Agrochemical Analysis Sub-group Report

November 8th, 2011

Santiago - Chile

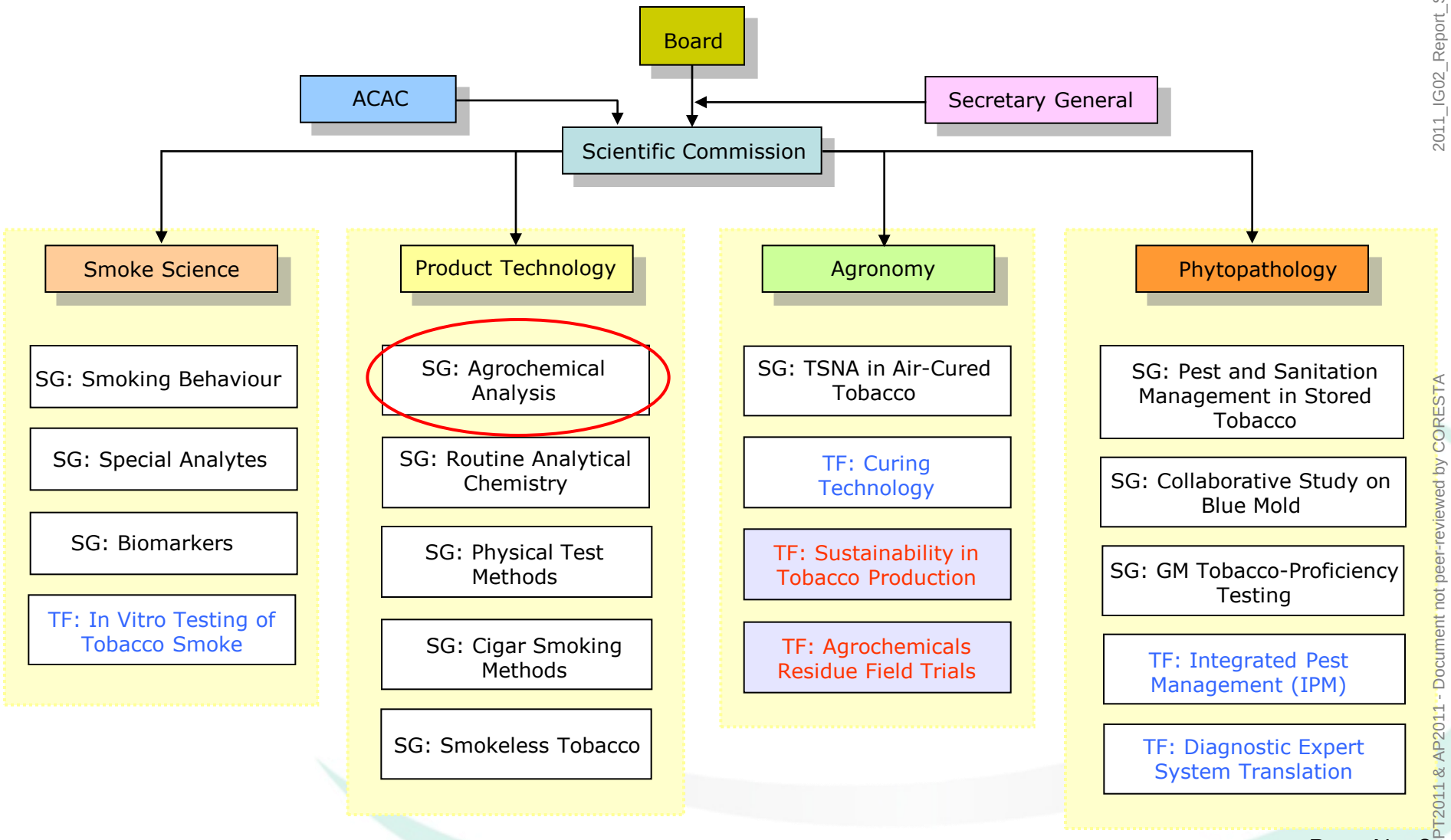
Objectives

1) To perform regular Proficiency Testing of Multi-Residue Methods for the analysis of agrochemical residues on tobacco

2) Undertake Joint Experiments to resolve unanswered questions arising from proficiency tests; to expand knowledge base on agrochemical residues and their analysis

3) Produce and maintain a series of Technical Notes (on different agrochemical residue classes and selected individual compounds) to supplement the Technical Guideline and aid method development and improvement

CORESTA Agrochemical Analysis Sub-group



2011 Activity

- CORESTA-FAPAS Proficiency test FT07
- Joint Experiment Test Study
- Degradation Study
- Technical Notes
- Technical Guideline review (CORESTA Guide N° 5)
- Sub-group meeting Bergerac (France) July 19-20, 2011

Objective 1: MRM Proficiency Testing

CORESTA-FAPAS Proficiency Test

- FAPAS = Food Analysis Performance Assessment Scheme ("The Food and Environmental Research Agency", York, UK)
- Proficiency Test N° 7
- 24 laboratories from 15 countries
- 20 spiked agrochemicals on blank tobacco residue free
- FAPAS Report FT07
- Collection of analytical methods used by the participant laboratories

Objective 1: MRM Proficiency Testing

FT07 Participant Laboratories

	Laboratory	Country
1	Analytica Alimentaria GmbH	Spain
2	Bioensaios	Brazil
3	CHELAB S.R.L.	Italy
4	China National Tobacco Quality Supervision & Test Center	China
5	Eurofins Analytik GmbH - Dr. Specht Lab	Germany
6	Eurofins Food & Agro Sweden AB	Sweden
7	Food and Consumer Product Safety Authority	Holland
8	Fytolab	Belgium
9	Global Laboratory Services, Inc.	USA
10	ITC R&D Centre	India
11	Japan Tobacco, Inc.- Tochigi	Japan
12	KT&G	Korea
13	Kutzaga Tobacco Insitute	Zimbabwe
14	Labor Friedle GmbH	Germany
15	LAnaRT	Argentina
16	Microbac Laboratories, Inc.	USA
17	Oekolab-JTI	Austria
18	Premium Tobacco (Thailand) Ltd	Thailand
19	PT H.M. Sampoerna Tbk.	Indonesia
20	Reemtsma Cigarettengabriken GmbH	Germany
21	Souza Cruz SA	Brazil
22	UFAG Laboratorien AG	Switzerland
23	Unviersidade Federal de Santa Maria	Brazil
24	USDA-AMS-S&T-FLS	USA
25	Zhengzhou Tobacco Research Institute of CNTC	China

