



Melamine in pet food – an issue for laboratory networking

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Overview

- **Context**
- **SOFIA as a node of the Eurofins Network**
- **Time scale**
- **Methodology**
- **Results**
- **Summary**
- **Conclusion**



The context

12/04 10 am

In South Africa, many dogs and cats died and suffer from very important health disorders (kidney disease).

A French pet food company, an historical customer of Eurofins is looking for a lab which is able to look for ethylene glycol in dry pet food samples.

They think about an intentional contamination by a local competitor or a problem of production.

12/04 11 am

Sofia GmbH is ok to perform the analysis with very short TATs

12/04 12 am

New customer request after internal investigations :

Which kind of contaminants should be at the origin of such health disorders ?



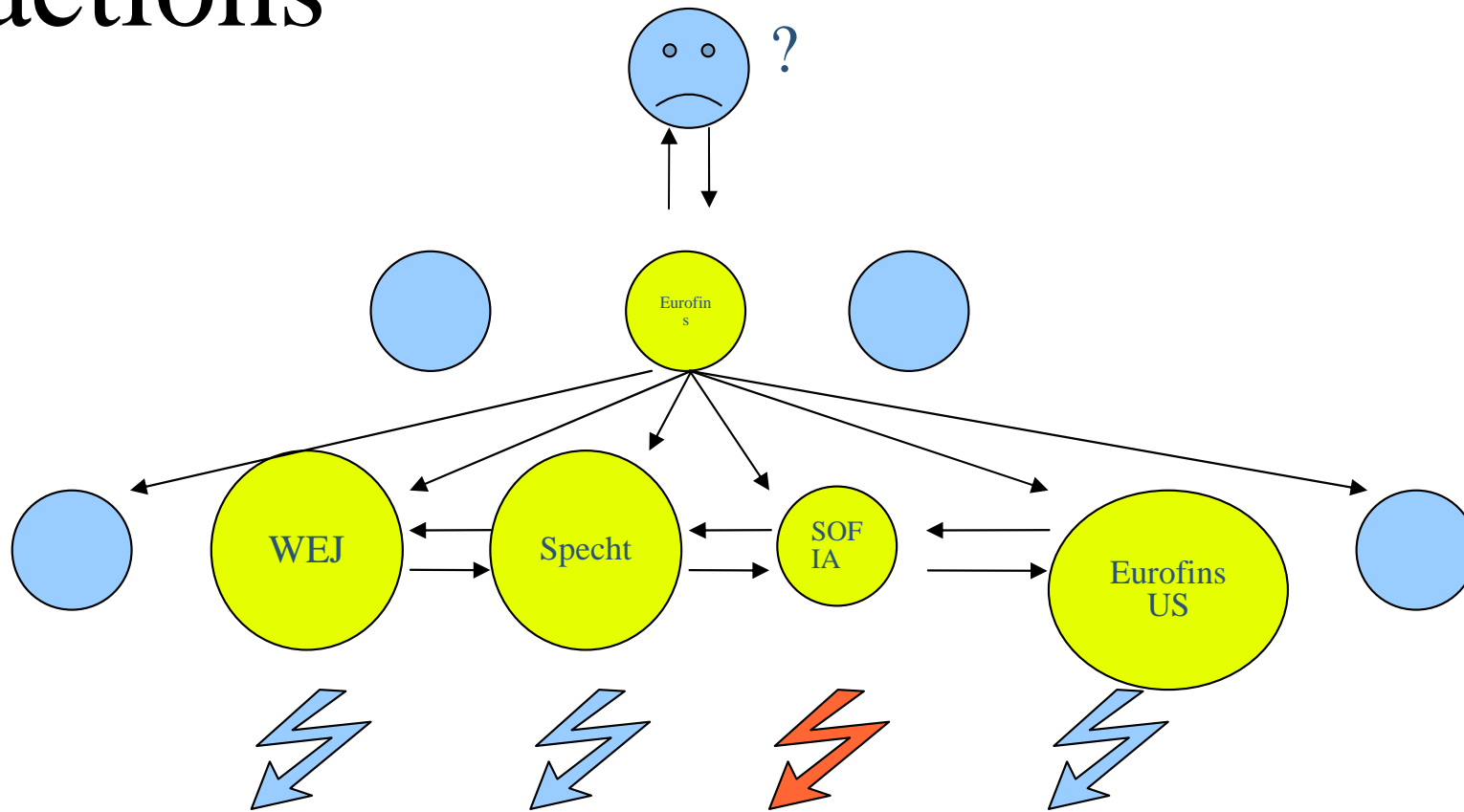
Are they concerned ?

They ask for a whole screening, including melamine, pesticides, mycotoxins to find out what's going on for the first 3 samples.

We needed to help them as soon as possible with the shortest TAT.

Feed back to the customer with solutions and instructions for logistic

The Eurofins network and it's reactions





Melamine – time scale

Th. 12.04. 11.00 / 19.00	Fr. 13.04.	Sa. 14.04.	Su. 15.04.	Mo. 16.04.	23.04	25.04
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first request on ethyleneglycol in PET food

request on ethyleneglycol and melamine

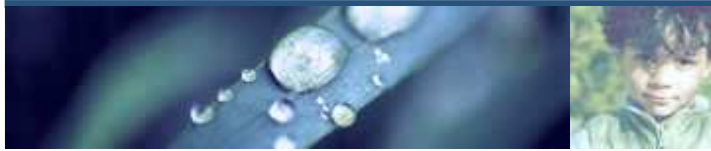
first three samples from the French customer
method validation (FDA-method)
method validation (LC/MS/MS-method)

