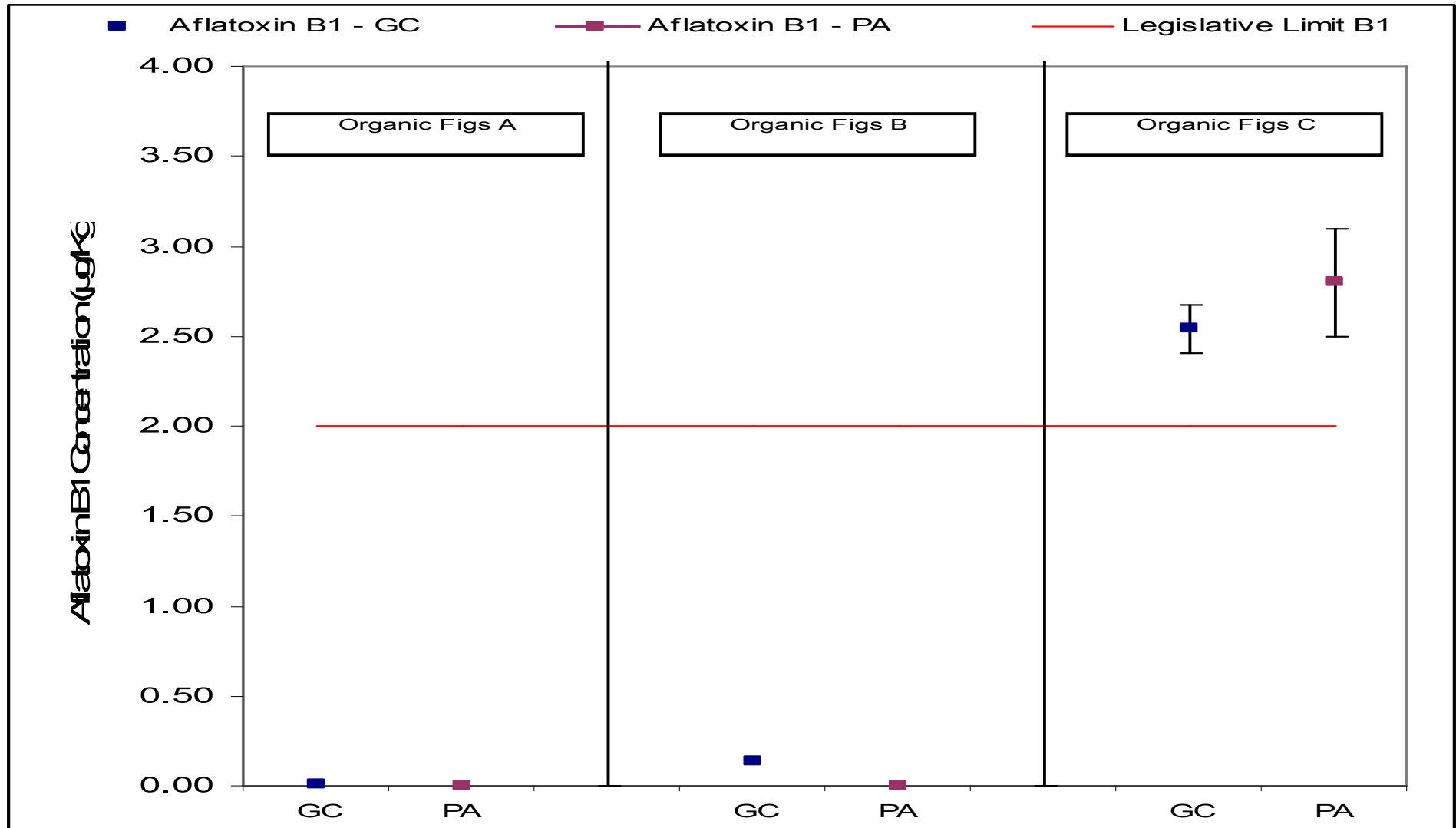
- PA analysed sample and failed consignment
- Owner requested 2<sup>nd</sup> expert opinion under 882/2004/EC



# American Almonds – Aflatoxins



<b>Sample: Organic Figs</b>  <b>Non Compliant Re-exported</b>	<b>Owner Analysis</b>	<b>Dispute</b>	<b>GC Resolution</b>
	No	2nd Expert Opinion	Confirms Public Analyst Results – Consignment re-exported