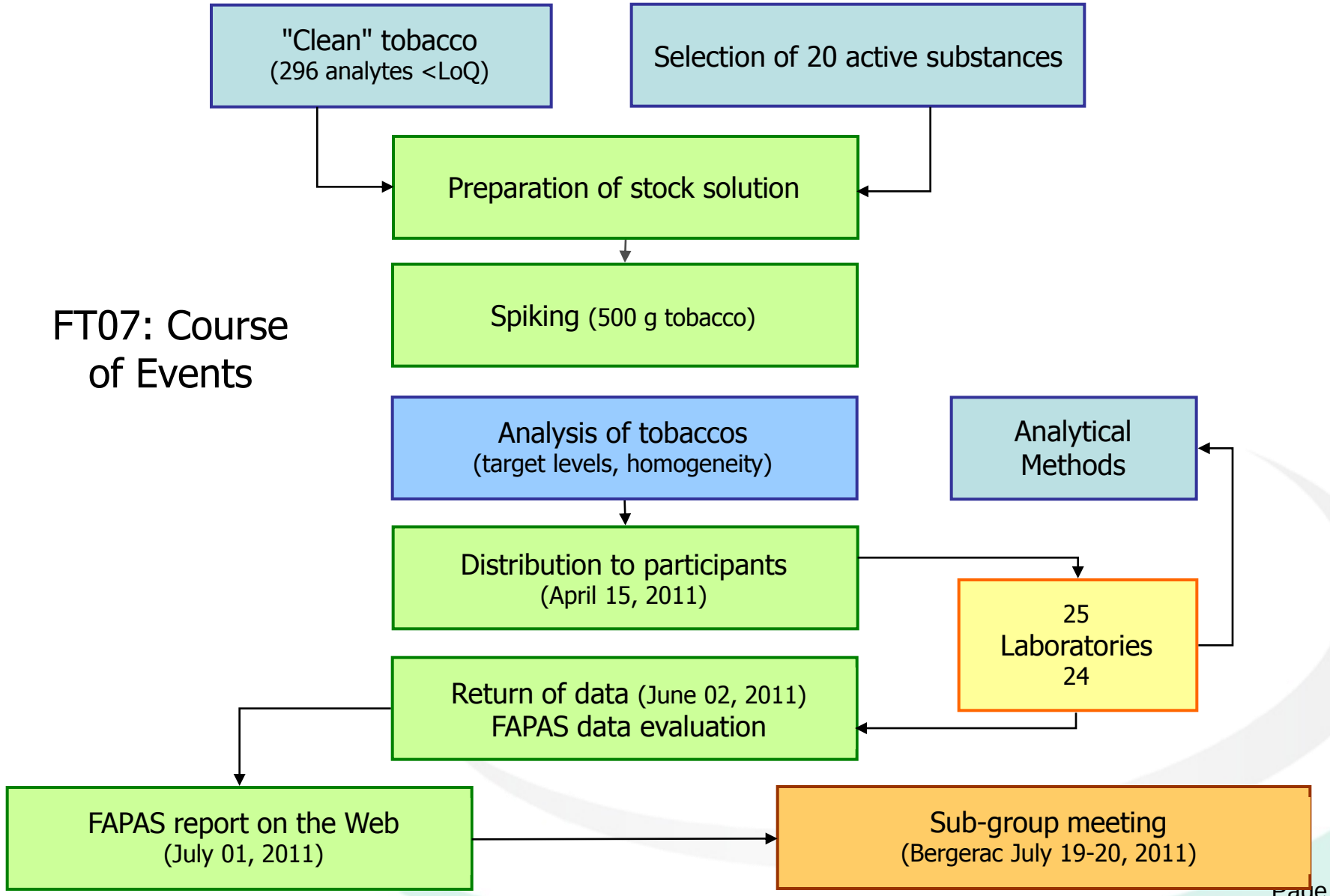
Objective 1: MRM Proficiency Testing

FT07: Agrochemical selection

Round 7: CPA List					
#	CPA	Spiking ppm	Reported as	GRL ppm	Type
1	2,4-D	0,20	2,4-D	0,20	Herbicide
2	Axoxystrobin	0,10	Axoxystrobin	n.a.	Fungicide
3	Benalaxyl	0,50	Benalaxyl	2,00	Fungicide
4	Captan	0,20	Captan	0,70	Fungicide
5	Chlorantraniliprole	5,00	Chlorantraniliprole	n.a.	Insecticide
6	Chlorpyrifos	0,40	Chlorpyrifos	0,50	Insecticide
7	Dicloran	0,80	Dicloran	1,00	Fungicide
8	Diflubenzuron	0,20	Diflubenzuron	0,10	Insecticide
9	Dimethomorph (Σ)	1,00	Σ of (E)-Dimethomorph and (Z)-Dimethomorph	2,00	Fungicide
10	Famoxadone	3,00	Famoxadone	5,00	Fungicide
11	Fenamidone	0,40	Fenamidone	n.a.	Fungicide
12	Iprobenfos	0,10	Iprobenfos	n.a.	Fungicide
13	Deltamethrin (Σ)	0,50	Σ of Deltamethrin + Tralomethrin	1,00	Insecticide
14	Flumetralin	2,50	Flumetralin	5,00	Growth Regulator
15	Indoxacarb (Σ)	2,00	Σ Indoxacarb (isomers S and R)	n.a.	Insecticide
16	Metalaxyl	0,50	Σ of all isomers including Metalaxyl-M / Mefenoxam	2,00	Fungicide
17	Methamidophos	1,00	Methamidophos	1,00	Insecticide
18	Pendimethalin	1,00	Pendimethalin	5,00	Herbicide
19	Triazophos	0,50	Triazophos	n.a.	Insecticide
20	Triflumuron	0,20	Triflumuron	n.a.	Insecticide

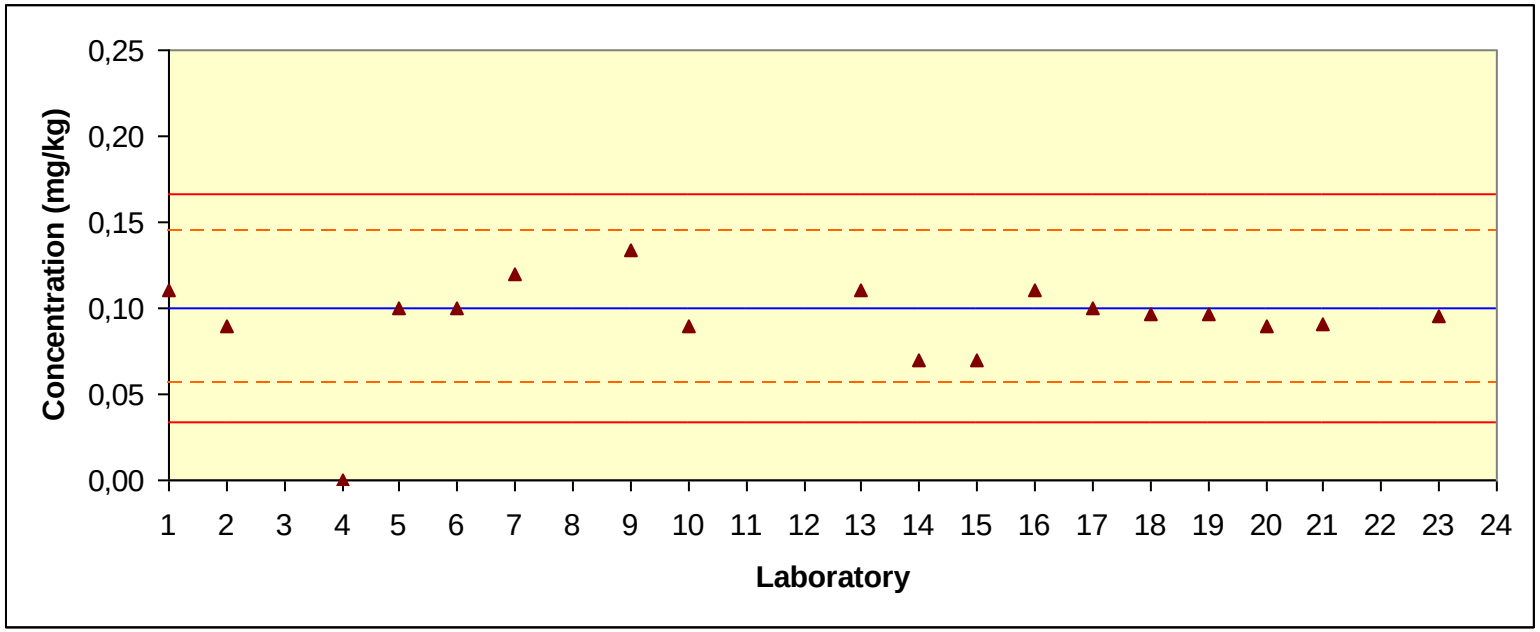
Objective 1: MRM Proficiency Testing

FT07: Course of Events



Objective 1: MRM Proficiency Testing

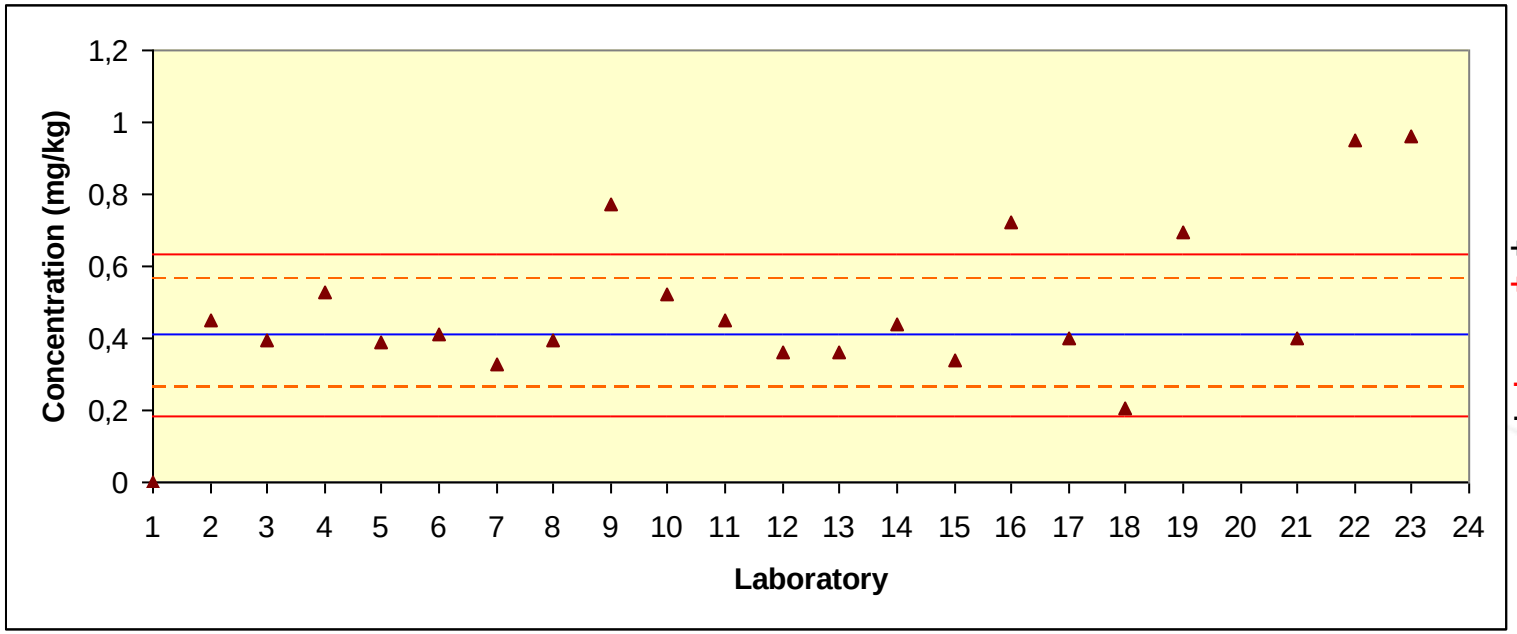
Azoxystrobin: laboratory results, z-score and assigned value