Measurement of the samples (LC/MS/MS)

report

Cyanuric acid requested

Cyanuric acid
integrated into
the FDA-method



Basics of SOFIA GmbH

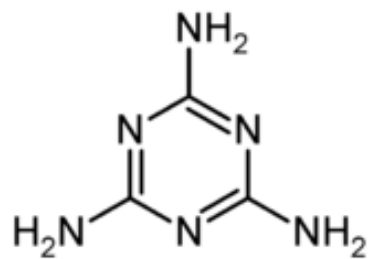
Personal / Equipment

- Food chemists
 - Chemists
 - laboratory stuff
 - IT
 - other
- GC/MS/NPD/FPD
 - GC/MS/EI
 - GC/MS/CI (pos./neg)
 - LC/UV/VIS/FD
 - LC/MS/MS
 - GC/HRMS (TOF)

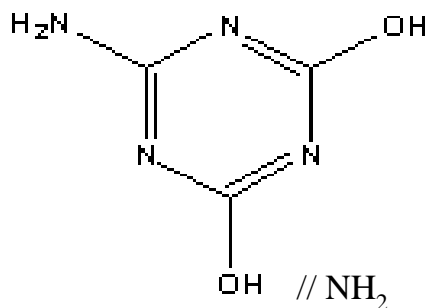


sum: 36 //academics: 19

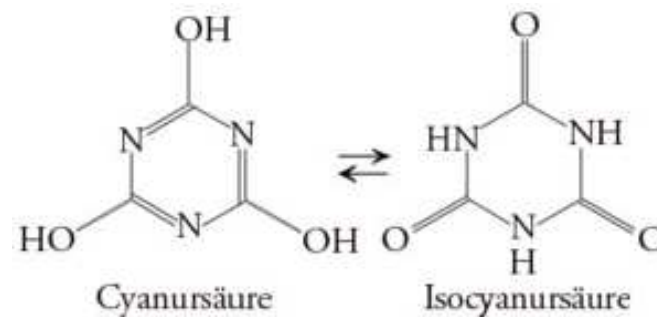
Analytics of Melamine and derived compounds



Melamine



Ammelide // Ammeline



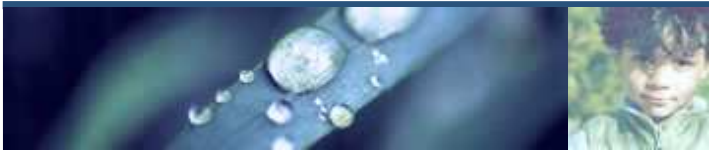
Cyanuric acid



FDA – method (GC/MS after silylation)

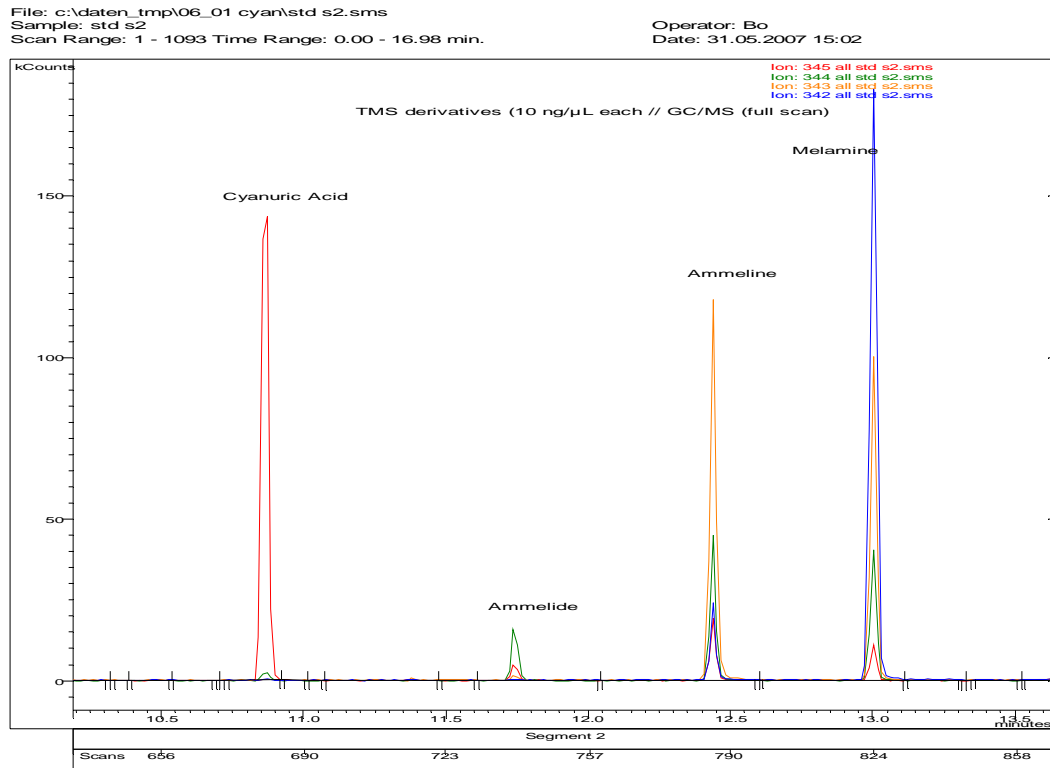
Method description (FDA Version Apr./May 2007)

- Extraction 0,5 to 1 g with DEA / Water / Acetonitrile (10/40/50)
- Sonication (30 min)
- Centrifugation
 - 200 µL evaporated to dryness for derivatization
 - Internal standard Benzoguanamine
- + 200 µl silylating reagent (BSTFA with 1 % TMCS) (bis(trimethylsilyl)trifluoroacetamide)
- analysis (GC/MS)

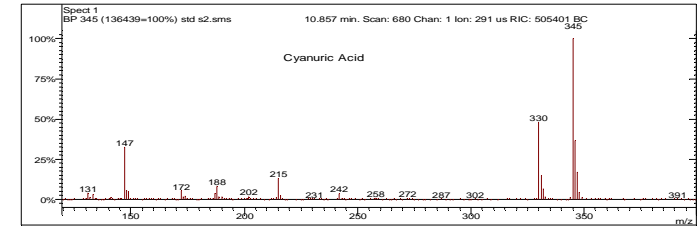


FDA – method (GC/MS after silylation)