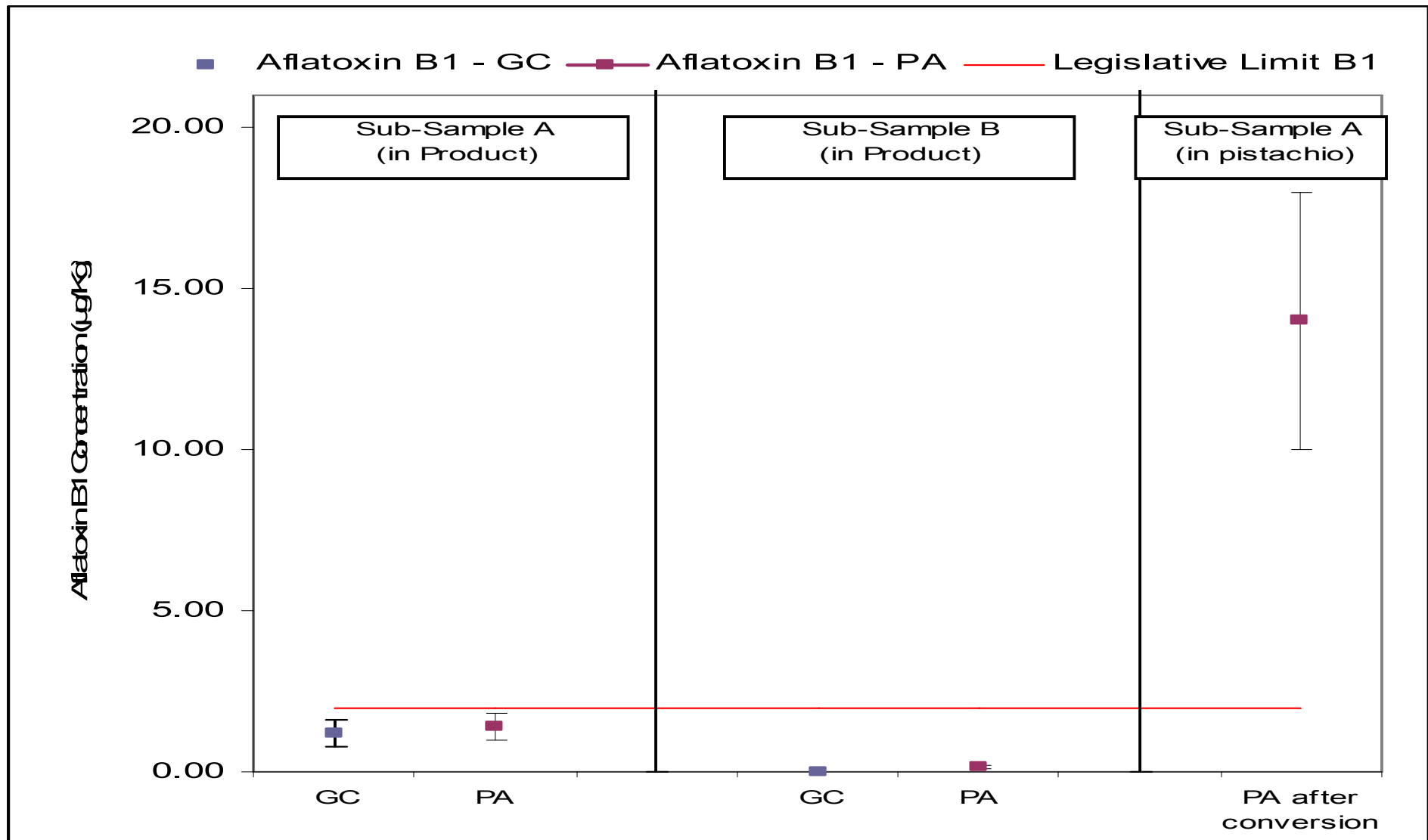
# Halawa With Pistachio

- We obtained as slurried whole product
- Aflatoxins could be present in the Tahina as it is made from sesame

