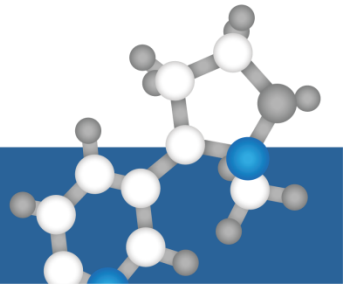
Stability of Pesticide Stock Solutions

Experiments Conducted by EURL-SRM

European Commission

EURL-SRM



Hubert Zipper

11.09.2014

EURL-SRM

Outline

- Introduction
- Stability Tests of Stock Solutions - Experiments by EURL-SRM
- Template for Submission of Compound Stability Data to EURL DataPool
- Testing the Stability of Pesticides in Stock Solutions by Quantitative NMR
- Summary

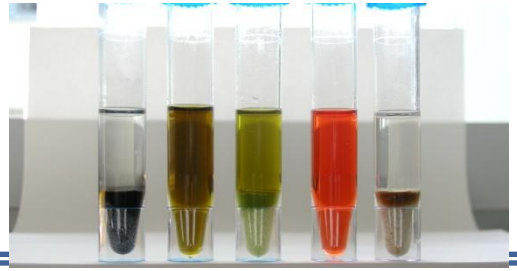
The of analytical pesticides-standards is a very “old issue”, but pesticide residue analysts have always to deal with it!

Reason:

One major source of errors

→ **degradation of standards** in

- stock solutions,
- working solutions (e.g. mixtures),
- matrix extracts.



Stability Tests of Stock Solutions - Project

- Start of project: 2008
- 137 compounds tested so far
- Procedure:

Preparation of stock solutions (1 mg/ml)

↓

Storage

↓

Preparation of working solution (10 µg/ml; mixture)

↓

Preparation of injection-solution (0.1 µg/ml)

↓

Measurement by GC-MS/MS / LC-MS/MS

↓

Data Evaluation and Storage

Preparation of stock solutions (1 mg/ml)

- Concept for stability project
- **One person responsible** for project

- **New certified standards** were purchased for the **entire period of stability project**
  => same batch was used for stability experiments of compound

- Weight: 25 + X mg of pure standard for almost all compounds;
  expensive compounds: not less than 10 mg
- Corrected for purity of standard

Preparation of stock solutions (1 mg/ml)

- Dissolved in 25 + X ml **acetonitrile** / **acetonitrile (+0.4 Vol% acetic acid)** / other solvent (e.g. carbendazim in ACN:DMF = 1:1)
- Base-labile pesticides:
  50 + X mg standard + 50 + X ml acetonitrile: 25 ml were acidified with acetic acid
- 1,5 ml vessels: glass, brown transparent;
  plastic snap cap with seal;
  cap inner material: TEF
- Check for precipitation during storage:
  one white glass vessel was filled with solution

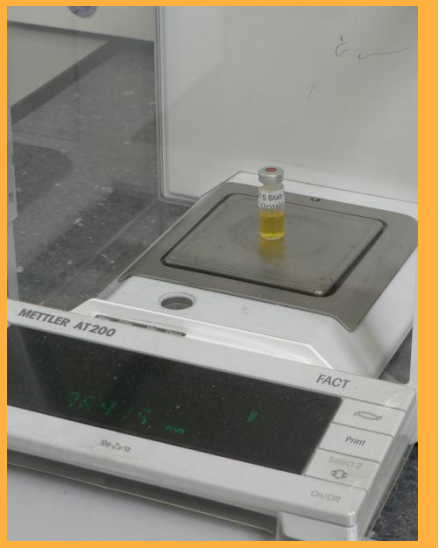
Storage

- Temperature: 4°C (refrigerator) / -20°C
- Period: 6 months, 1, 2 and 4 or 5 years

=> 9 vessels for each pesticide

Check for Solvent Loss

- Possible loss of solvent during the storage period was monitored for each vessel by weighing.
- The weight deviation must not be more than 1%. Otherwise vessel was discarded or result corrected for solvent loss.



Preparation of Working-Solution (10 µg/ml)

- As mixture
- Dilution to 10 µg/ml **acetonitrile** / **acetonitrile (+0.4 Vol% acetic acid)** / other solvent

Preparation of NEW Solutions

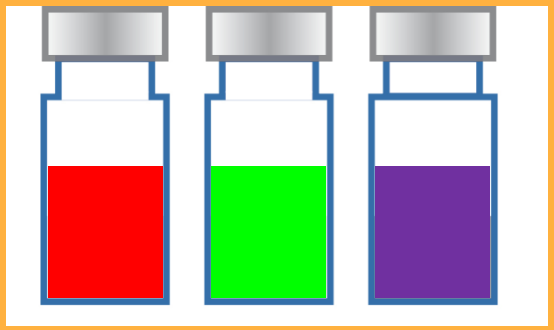
- NEW stock solution: 10 + X mg of pure standard of the same batch + solvent
- NEW working solution: mixture

Preparation of Injection-solutions

- Dilute working-standard solution to 0,1 µg/ml

Measurement by GC-MS/MS / LC-MS/MS

- Sequence (alternate injections):
  (**stored-sln (4°C)** - **stored-sln (-20°C)** - **reference-sln**) x5



- 5 times injection of stored and new solutions
- ISTD: Chlorpyrifos-D10

Data Evaluation

- The response of the stored solution was compared with the response of the new solution of the same standard batch
  - Quotient X

$$\text{Quotient X} = \frac{\text{Response of standard solution}}{\text{Response of ISTD}}$$