Overlaid Chromatogram Plots

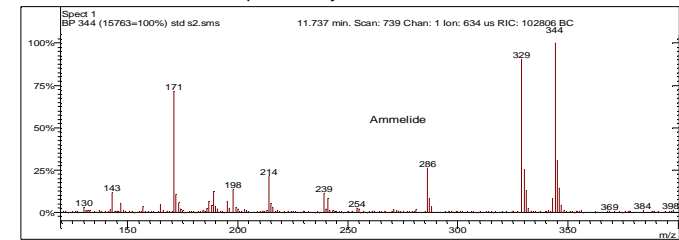


Scan 680 from c:\daten_tmp\06_01 cyan\std s2.sms



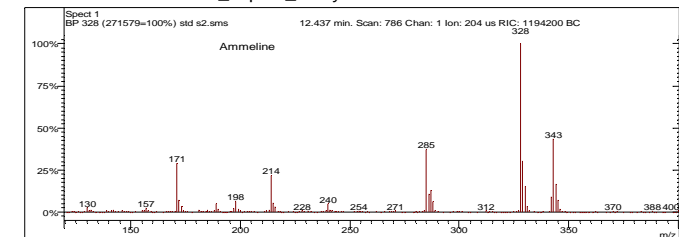
Spectrum from c:\daten_tmp\06_01 cyan\std s2.sms
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 No averaging, Background corrected.
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 Pair Count: 197 MW: 0 Formula: None CAS No: None Acquired Range: 120 - 400

Scan 739 from c:\daten_tmp\06_01 cyan\std s2.sms



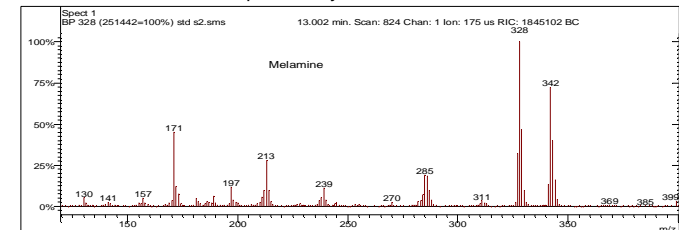
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 Scan No: 739, Time: 11.737 minutes
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 Comment: 11.737 min. Scan: 739 Chan: 1 Ion: 634 us RIC: 102806 BC
 Pair Count: 194 MW: 0 Formula: None CAS No: None Acquired Range: 120 - 400

Scan 786 from c:\daten_tmp\06_01 cyan\std s2.sms



Spectrum from c:\daten_tmp\06_01 cyan\std s2.sms
 Scan No: 786, Time: 12.437 minutes
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 Pair Count: 212 MW: 0 Formula: None CAS No: None Acquired Range: 120 - 400

Scan 824 from c:\daten_tmp\06_01 cyan\std s2.sms

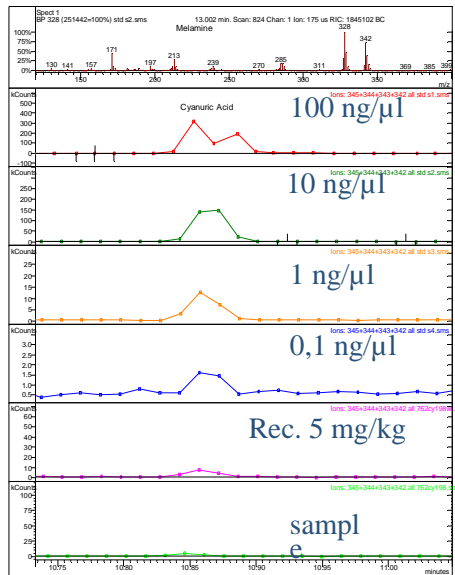


Spectrum from c:\daten_tmp\06_01 cyan\std s2.sms
 Scan No: 824, Time: 13.002 minutes
 No averaging, Background corrected.
 Comment: 13.002 min. Scan: 824 Chan: 1 Ion: 175 us RIC: 1845102 BC
 Pair Count: 250 MW: 0 Formula: None CAS No: None Acquired Range: 120 - 400

Sensitivity (LOQ)

Chromatogram Plots

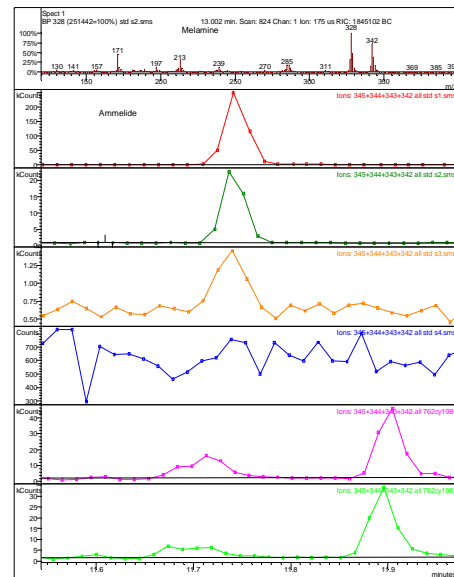
Plot 1: c:\daten_tmp\06_01 cyanistd s1.sms Ions: 345+344+343+342 all
 Plot 2: c:\daten_tmp\06_01 cyanistd s2.sms Ions: 345+344+343+342 all
 Plot 3: c:\daten_tmp\06_01 cyanistd s3.sms Ions: 345+344+343+342 all
 Plot 4: c:\daten_tmp\06_01 cyanistd s4.sms Ions: 345+344+343+342 all
 Plot 5: c:\daten_tmp\06_01 cyan/762cy198wf.sms Ions: 345+344+343+342 all
 Plot 6: c:\daten_tmp\06_01 cyan/762cy198.sms Ions: 345+344+343+342 all