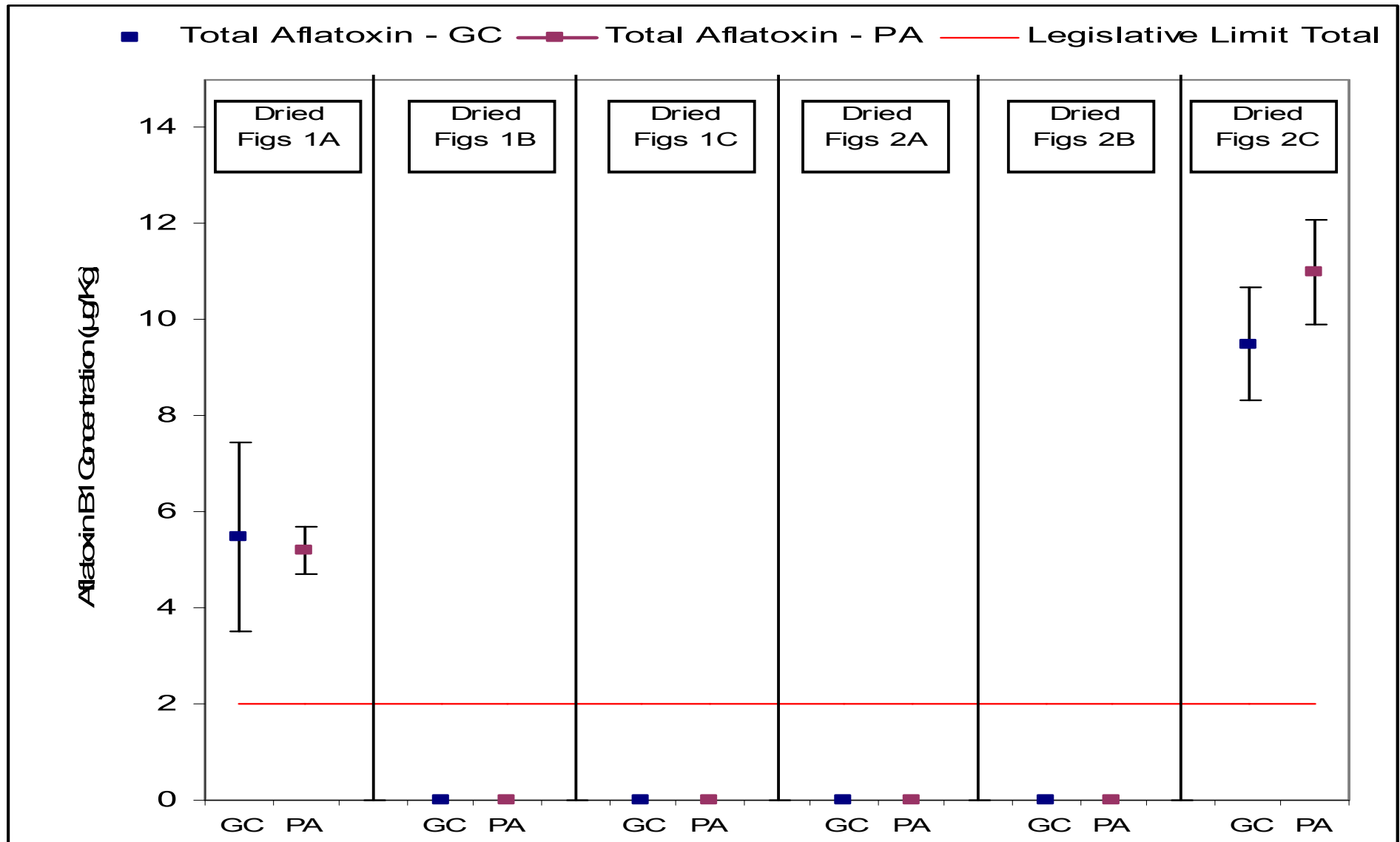


- It was possible to separate pistachios from the majority of the tahina
- A nut enriched slurry could have been analysed

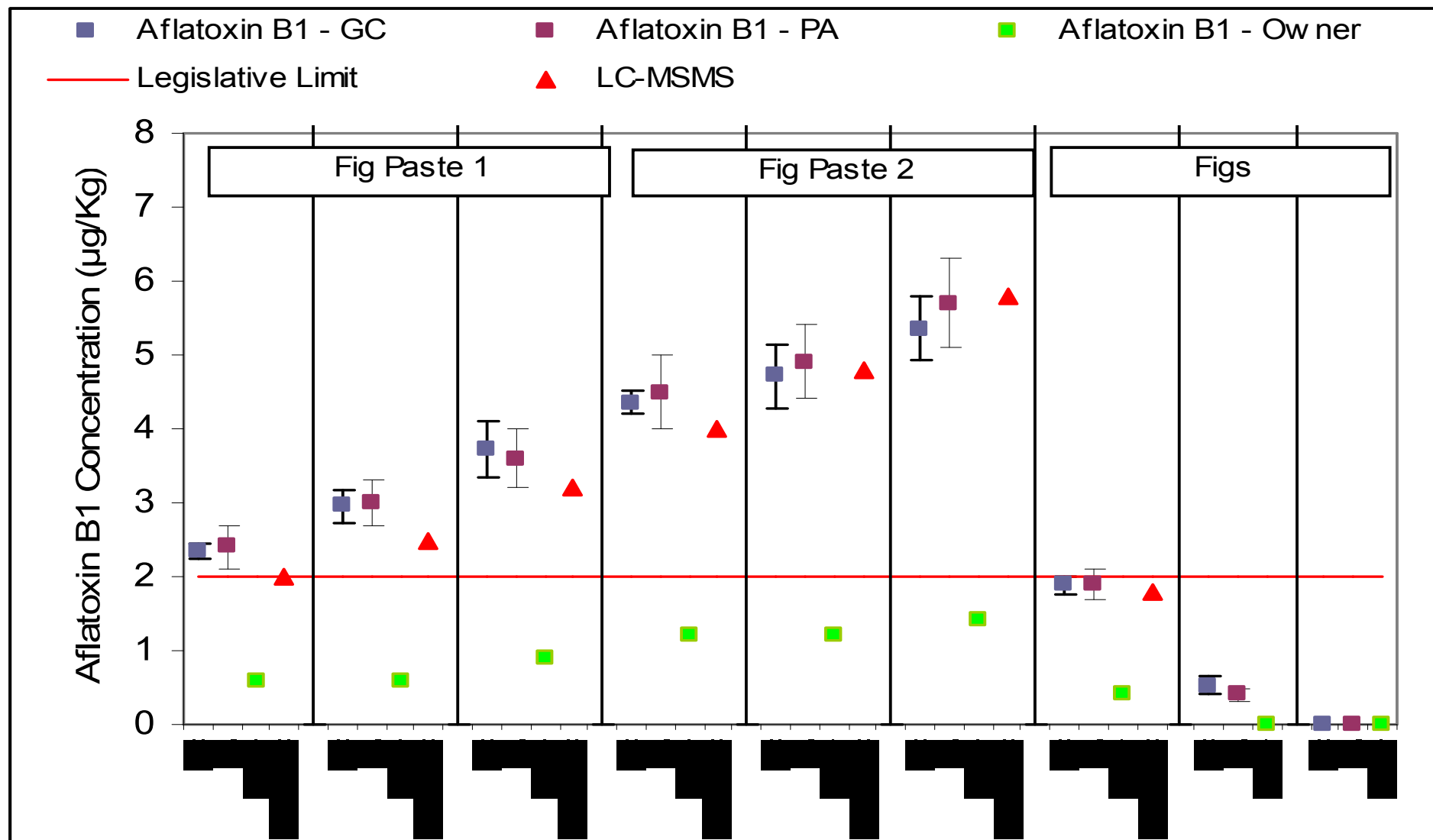
<b>Sample: Halawa with Pistachio</b> Report & Outcome Non-compliant GC Recommended further analysis (after separation of pistachios) PA found no evidence of Aflatoxins in sesame Tahini	<b>Owner Analysis</b>	<b>Dispute</b>	<b>GC Resolution</b>
	No	2nd Expert Opinion	Recommended further PA analysis