Azoxystrobin	Total laboratories	Total n° of scores	Scores %	N° of satisfactory scores	Satisfactory %
	24	17	71	17	100

Objective 1: MRM Proficiency Testing

Deltamethrin: laboratory results, z-score and assigned value



Deltamethrin	Total laboratories	Total n° of scores	Scores %	N° of satisfactory scores	Satisfactory %
	24	21	88	14	67

Objective 1: MRM Proficiency Testing

Round 7: z-scores satisfactory %

	2,4-D	Azoxystrobin	Benalaxyl	Captan	Chlorantraniliprole	Chlorpyriphos	Deltamethrin	Dicloran	Diflubenzuron	Dimethomorph	Famoxadone	Fenamidone	Flumetralin	Indoxacarb	Iprobenfos	Metalaxyl	Methamidiphos	Pendimethalin	Triazophos	Triflumuron	Total n° of score	N° of satis. scores	Satisfactory %
1				no z-score			-5,5				2,2										19	17	**89**
2														-2,3			-3,4				18	17	**94**
3	no z-score					-2,1															3	1	**33**
4		no z-score																			11	10	**91**
5																					19	19	**100**
6											3,2										18	17	**94**
7	3,8		3,4					7,2		3,0	6,1		-4,7	3,0		2,9	9,7	2,7			19	8	**42**
8			5,6							3,1	-2,4		4,3				-2,3	2,9			12	6	**50**
9						2,5	4,9		-4,8		-7,4			2,2			-5,8		2,4	-4,9	18	10	**56**
10						3,1		4,5	-4,8									3,8			19	15	**79**
11						no z-score												no z-score			4	2	**50**
12	-4,8												-5,4					-2,6			5	2	**40**
13																					19	19	**100**
14	-3,5																				12	11	**92**
15																					14	14	**100**
16							4,1		-4,8		-7,4						-5,8			-4,9	18	13	**72**
17																					19	19	**100**
18							-2,8														19	18	**95**
19							3,8		-2,1					6,9	no z-score						19	15	**79**
20					-2,2			-5,8					-6,9			-2,2					14	10	**71**
21																					19	19	**100**
22						no z-score	7,2														2	0	**0**
23							7,3				4,9				-2,4		6,9				19	15	**79**
24						2,6															3	2	**67**
Total number of score	15	17	19		15	22	22	19	18	19	16	15	21	16	12	19	18	21	16	15			
Number of satisfactory scores	12	17	17		14	18	14	16	14	17	9	15	17	12	11	17	12	17	15	13			
Satisfactory %	**80**	**100**	**89**		**93**	**82**	**64**	**84**	**78**	**89**	**56**	**100**	**81**	**75**	**92**	**89**	**67**	**81**	**94**	**87**			

= satisfactory
= not found / <LoQ
= unsatisfactory
= no tested for
underline = for information only

Objective 1: MRM Proficiency Testing

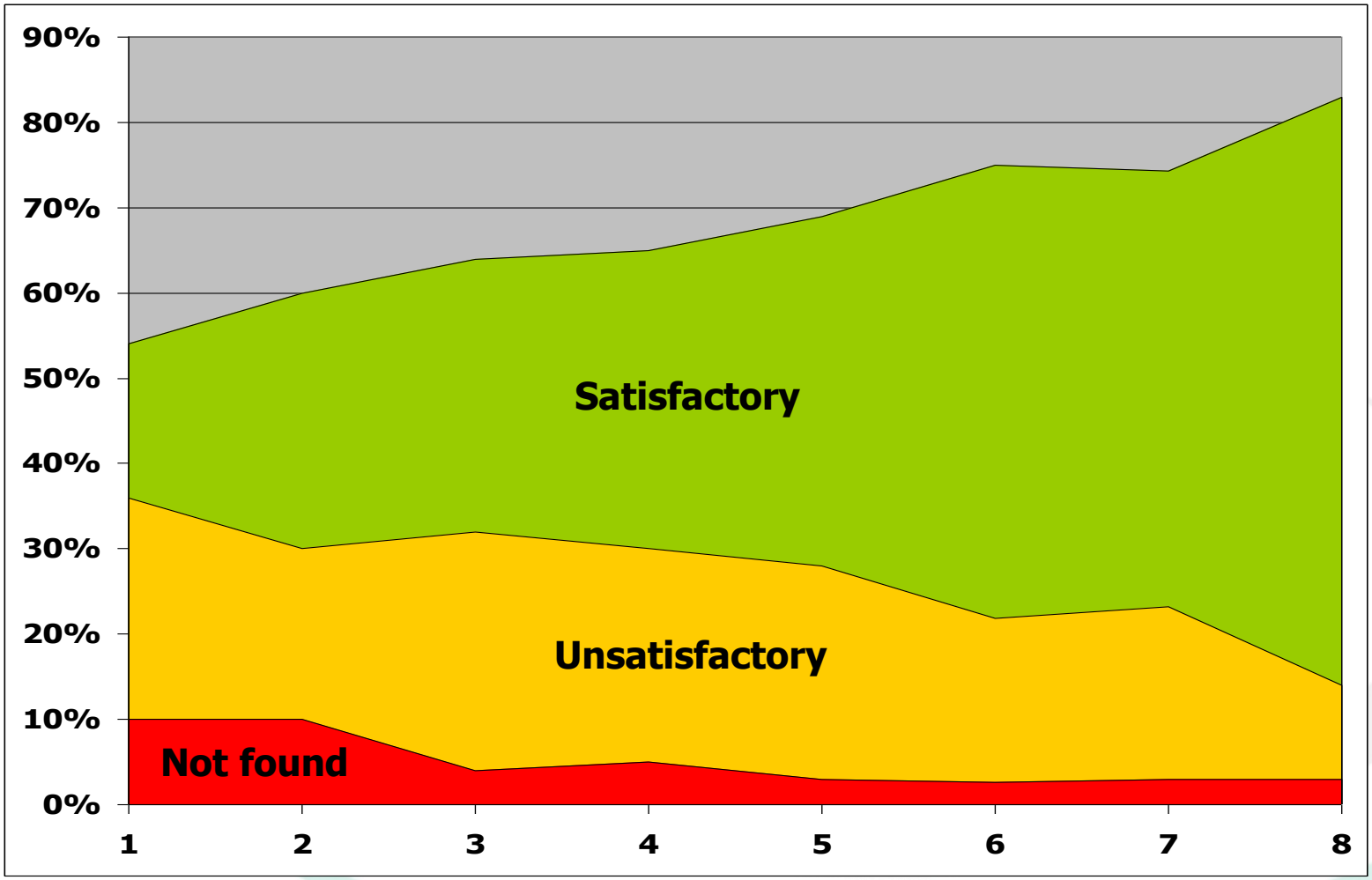
z-scores satisfactory % comparison between PTs

	2004	2005	2006	2007	2008	2009	2010	2011
	Pilot Run	Round 1	Round 2	Round 3	Round 4	Round 5	Round 6	Round 7
Analytes not tested for	42%	16%	15%	23%	33%	27%	17%	25%
Analytes within battery	58%	84%	85%	77%	67%	73%	83%	75%
Results satisfactory	54%	60%	64%	65%	69%	75%	74%	83%
Results unsatisfactory	36%	30%	32%	30%	28%	22%	23%	14%
Analytes not found	10%	10%	4%	5%	3%	3%	3%	3%

Results satisfactory	Pilot Run	Round 1	Round 2	Round 3	Round 4	Round 5	Round 6	Round 7
Aldicarb	-	-	-	59%	-	-	79%	-
Butralin	-	-	-	78%	-	-	70%	-
Carbaryl	45%	-	-	-	80%	-	83%	-
Carbendazim	-	-	-	67%	-	76%	52%	-
Chlorothalonil	-	-	44%	43%	-	no z-score	-	-
Chlorpyriphos (-ethyl)	-	83%	-	-	-	76%	-	82%
Chlorthal-dimethyl	50%	-	-	-	-	75%	-	-
Cypermethrin	79%	-	71%	-	-	68%	73%	-
Benalaxyl	-	64%	-	-	-	-	-	89%
Deltamethrin	-	44%	59%	-	-	-	60%	64%
Dimethomorph	-	-	-	-	60%	-	-	89%
Endosulfan (Σ)	-	59%	67%	-	-	68%	-	-
Famoxadone	-	-	-	-	-	no z-score	-	56%
Fenamiphos	-	-	-	-	79%	-	90%	-
Flumetralin	-	-	61%	-	-	-	71%	81%
Heptachlor	81%	77%	-	-	-	-	-	-
Imidacloprid	-	54%	64%	-	-	-	-	-
Indoxacarb	-	-	-	-	-	-	83%	75%
Iprodione	-	-	-	-	58%	85%	-	-
Metalaxyl	33%	-	63%	-	-	-	-	89%
Methamidophos	8%	25%	44%	48%	-	55%	no z-score	67%
Permethrin	-	-	-	81%	-	-	71%	-
Pendimethalin	-	87%	76%	-	-	-	62%	81%
Profenophos	54%	-	76%	-	-	-	70%	-
Trifluralin	62%	-	-	87%	-	-	90%	-