Cyanuric Acid
10 mg/kg

Chromatogram Plots

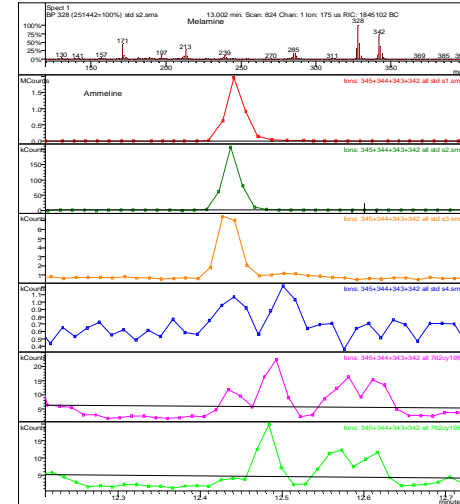
Plot 1: c:\daten_tmp\06_01 cyanistd s1.sms Ions: 345+344+343+342 all
 Plot 2: c:\daten_tmp\06_01 cyanistd s2.sms Ions: 345+344+343+342 all
 Plot 3: c:\daten_tmp\06_01 cyanistd s3.sms Ions: 345+344+343+342 all
 Plot 4: c:\daten_tmp\06_01 cyanistd s4.sms Ions: 345+344+343+342 all
 Plot 5: c:\daten_tmp\06_01 cyan/762cy198wf.sms Ions: 345+344+343+342 all
 Plot 6: c:\daten_tmp\06_01 cyan/762cy198.sms Ions: 345+344+343+342 all



Ammelide
20 mg/kg

Chromatogram Plots

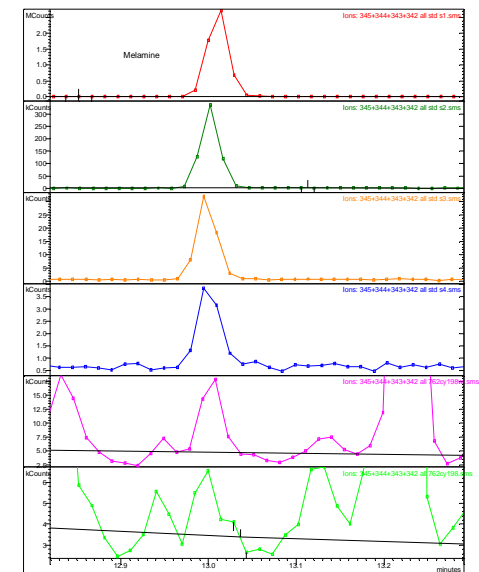
Plot 1: c:\daten_tmp\06_01 cyanistd s1.sms Ions: 345+344+343+342 all
 Plot 2: c:\daten_tmp\06_01 cyanistd s2.sms Ions: 345+344+343+342 all
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 Plot 4: c:\daten_tmp\06_01 cyanistd s4.sms Ions: 345+344+343+342 all
 Plot 5: c:\daten_tmp\06_01 cyan/762cy198wf.sms Ions: 345+344+343+342 all
 Plot 6: c:\daten_tmp\06_01 cyan/762cy198.sms Ions: 345+344+343+342 all



Ammeline
10 mg/kg

Chromatogram Plots

Plot 1: c:\daten_tmp\06_01 cyanistd s1.sms Ions: 345+344+343+342 all
 Plot 2: c:\daten_tmp\06_01 cyanistd s2.sms Ions: 345+344+343+342 all
 Plot 3: c:\daten_tmp\06_01 cyanistd s3.sms Ions: 345+344+343+342 all
 Plot 4: c:\daten_tmp\06_01 cyanistd s4.sms Ions: 345+344+343+342 all
 Plot 5: c:\daten_tmp\06_01 cyan/762cy198wf.sms Ions: 345+344+343+342 all
 Plot 6: c:\daten_tmp\06_01 cyan/762cy198.sms Ions: 345+344+343+342 all



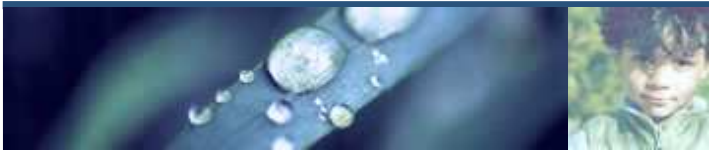
Melamine
5 mg/kg



LC/MS/MS method for confirmation and high speed analysis

Method description

- *Extraction 0,5 to 1 g with 20 mL Methanol/Water (50/50)*
- *Sonication (30 min)*
- *Freezing at -18 °C to separate the fat*
- *Dilution*
- *Membrane filtration*
 - *LC/MS/MS (5 µL, UPCL//QP-XE (C18 (1,7µm, 2,1 mm x 100 mm))*



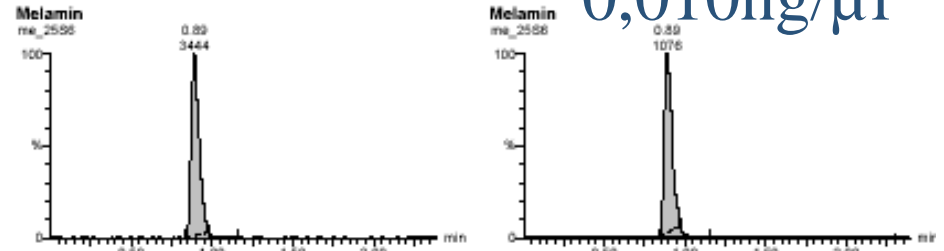
sensitiv

LOQ: 5 mg/kg

Method: Untitled 18 Feb 2008 16:19:48
 Calibration: 18 Feb 2008 16:21:27

Name: me_26S6, Date: 02-Aug-2007, Time: 10:22:56, Vial: 1:5, Description:

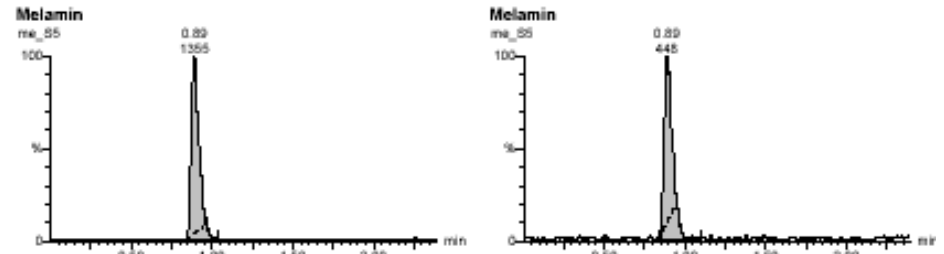
0,010ng/μl



#	Name	Sample Text	RT	1 st Trace	1 st Area	1 st Ratio (Actual)	Trace	Area
1	Melamin		0.89	127.03 > 68.05	1076	3.20	127.03 > 85	3444

Name: me_S6, Date: 02-Aug-2007, Time: 10:32:56, Vial: 1:6, Description:

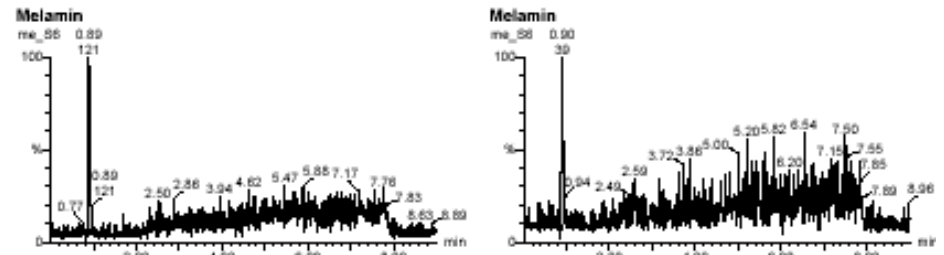
0,005ng/μl



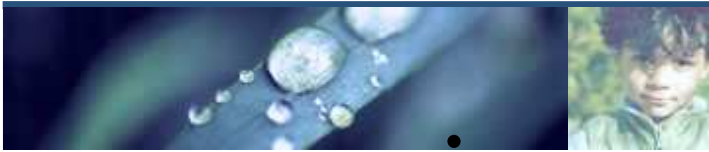
#	Name	Sample Text	RT	1 st Trace	1 st Area	1 st Ratio (Actual)	Trace	Area
1	Melamin		0.89	127.03 > 68.05	448	3.03	127.03 > 85	1355

Name: me_S6, Date: 02-Aug-2007, Time: 10:42:56, Vial: 1:7, Description:

0,001ng/μl



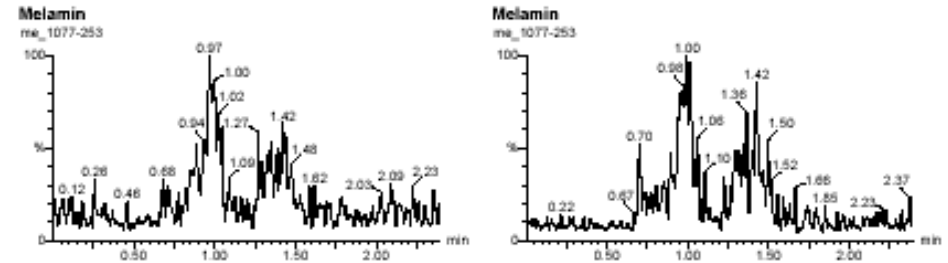
#	Name	Sample Text	RT	1 st Trace	1 st Area	1 st Ratio (Actual)	Trace	Area
1	Melamin		0.89	127.03 > 68.05	39	3.08	127.03 > 85	121



recovery rates

80 – 100 % for Glutene
70 – 90 % for pet food

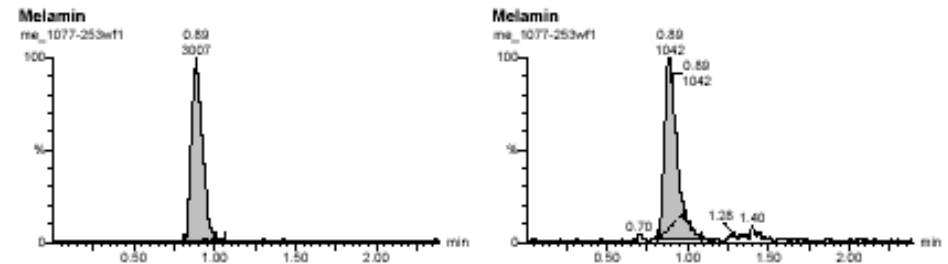
Name: me_1077-253, Date: 02-Aug-2007, Time: 11:12:51, Vial: 1:10, Description:



#	Name	Sample Text	RT	1 st Trace	1 st Area	1 st Ratio (Actual)	Trace	Area
1	Melamin				127.03	> 85		

5 mg/kg

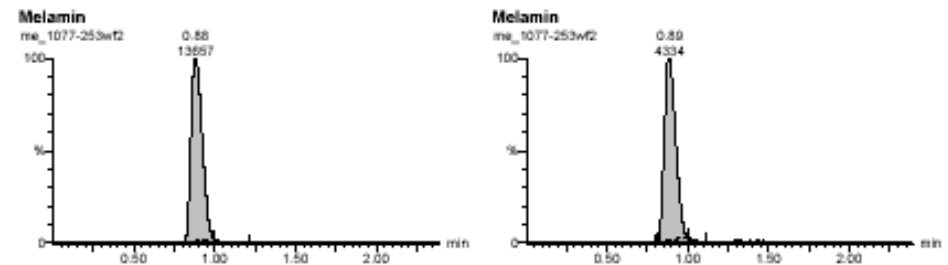
Name: me_1077-253wf1, Date: 02-Aug-2007, Time: 11:22:50, Vial: 1:11, Description: 5 ppm