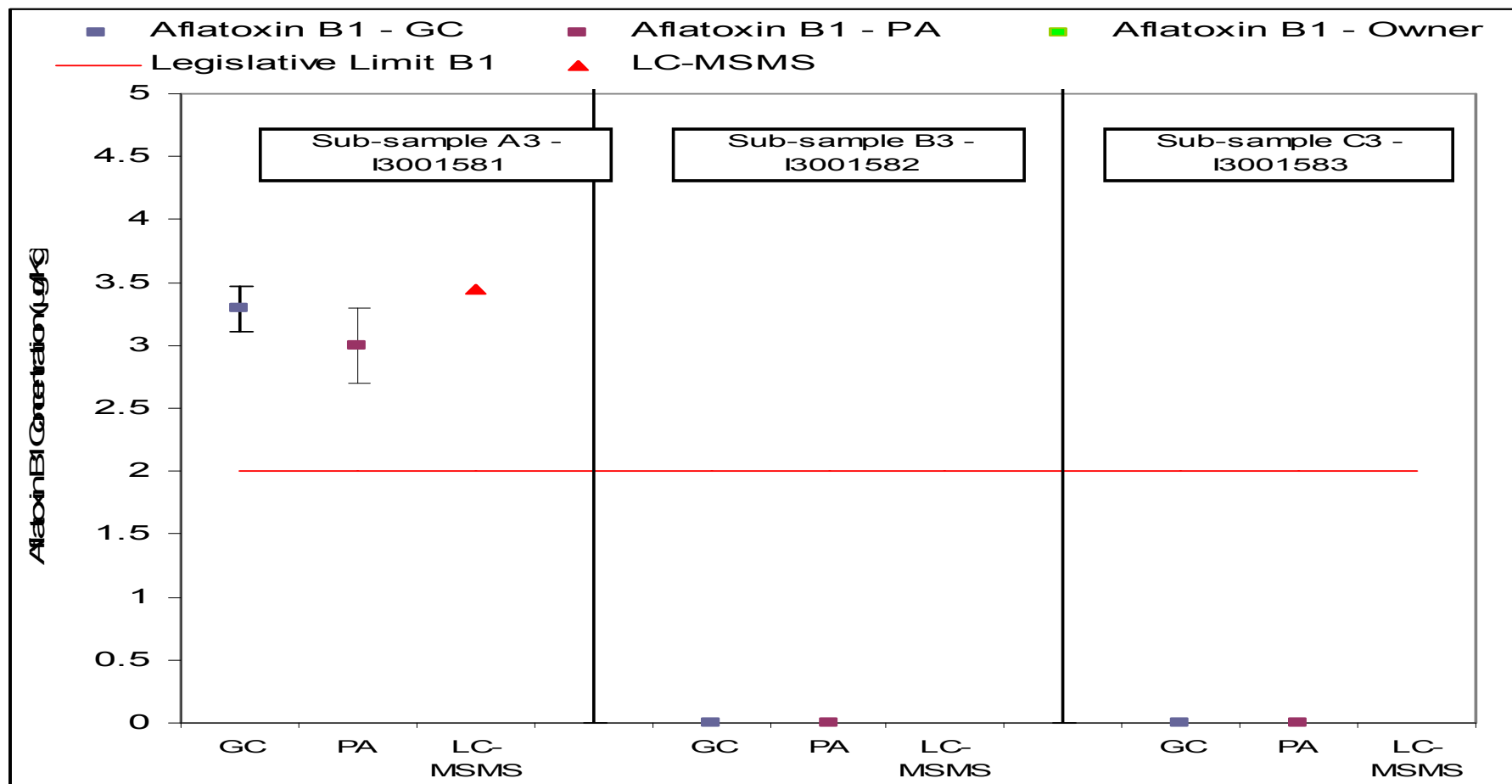
<b>Sample: Dried Figs (2 samples)</b> <b>Report &amp; Outcome</b> <b>Non Compliant</b> <b>Awaiting Outcome</b>	<b>Owner Analysis</b>	<b>Dispute</b>	<b>GC Resolution</b>
	No	2nd Expert Opinion	Confirms Public Analyst Results