Objective 1: MRM Proficiency Testing

z-scores satisfactory % comparison between PTs

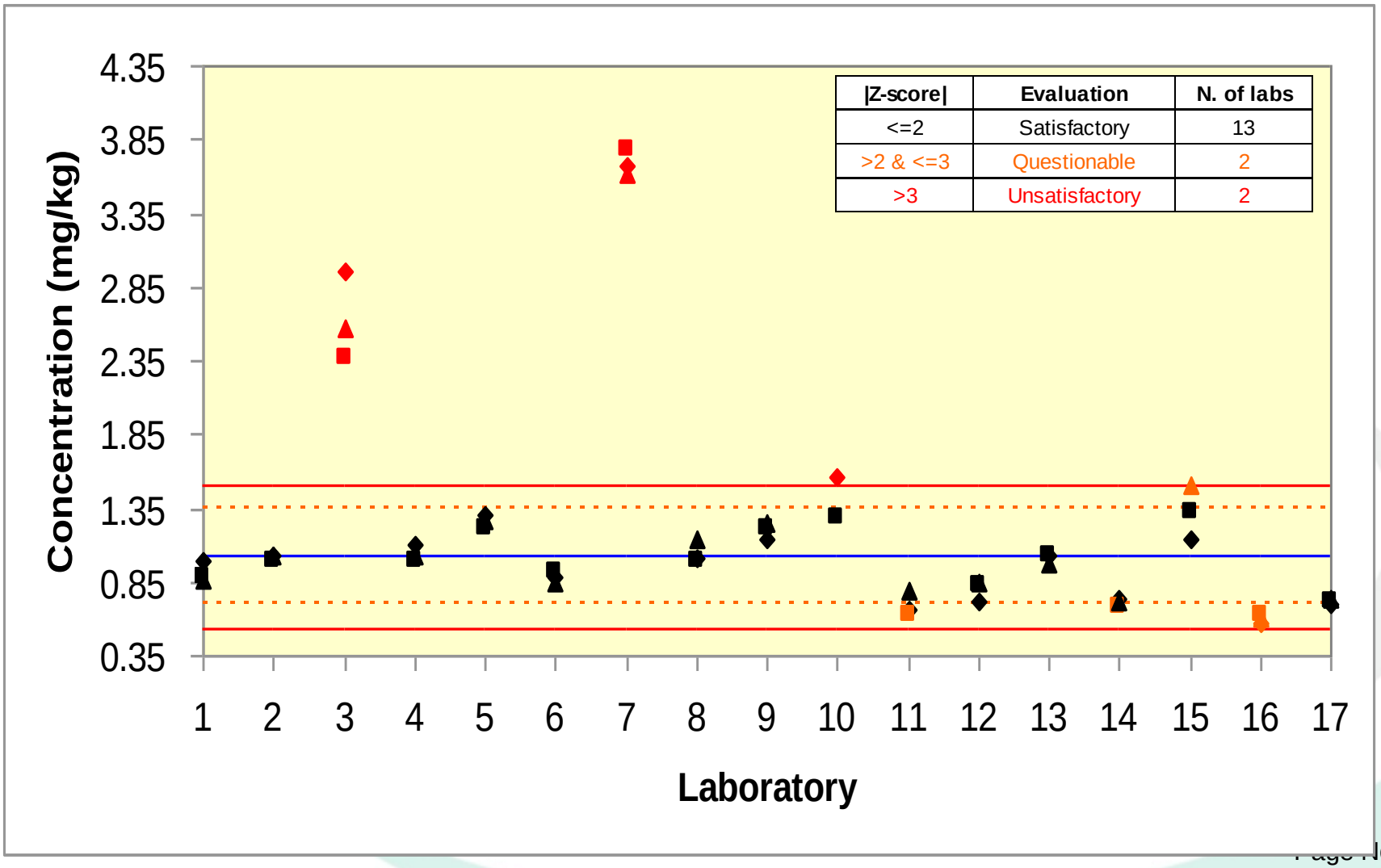


Joint Experiment Test Study

- Objective: To identify analytical issues for specific CPAs arising from the proficiency test
- Performed: Cyfluthrin (coordinator **Masahiro Miyoshi**, JT LTRC Oyama)
  - 17 participant laboratories
  - Test material:
    - Sample 1: naturally incurred tobacco (Turkish Oriental crop 2008)
    - Sample 2: artificially spiked tobacco
    - Sample 3: blank tobacco for recovery and/or matrix-matched calibration
    - Homogeneity test sample 1 and 2
  - Test procedure:
    - Each sample was analyzed in triplicate
    - Laboratories were free to choose the analytical method
    - Two recoveries (fortification level 0.50 mg/kg) were measured

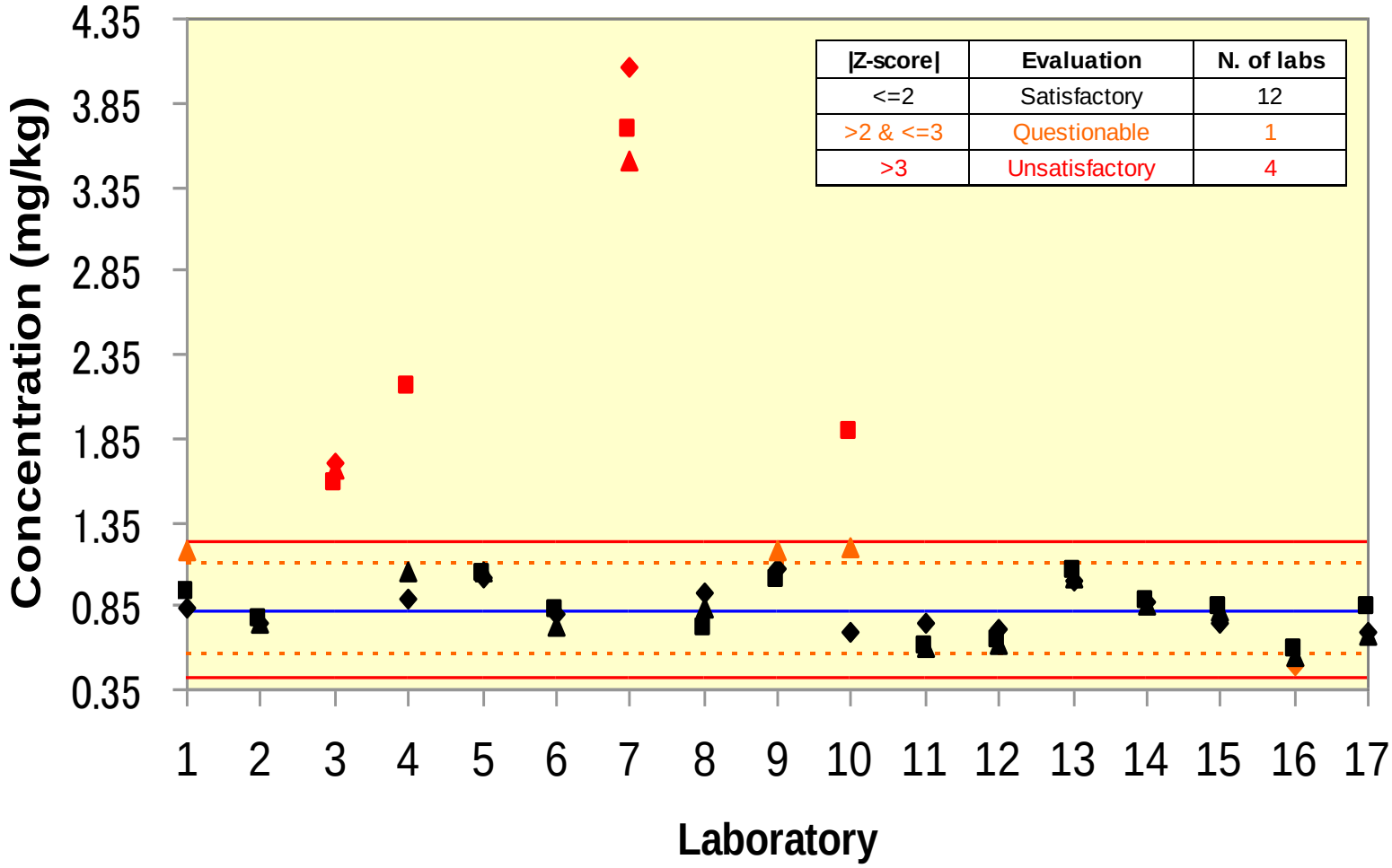
Objective 2: Joint Experiment Test Study

Sample 1 – naturally incurred



Objective 2: Joint Experiment Test Study

Sample 2 – artificially spiked



|Z-score|	Evaluation	N. of labs
<=2	Satisfactory	12
>2 & <=3	Questionable	1
>3	Unsatisfactory	4

Objective 2: Joint Experiment Test Study

Sample 1 & Sample 2

Lab number	Z-score	
	Sample 1	Sample 2
1	-0.6	1.2
2	0.0	-0.5
3	9.9	6.1
4	0.1	4.1
5	1.5	1.6
6	-0.9	-0.3
7	16.5	21.7
8	0.2	0.0
9	1.0	1.9
10	2.5	3.3
11	-2.0	-1.3
12	-1.4	-1.2
13	0.0	1.5
14	-1.9	0.4
15	1.9	-0.1
16	-2.6	-2.1
17	-1.9	-0.7

Except Lab 4, each laboratory obtained almost the same Z-score for both samples, regardless of the type of samples, i.e. incurred or spiked.