#	Name	Sample Text	RT	1 st Trace	1 st Area	1 st Ratio (Actual)	Trace	Area
1	Melamin	5 ppm	0.89	127.03	> 88.05	1042	2.89	127.03 > 85
								3007

25 mg/kg

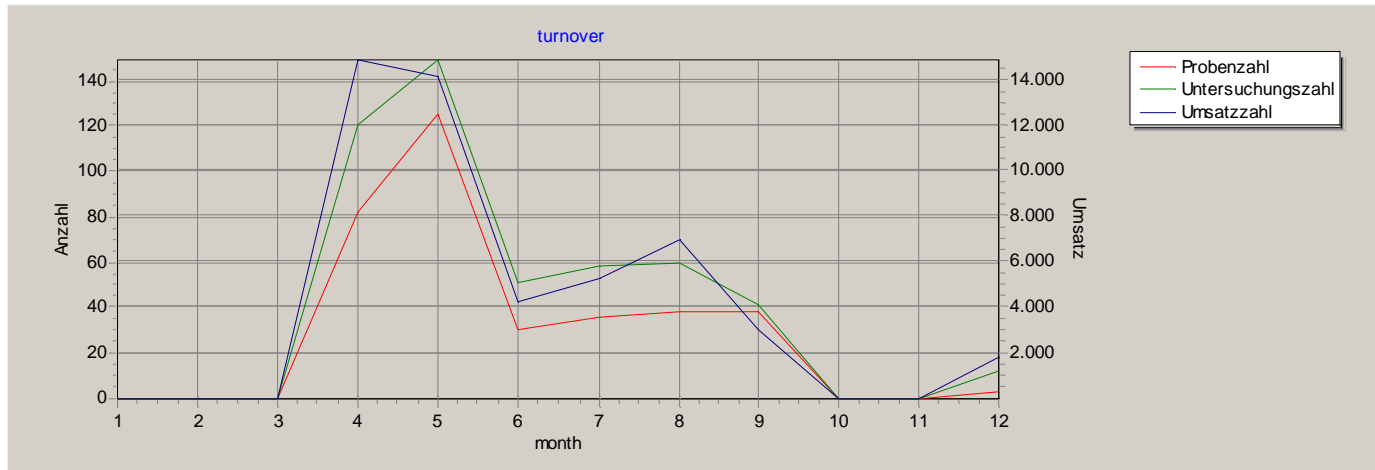
Name: me_1077-253wf2, Date: 02-Aug-2007, Time: 11:32:49, Vial: 1:12, Description: 25 ppm



#	Name	Sample Text	RT	1 st Trace	1 st Area	1 st Ratio (Actual)	Trace	Area
1	Melamin	25 ppm	0.88	127.03	> 88.05	4334	3.15	127.03 > 85
								13657