<b>Sample: Figs &amp; Fig Paste (3 samples)</b> <b>Report &amp; Outcome: Non Compliant</b> <b>Case closed – goods redispached to country of origin</b>	<b>Owner Analysis</b>	<b>Dispute</b>	<b>GC Resolution</b>
	Yes	Disputed Results	Confirms Public Analyst Results

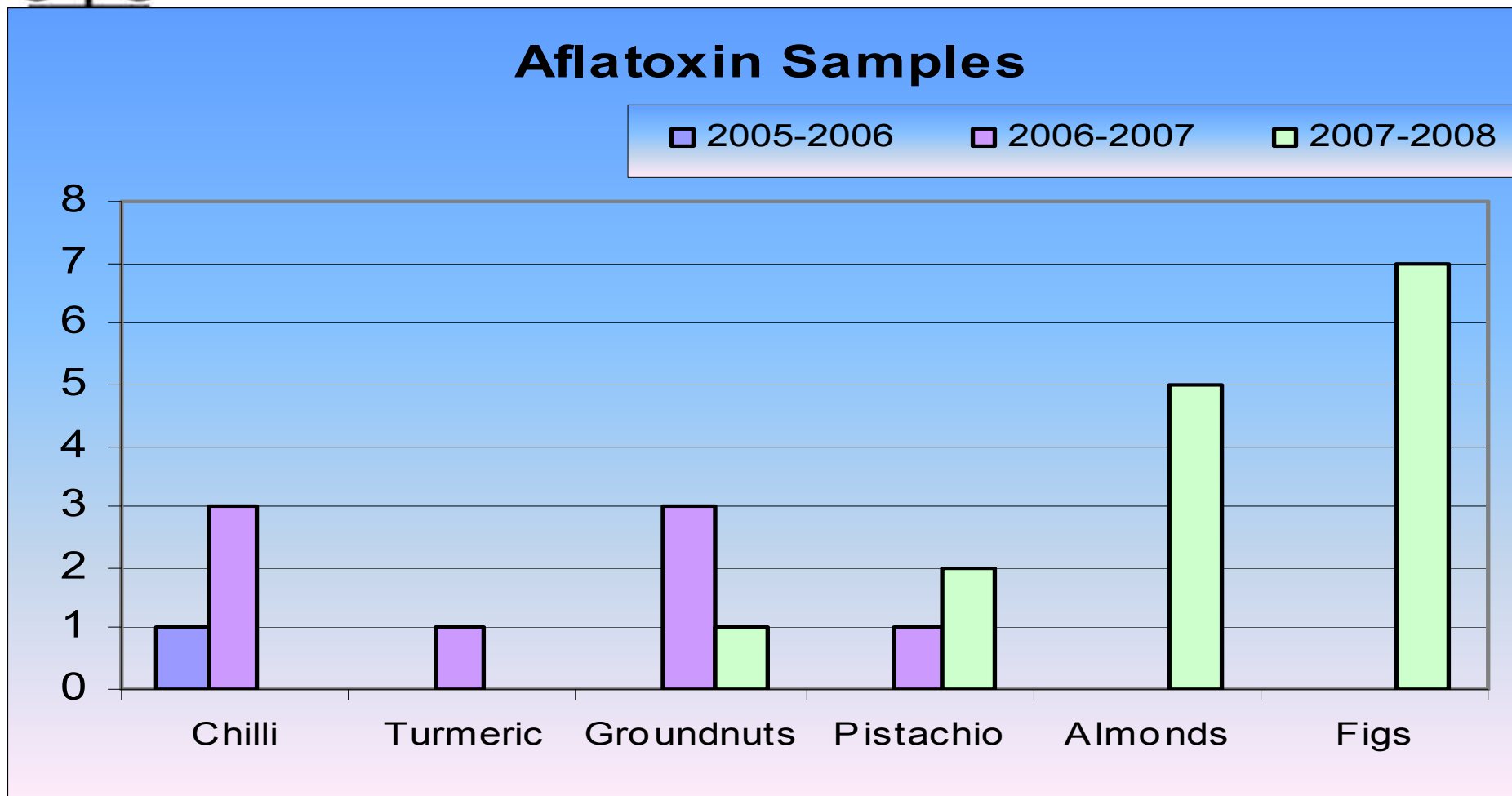


<b>Sample: Figs</b> <b>Report &amp; Outcome:</b> <b>Case closed – goods redispached to country of origin</b>	<b>Owner Analysis</b>	<b>Dispute</b>	<b>GC Resolution</b>
	No	2nd Expert Opinion	Confirmed PA Results





# Aflatoxin samples by matrix





# Summary of Aflatoxin Cases

Sample	Owner Analysis	Dispute	GC Resolution
Californian Almonds	No	2nd Expert Opinion	Confirmed Public Analyst result
Groundnuts	Yes (not OFCL)	2nd Expert Opinion	Confirmed Public Analyst result
Blanched Almond Pieces	No	2nd Expert Opinion	Confirmed Public Analyst result
Whole Almonds Natural	No	2nd Expert Opinion	Confirmed Public Analyst result
Australian Almonds	No	2nd Expert Opinion	Confirmed Public Analyst result
Roasted Pistachio Nuts	Yes (OFCL)	Disputed Uncertainty	Confirmed Public Analyst result
American Almonds	No	2nd Expert Opinion	Confirmed Public Analyst result



# Summary of Aflatoxin Cases

Sample	Owner Analysis	Dispute	GC Resolution
Organic Figs	No	2nd Expert Opinion	Confirms Public Analyst Results – Consignment re-exported
Halawa with Pistachio	No	2nd Expert Opinion	Recommended further PA analysis
Dried Figs (2 samples)	No	2nd Expert Opinion	Confirms Public Analyst Results
Figs & Fig Paste (3 samples)	Yes	Disputed Results	Confirms Public Analyst Results
Figs	No	2nd Expert Opinion	Confirms Public Analyst Results



# Lamb Mince – Meat Speciation

- Public Analyst reported
  - ELISA Beef present at least 8 %
  - ELISA Pork present at least 2 %
- Owner's analysis
  - ELISA Raw species test – Beef (negative)
  - ELISA Raw species test – Pork (negative)