Objective 2: Joint Experiment Test Study

Degradation Study: coordinator **Dr. Thomas Anspach** - Eurofins Dr. Specht

#	CPA	Spiking ppm	Reported as	GRL ppm	Notes
1	Aldicarb (Σ)	0,40	Σ of Aldicarb + Aldicarb sulfoxide + Aldicarb sulfone	0,50	
2	Carbendazim (Σ)	3,00	Σ of Benomyl + Carbendazim + Thiophanate-methyl	2,00	Degradation study
3	Butralin	2,00	Butralin	5,00	Degradation study
4	Carbaryl	0,50	Carbaryl	0,50	
5	Cyfluthrin (Σ)	0,50	Σ Cyfluthrin (all isomers)	0,50	Degradation study
6	Cypermethrin (Σ)	1,50	Σ Cypermethrin (all isomers)	1,00	Degradation study
7	Deltamethrin (Σ)	1,00	Σ of Deltamethrin + Tralomethrin	1,00	
8	Difenconazole	0,50	Difenconazole	na	
9	Fenamiphos (Σ)	0,30	Σ of Fenamiphos + Fenamiphos sulfoxide + Fenamiphos sulfone	0,50	
10	Flumetralin	6,00	Flumetralin	5,00	Degradation study
11	Indoxacarb (Σ)	1,00	Σ Indoxacarb (isomers S and R)	15,00	
12	Methamidophos	2,00	Methamidophos	1,00	
13	Pendimethalin	3,00	Pendimethalin	5,00	Degradation study
14	Permethrin (Σ)	0,60	Σ Permethrin (all isomers)	0,50	Degradation study
15	Profenofos	0,20	Profenofos	0,10	
16	Trifluralin	0,20	Trifluralin	0,10	Degradation study

- Test material: FAPAS Round 6 samples → 8 CPAs identified for the degradation study
- The CPAs for the degradation study have been communicated to the laboratories at the end of the PT
- Some laboratory have additionally tested: Aldicarb, Aldicarb sulfoxide, Carbaryl, Deltamethrin, Indoxacarb, Fenamiphos, Fenamiphos sulfoxide, Methamidophos, Profenofos
- 7 participant laboratories: GLS, Oekolab, Microbac, LAnaRT, Eurofins Dr. Specht, Eurofins Sweden, JT LTRC

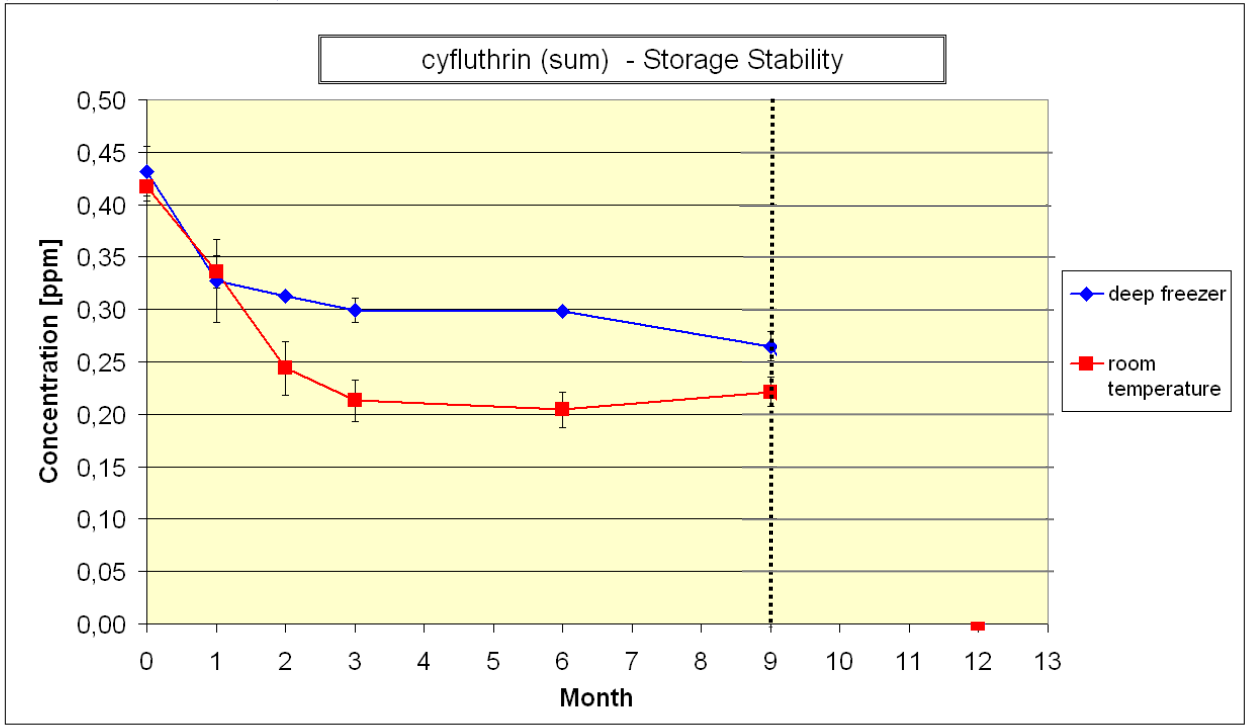
Objective 2: Joint Experiment Test Study

Degradation Study

cyfluthrin (sum)

		deep freezer					room temperature						
	date of analysis	replicate 1 [ppm]	replicate 2 [ppm]	replicate 3 [ppm]	mean [ppm]	SD [ppm]	replicate 1 [ppm]	replicate 2 [ppm]	replicate 3 [ppm]	mean [ppm]	SD [ppm]	fortification level [ppm]	fortification recovery [%]
Month 0	21. Mai 10	0,429	0,409	0,457	0,432	0,024	0,418	0,430	0,403	0,417	0,014		
Month 1	21. Jun 10	0,289	0,325	0,368	0,327	0,039	0,321	0,352	0,335	0,336	0,016		
Month 2	21. Jul 10	0,313	0,313	0,313	0,313	0,000	0,216	0,267	0,248	0,244	0,026		
Month 3	21. Aug 10	0,296	0,290	0,312	0,299	0,011	0,200	0,236	0,204	0,213	0,020		
Month 6	21. Nov 10	0,299	0,301	0,296	0,299	0,003	0,186	0,207	0,219	0,204	0,017		
Month 9	21. Feb 11	0,250	0,267	0,277	0,265	0,014	0,206	0,223	0,234	0,221	0,014		
Month 12					#DIV/0!	#DIV/0!				#DIV/0!	#DIV/0!		

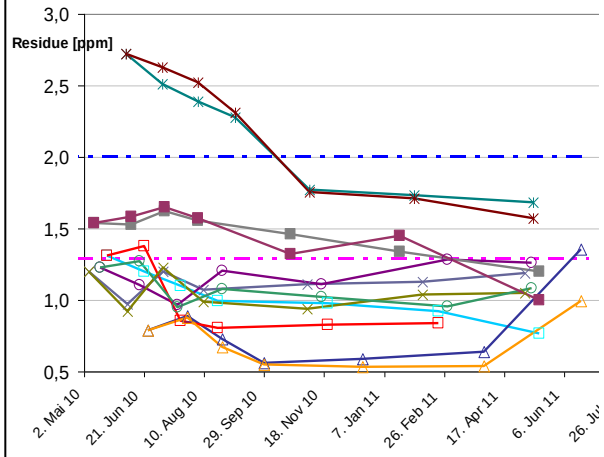
Note: deep freezer results for Month 2 were extrapolated