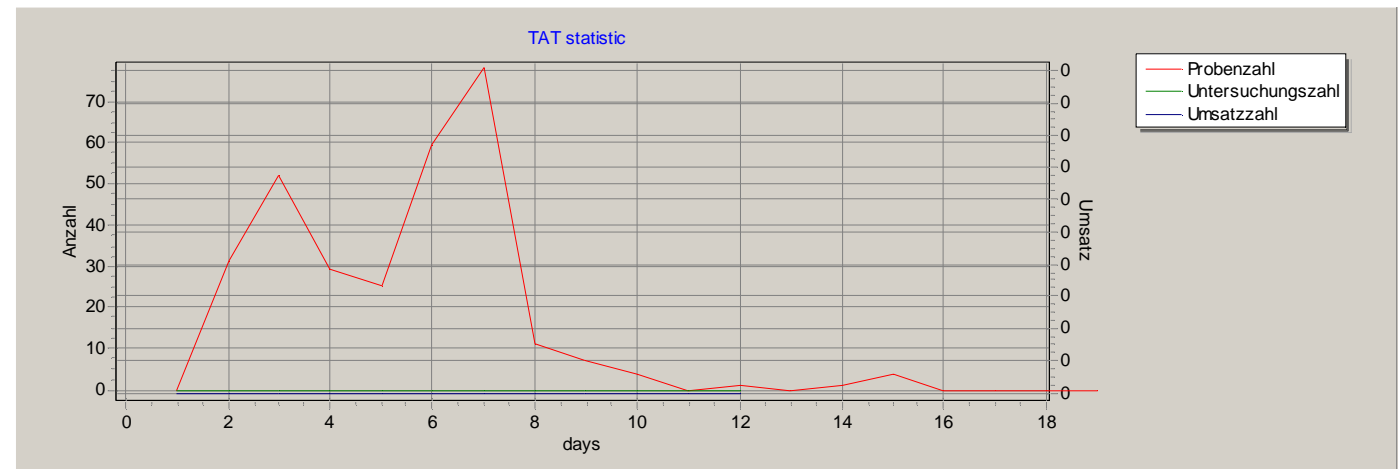


Turnover with Melamine / TATs



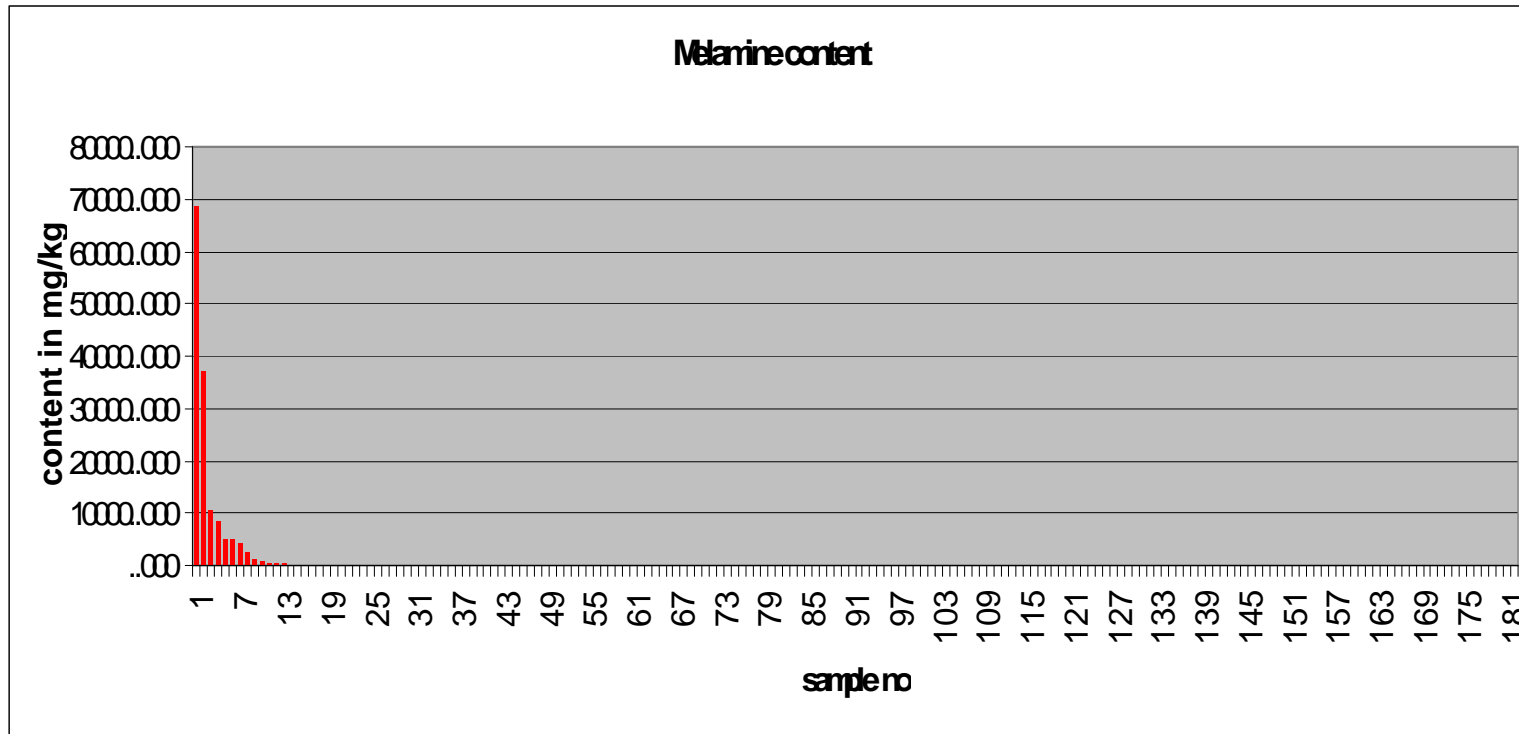
350 samples with 490 tests

Nearly 30% as express



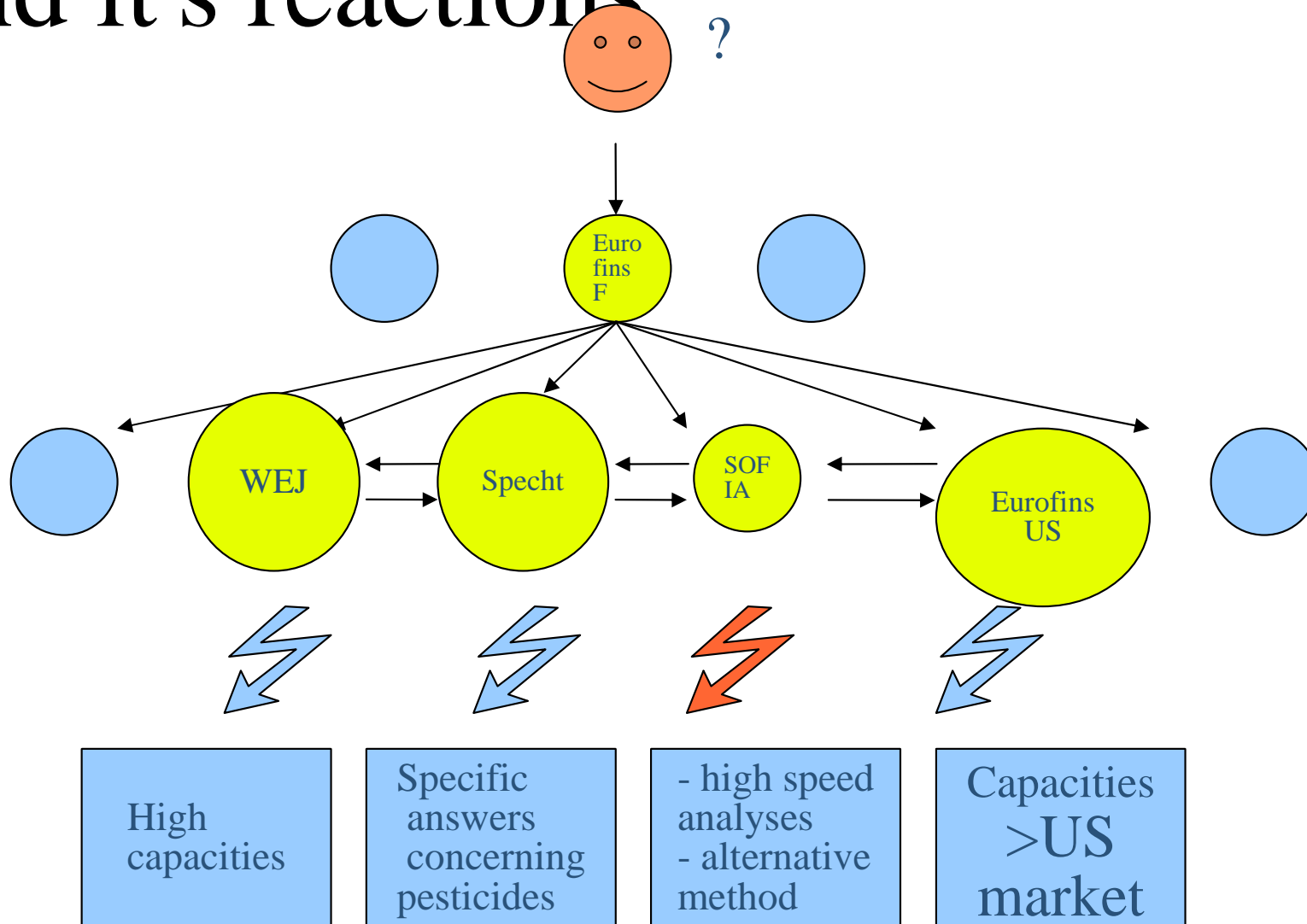


Melamin contents in pet food and gluten

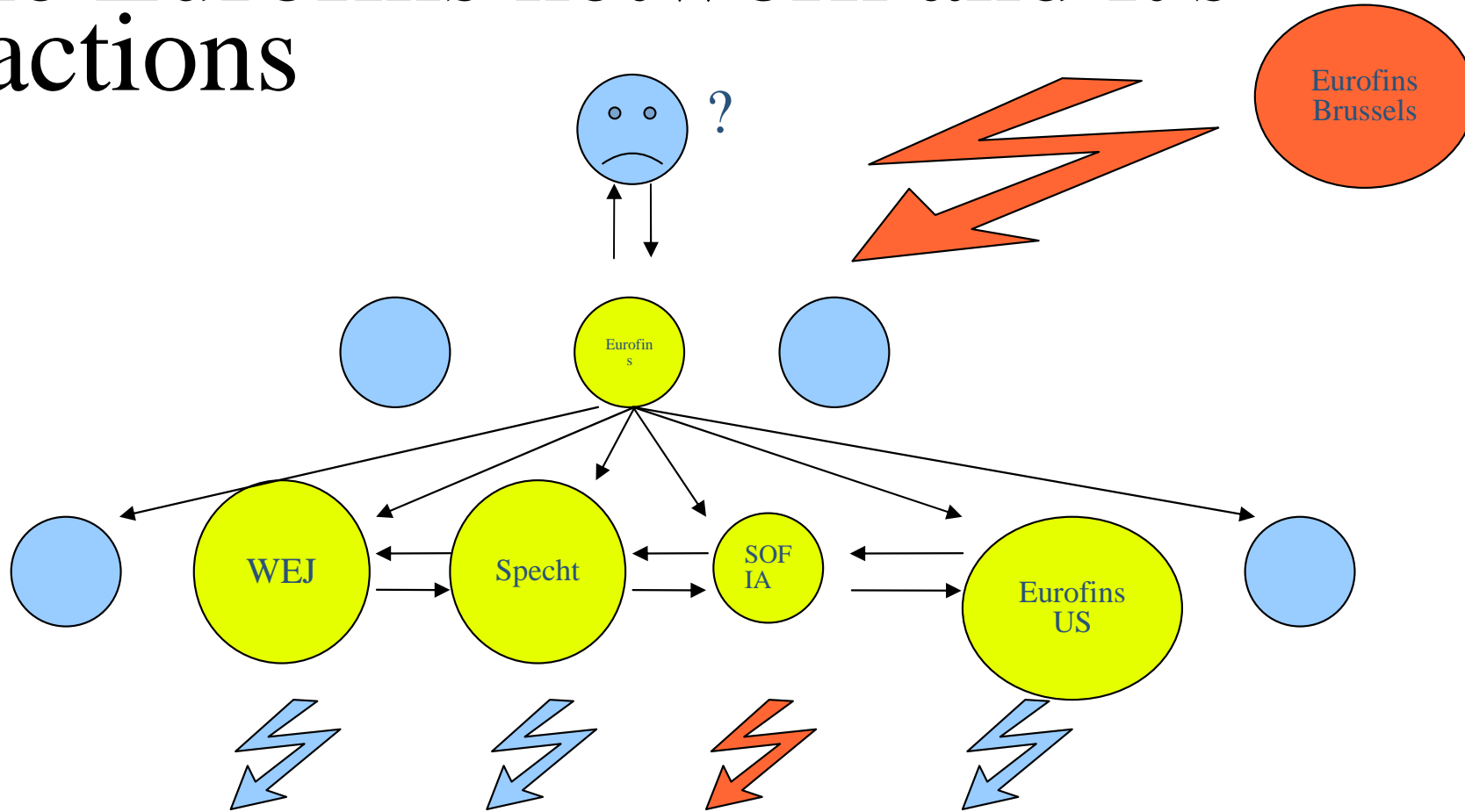


	Max (mg/kg)	count	% pos	comment
Ammelide	6800	86	3	highest values in glutene
Ammeline	711	86	10	highest values in glutene
Cyanuric acid	10000	171	24	highest values in glutene
Melamine	68000	242	10	highest values in glutene

Summary: The Eurofins network and it's reactions



The Eurofins network and it's reactions





Assessment :

The origin of the contamination.

More than 400 samples till the crisis beginning at the SOFIA site and several hundreds of samples at the WEJ site.

Our strong points according to the customer :

Local partner drawing on global expertise with a worldwide presence.

Complete offer, with a very high level of knowledge.

Real-time data and information exchange.

Expert in contaminant field.

Additional benefit for :

Client: They have now their dedicated lab for crisis management.

Dogs and cats: save feed



Conclusion

Eurofins can give a global answer to a global analytical question considering all specific requirements



Thank you for your attention

**Please find this presentation at
www.sofia-gmbh.de/melamine_EFS2008.pdf**