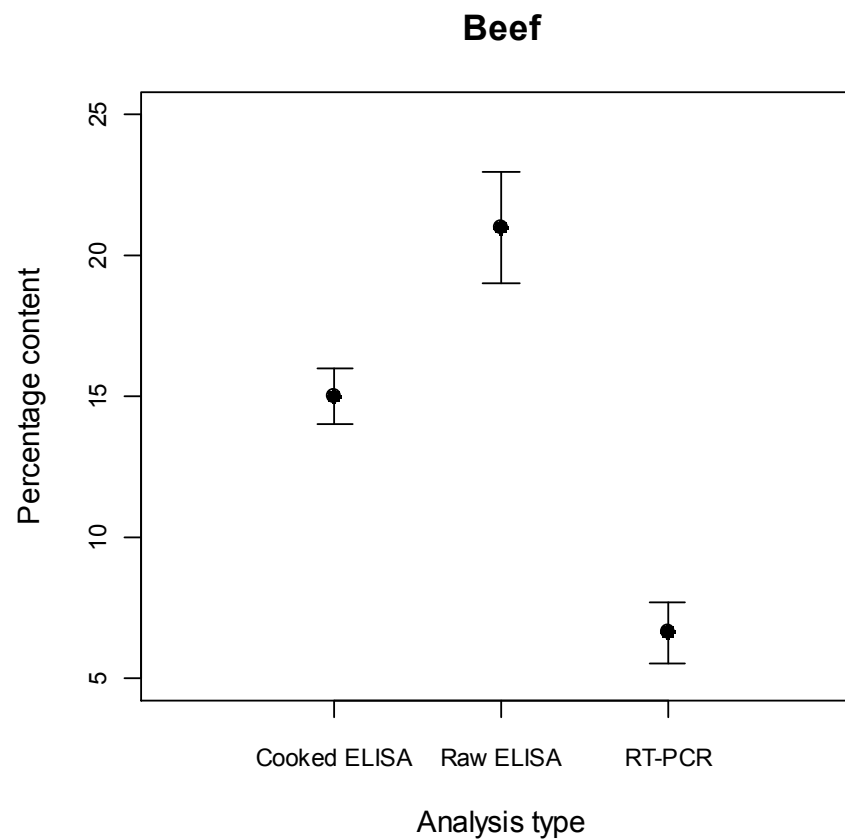
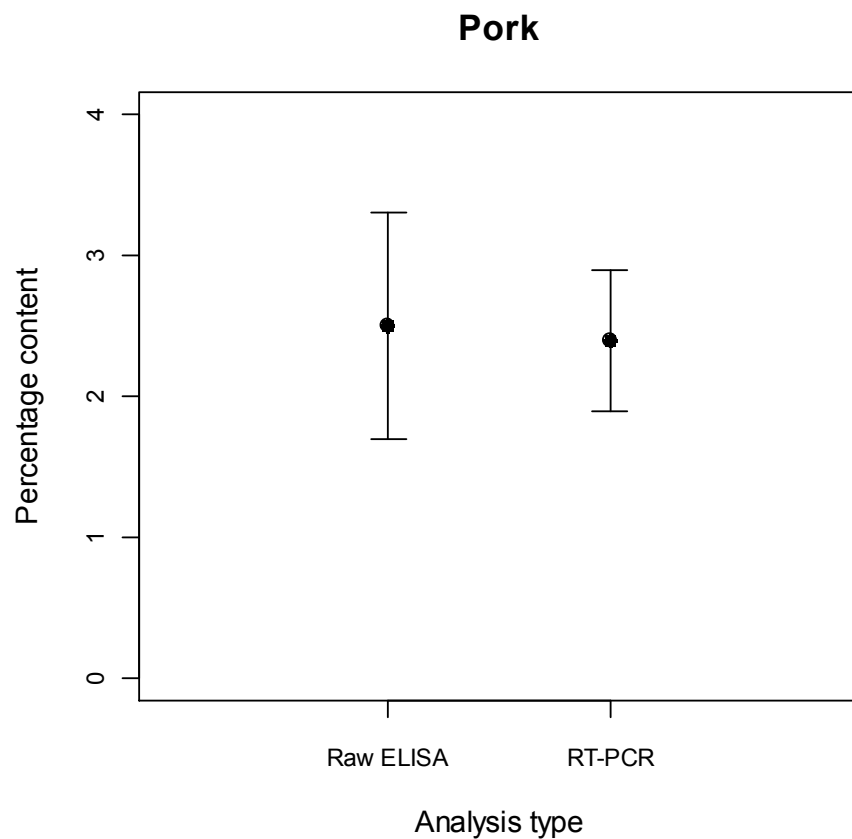


# Lamb Mince – Analysis

- GC analysis by;
  - ELISA Raw species test
  - ELISA Cooked species test
  - RT-PCR
    - Test kit (Surefood)
    - In-house RT-PCR method of N Harris et al. 2003



# Lamb Mince – Findings confirmed Public Analyst's results





# Extract from Wording on the Certificate

- .....
- The presence of ovine, bovine and porcine protein and DNA in sample H3007845 confirm that it contains beef and pork in addition to lamb.
- By way of illustration, assuming average fat contents' of 16% and 10% in the beef and pork respectively the results detailed in section 5 below are consistent with the presence in the sample of between 5 and 23 per cent beef and between 1 and 3 per cent pork.
- Had the contamination by beef occurred only through ingress of beef drip approximately 8 to 10% could have been added, based on experimental work carried out as part of a Government Chemist project
- .....



# Lamb Mince – Outcome

- Trading Standards sent a warning letter to the butcher and discussed with them methods for improving procedures.
- The TSO felt a prosecution would have been problematic due to the range of results obtained.
- The explanation lies in the nature of the antibodies used in the lab and how they were developed and the amount of blood retained in the carcass at slaughter.
- A procedure for successive extractions is under investigation



# Organic Oregano - Irradiation

- Formal sample of oregano taken as part of a survey of herbs and spices
- Using PSL and TL, the PA found sample was irradiated
  - TL performed by  
Scottish Universities Environmental Research Centre (SUERC)
- Owner found samples not irradiated
  - TL performed by Eurofins



# Organic Oregano - Irradiation

- **Analysis**
  - GC analysis at a third laboratory (Finland)
  - GC provided blank material and irradiated controls and blends and witnessed the analysis
- **Results**
  - PL 6 replicates 2 –ve & 4 intermediate
  - TL 13 replicates – all ‘non-irradiated’
- **Opinion**
  - ... that sample H3009873 does not contain irradiated silicate minerals
  - and therefore there is no evidence, beyond reasonable doubt, that the referee sample was irradiated or contained irradiated material.