Objective 2: Joint Experiment Test Study

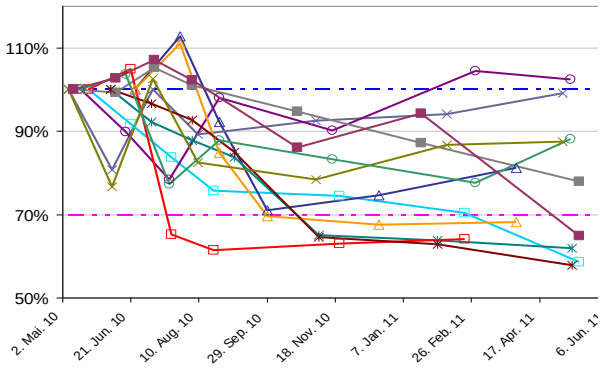
Degradation Study: graphical set-up of results

individual absolute mg/kg results



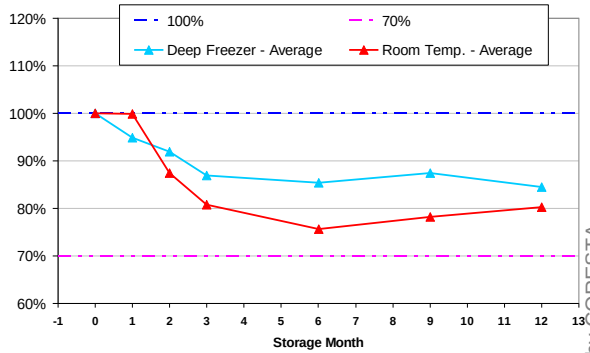
The mg/kg results of each lab are plotted versus the concrete date of month (no correction for recovery was made)

individual relative % results



Day 0 of each lab was set to 100% and percentage of the following monthly results was calculated in regards to day 0

average degradation curve



Mean percentage value for each month interval for all lab results was calculated and plotted versus unified month x-scale

Objective 2: Joint Experiment Test Study

Assessment of degradation studies

ec.europa.eu/food/plant/protection/resources/app-h.pdf

COMMISSION OF THE
EUROPEAN COMMUNITIES

7032/VI/95 rev.5
22/7/97

Directorate General for Agriculture

VI B II-1

APPENDIX H

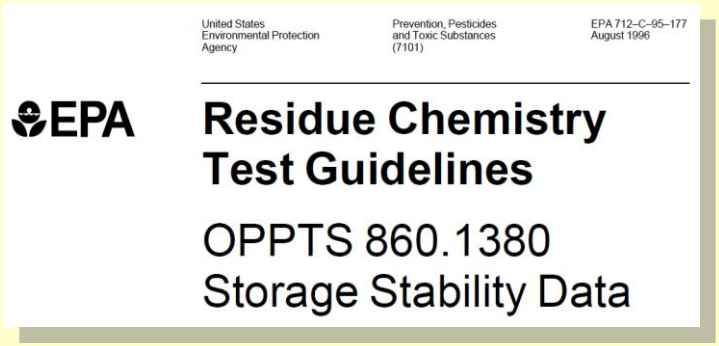
STORAGE STABILITY OF RESIDUE SAMPLES

Where the degradation during storage is significant (more than 30%) it may be necessary to use a shorter sample storage period prior to analysis.

Objective 2: Joint Experiment Test Study

Assessment of degradation studies

www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series860.htm


United States Environmental Protection Agency | Prevention, Pesticides and Toxic Substances (7101) | EPA 712–C–95–177 August 1996

EPA

Residue Chemistry Test Guidelines

OPPTS 860.1380
Storage Stability Data

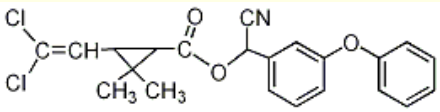
(8) **Use of storage stability results.** (i) If a storage stability study shows limited decline of residues during the storage period observed for the corresponding magnitude-of-the-residue study, correction factors will generally be used to determine the residue levels that were present at the time of sample collection in the study. However, if extensive dissipation of residues has occurred during storage, the study may need to be repeated with samples analyzed closer to their time of collection. As a rule of thumb, correction factors will be applied to losses in storage up to 30 percent. Beyond that point, the Agency will consider corrections on a case-by-case basis taking into account factors such as the absolute (parts per million) and relative (percent of TTR) residue levels of the component that is unstable in storage.

(ii) The degree of loss will normally be adjusted or corrected for analytical method recoveries before applying the 30 percent rule of thumb. In other words, the apparent residue level of an analyte after storage should be divided by the analytical method recoveries obtained for freshly fortified samples analyzed at the same time. For example, a storage stability sample was originally prepared by spiking at 1.0 ppm (level confirmed by zero-day analysis after correcting for method recovery of 75 percent on a freshly fortified sample). After a given period of storage, a portion of the sample is analyzed and found to contain only 0.63 ppm (an apparent loss of 37 percent). If the method recoveries for freshly fortified samples analyzed at the same time are 70 percent, the corrected residue level in the stored sample is 0.63 ppm/0.70 = 0.90 ppm. Thus, the corrected degree of loss in storage is 10 percent (or corrected recovery of 90 percent for the stored sample).

Objective 2: Joint Experiment Test Study

Cypermethrin – individual absolute results [ppm]