# Animal Feed Case – 2 samples



Heterogenous – crushed (P&M),  
Riffled and milled before analysis





# Animal Feed Case – 2 samples



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# Animal Feed Protein, N x 6.25

Decl'n	AA	Owner	Lower limit	GC Statutory Method	GC LECO
18 %	13.6 %	17.2 %	16.2 %	16.9 %	16.85 %
17 %	13.0 %	17.1 %	15.3 %	15.8 %	16.0 %



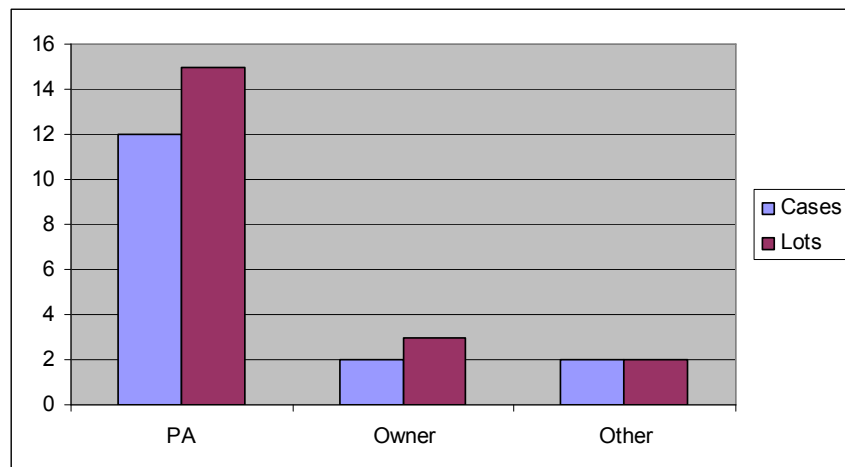


# Animal Feed Oil

Decl'n	AA	Owner	Lower limit	GC
4.0%	3.0%	Not done	3.2%	3.3
4.0%	3.2%	3.4%	3.2%	3.2

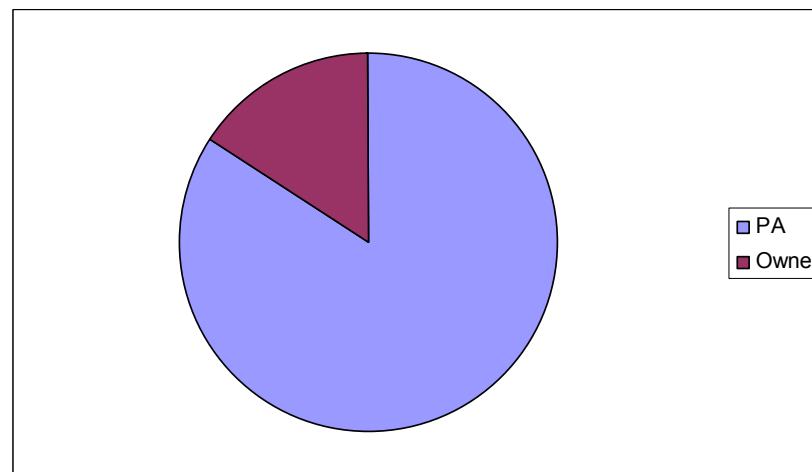


# Conclusions 1- Findings Confirmed



Original findings confirmed -  
Numbers of completed cases  
2007/08

% of lots where PA (84.2%) or  
Owners' (15.8%) analyses confirmed,  
completed Cases 2007/08





## Conclusions 2 – Issues raised

- Aflatoxins:
  - Compound products – nut/matrix, if matrix could contain aflatoxins separate if possible before slurring and analysis
  - Recovery – overnight spiking
  - Appropriate extraction solvents for spices
  - Conditions for optimum recovery from IAC
  - Blank material – often not straightforward to obtain and for almonds for optimum recovery needs to be well matched to sample
  - Non-slurried and Slurried – different results
  - Figs – co-extracted late eluting peak and interferences
  - LC-MS/MS successfully applied but LC F can be more sensitive



## Conclusions 3 – Issues raised

- Meat Speciation:
  - ELISA is semi quantitative at best and can yield wide range of results if contamination  $>2 - 3\%$
  - DNA should be used where possible as confirmation
- Animal Feed
  - Sampling and sample preparation is key
- Irradiation
  - Blends can be analysed – may need more work on conditions of extraction and handling of silicate minerals
- Statistical support



## Conclusions 4 – The Future

- Allergens?
- Other mycotoxins?
- Food Contact materials?
- Probiotics?
- Animal feed – vitamins.....
- Global food price rises – temptation to adulterate
  - 90% will be what we have seen before –
  - Added water (to meat, milk...)
  - Inferior substitution ... Fish, Basmati, Durum, ....
  - DNA based methods
  - possible disputes?



# Thank you

- Acknowledgements
  - **Department for Innovation, University and Skills**
  - The Government Chemist Function is funded by DIUS
  - **Food Chemistry Team**
    - Selvarani Elahi, Pete Colwell, Paul Lawrance, Kirstin Gray, Brian Stuart, Malcolm Burn, Dionisis Theodosios, Bhavna Bhatt, Kam Lee, Inaki Borja, Agata Bunia, Frank Torma and Huda Ibrahim.