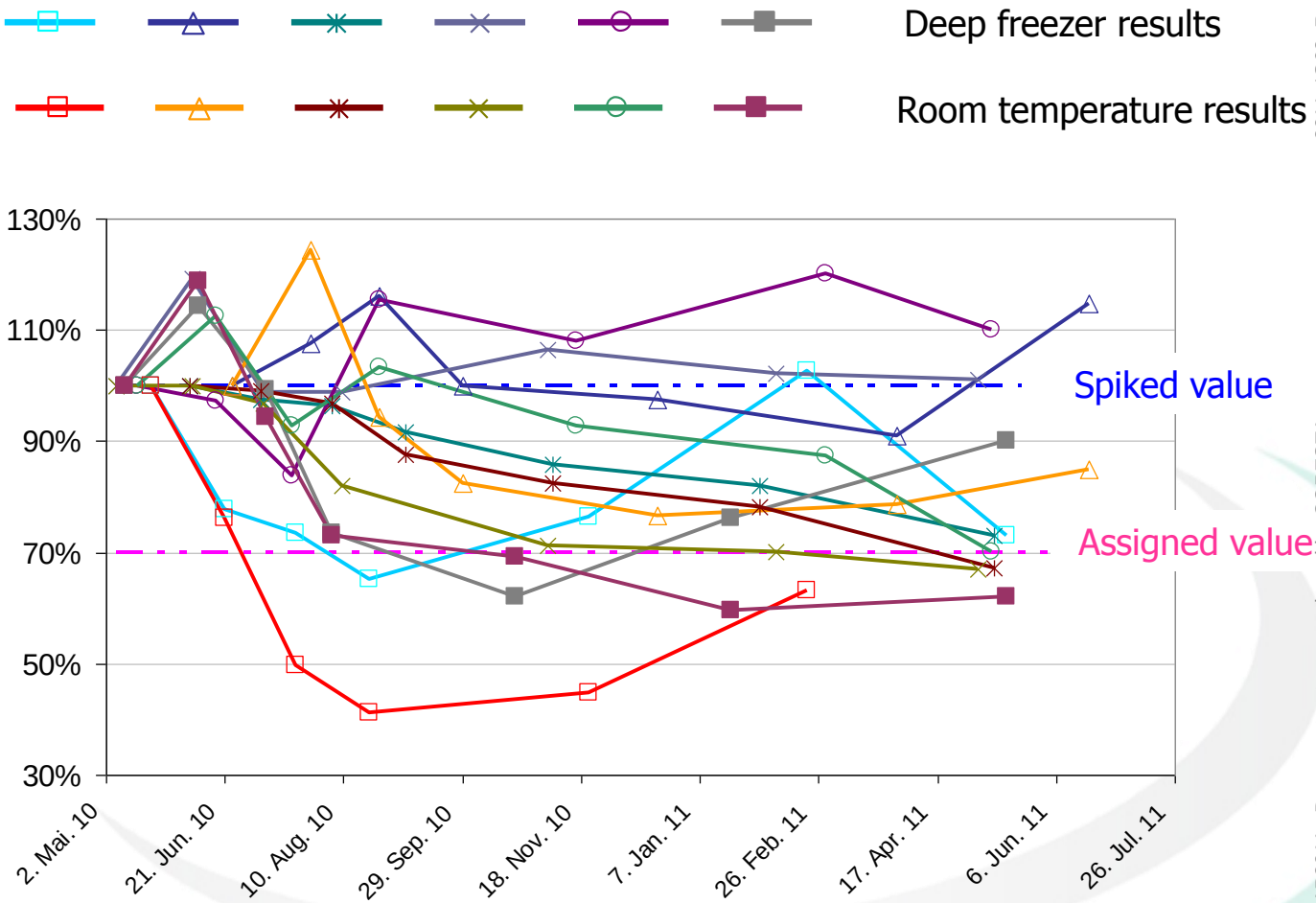
- **pyrethroid**



Comprises a mixture of cis- and trans-isomeres

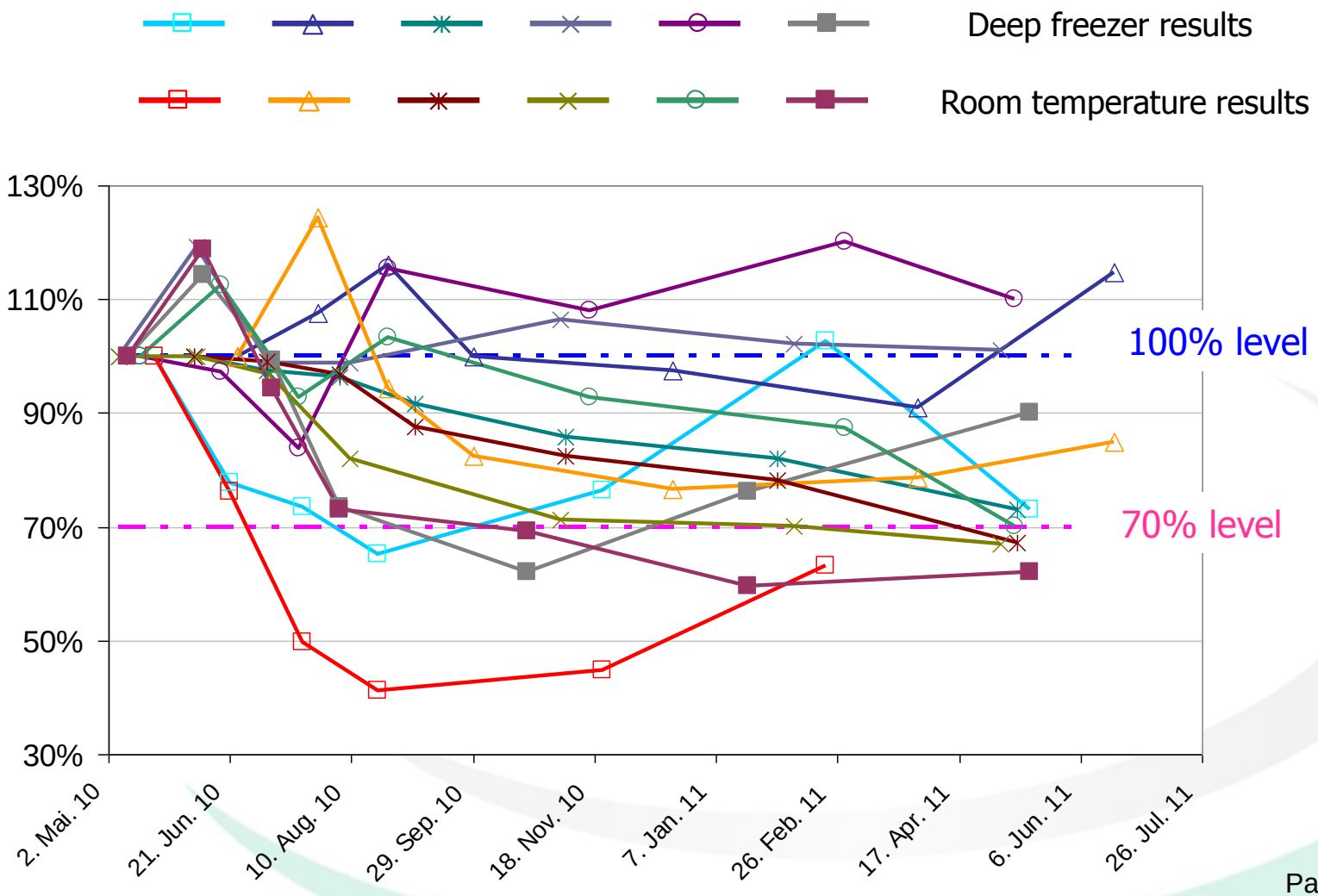
On the plant:

No information.



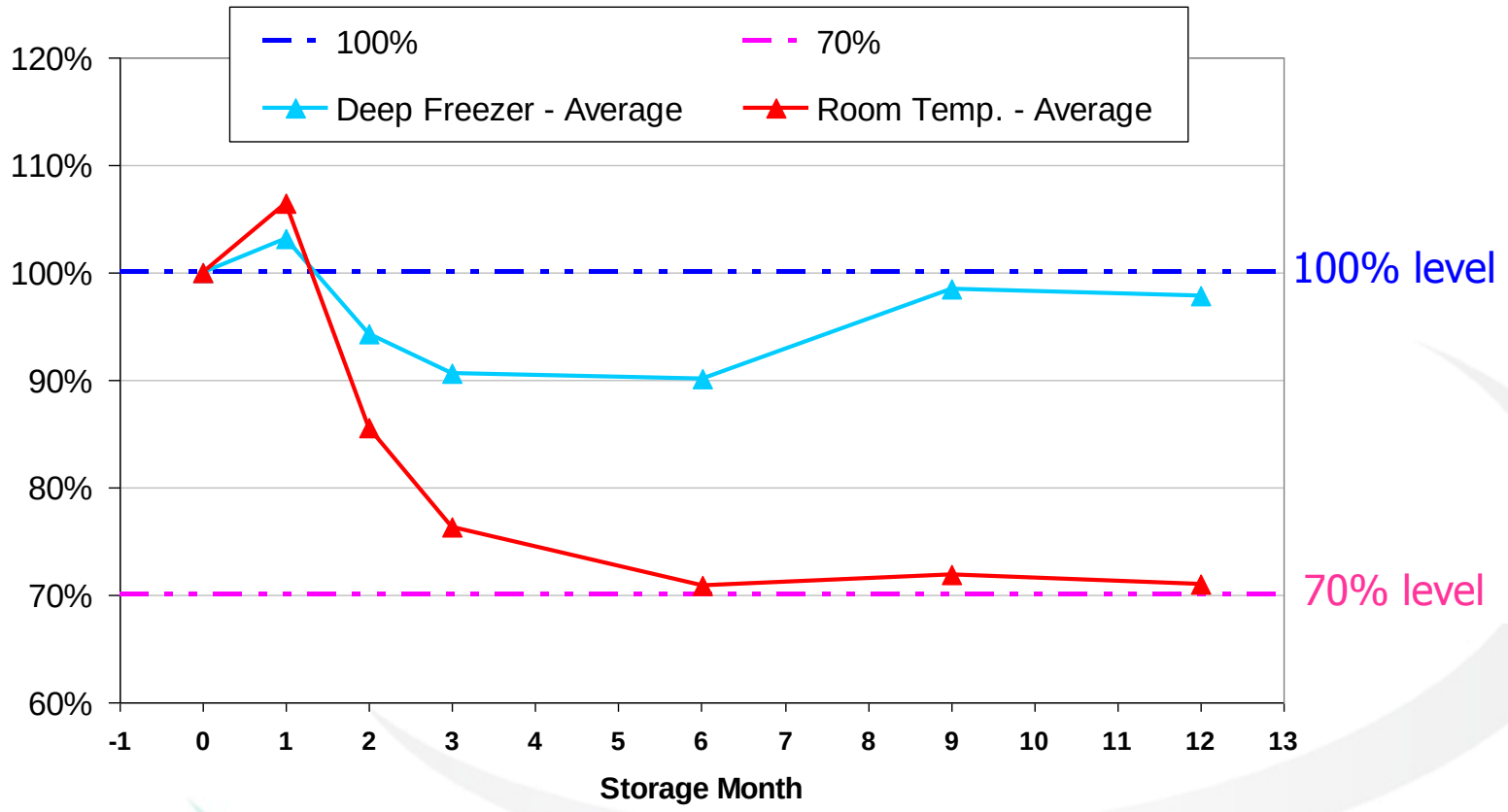
Objective 2: Joint Experiment Test Study

Cypermethrin – individual relative results [%]



Objective 2: Joint Experiment Test Study

Cypermethrin – average degradation curve

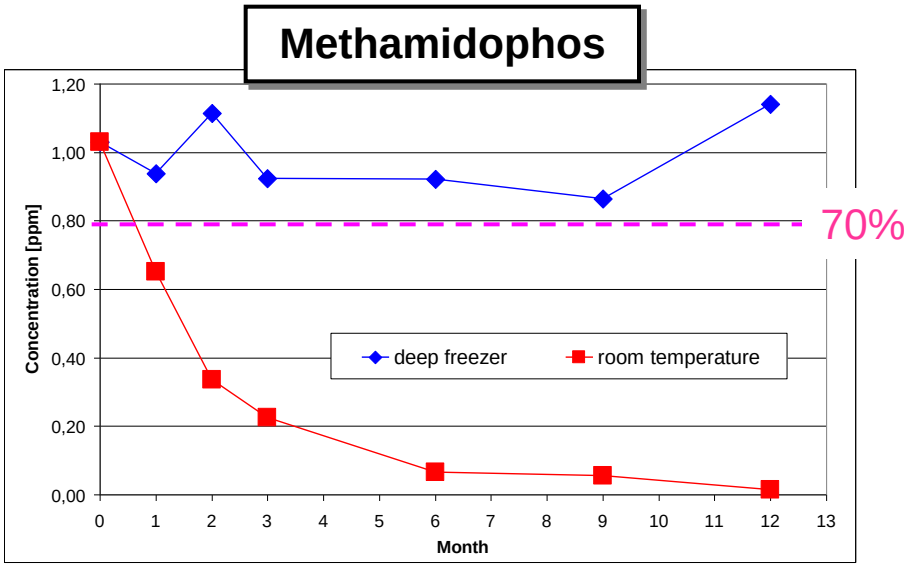


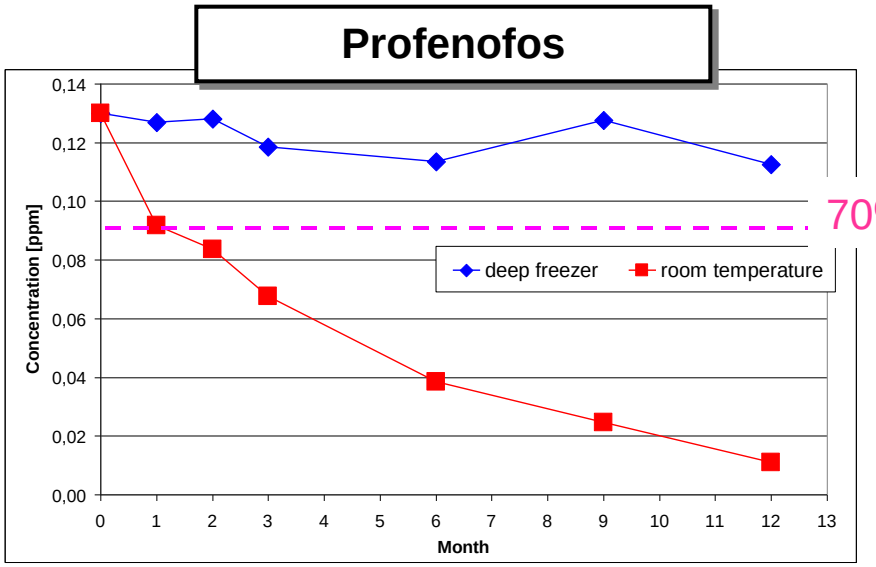
Objective 2: Joint Experiment Test Study

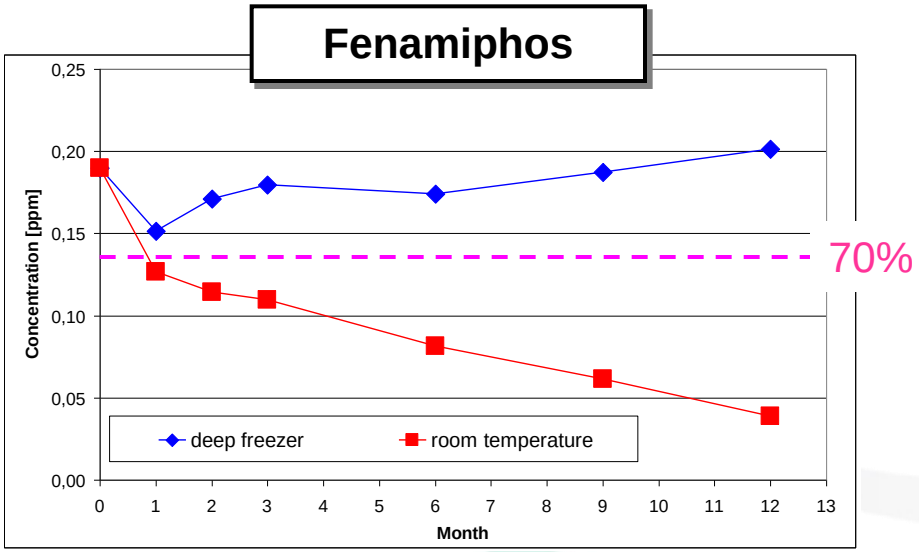
Degradation Study: Conclusion

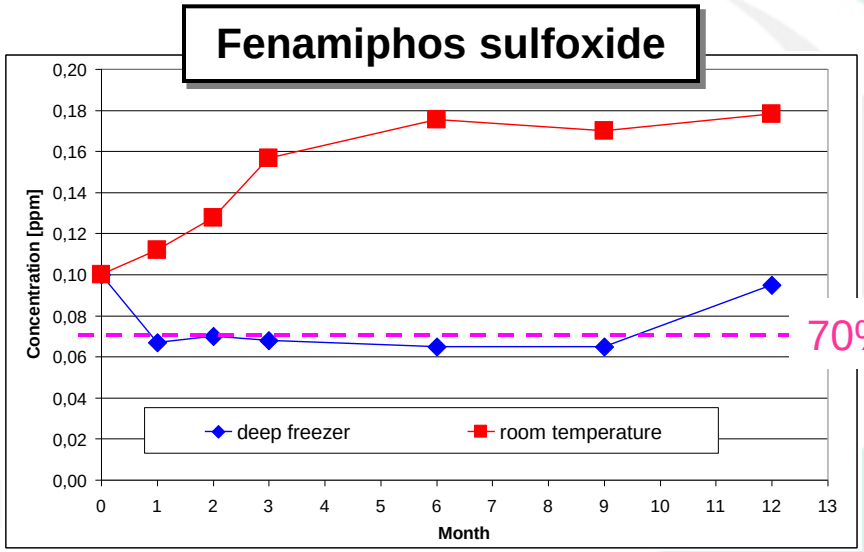
- No significant degradation losses for any of the investigated agrochemicals were found
- Only the pyrethroids **cyfluthrin** and **cypermethrin** showed degradation curves for room temperature storage with about 30% losses over the time
- Some laboratories have conducted the study with the additional agrochemicals in the spiked sample from the proficiency test "Round 6": Aldicarb, Aldicarb sulfoxide, Carbaryl, Deltamethrin, Indoxacarb, Fenamiphos, Fenamiphos sulfoxide, Methamidophos, Profenofos

Additional degradation data



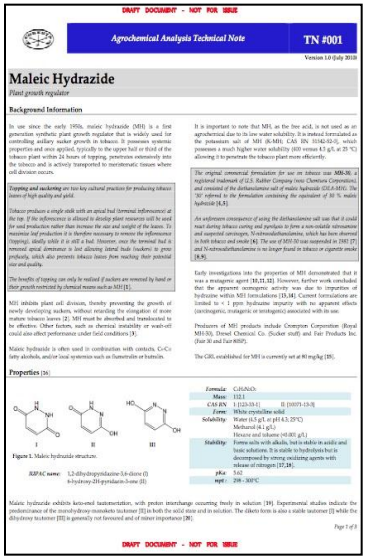






Objective 3: Technical Notes

Technical Note: Maleic Hidrazide (Fraser Williamson)


DRAFT DOCUMENT - NOT FOR ISSUE

Agrochemical Analysis Technical Note TN #001

Version 1.0 (July 2010)

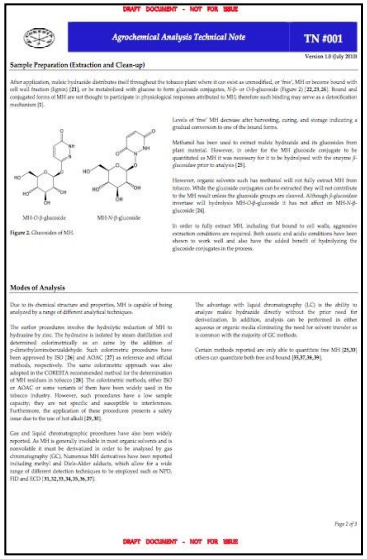
Maleic Hydrazide

Plant growth regulator

Background Information

Properties [1]

DRAFT DOCUMENT - NOT FOR ISSUE


DRAFT DOCUMENT - NOT FOR ISSUE

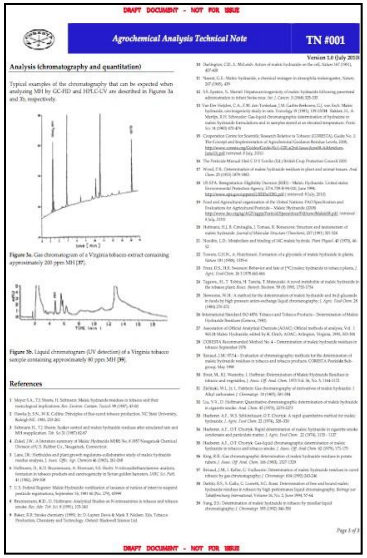
Agrochemical Analysis Technical Note TN #001

Version 1.0 (July 2010)

Sample Preparation (Extraction and Clean-up)

Modes of Analysis

DRAFT DOCUMENT - NOT FOR ISSUE


DRAFT DOCUMENT - NOT FOR ISSUE

Agrochemical Analysis Technical Note TN #001

Version 1.0 (July 2010)

Analysis (chromatography and quantitation)

References

DRAFT DOCUMENT - NOT FOR ISSUE

Sections:

- Background information
- Proprieties
- Sample preparation (extraction and clean-up)
- Modes of analysis
- Analysis (Chromatography and quantitation)
- References

Additional Technical Notes: Dithiocarbamates, Acid herbicides, Dinitroanilines, Pyrethroides, Methamidophos

Sub-group annual meeting

Bergerac - France July 19th-20th, 2011

Special thanks

- Fraser Williamson: Arista Laboratories, USA
- Thomas Anspach: Eurofins Dr. Specht, Germany
- Torbjörn Synnerdahl: Eurofins, Sweden
- Masahiro Miyoshi: JT LTRC, Japan

Thank you