  - Average of quotients for stored and reference solutions
  - Difference of stored versus new solution

$$\text{Difference in \%} = \left(\frac{\overline{\text{X}} \text{ of stored solution} * 100}{\overline{\text{X}} \text{ of new solution}} \right) - 100\%$$

Data Storage

- Compound Stability database at www.eurl-pesticides-test.eu
- Template for Submission of Compound Stability Data

EURL-SRM Download Excel-Submission-file from EURL DataPool

www.eurl-pesticides-test.eu

EURL-DataPool

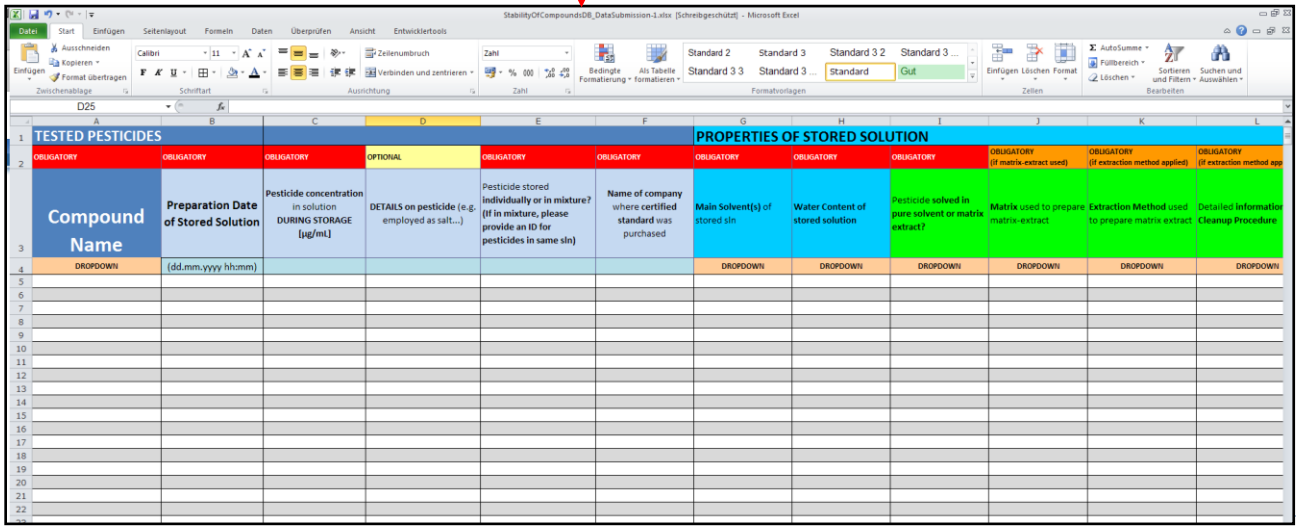
Home | Compound Data | myLab | myNRL Network | EURL Network | Administration | Reference Labs | Tutorials

Welcome to our Tutorial Page!

Whether you are a new visitor or a registered user of our EURL DataPool-website, our Tutorial is here to help you get the most out of our database services.

Category	Format	Title
Getting Started	Video	How to customize and filter the tables
Stability of Compounds	zip-File	Template for Data Submission: Submission of Compound Stability Data
Method Validation Data	Website	Template for Data Submission: Submission of Method Validation Results

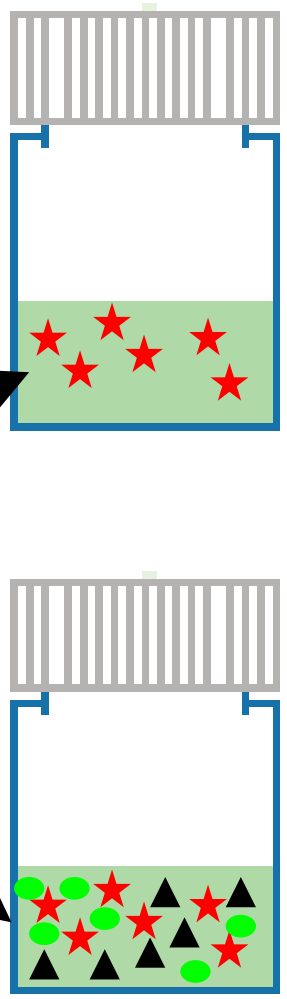
Download the Template for Submission of Compound Stability Data



EURL-SRM Template for Compound Stability Data: Tested Pesticides

The preparation date of the stock solution is needed to calculate the storage duration.

	A	B	C	D	E	F
1	TESTED PESTICIDES					
2	OBLIGATORY	OBLIGATORY	OBLIGATORY	OPTIONAL	OBLIGATORY	OBLIGATORY
3	Compound Name	Preparation Date of Stored Solution	Pesticide concentration in solution DURING STORAGE [µg/mL]	DETAILS on pesticide (e.g. employed as salt...)	Pesticide stored individually or in mixture? (If in mixture, please provide an ID for pesticides in same sln)	Name of company where certified standard was purchased
4	DROPDOWN	(dd.mm.yyyy hh:mm)				
5	Carbendazim	11.09.2010	1000		individually	Dr. Ehrenstorfer
6	2,4,5-T-Methylester	15.05.2010	1000		[illegible]	Dr. Ehrenstorfer
7	Acrinathrin (sum isomers)	01.07.2012	10		a3400	Dr. Ehrenstorfer
8	Captan	01.07.2012	10		a3400	Dr. Ehrenstorfer
9	Chlorthalonil	01.07.2012	10		a3400	Dr. Ehrenstorfer
10	Folpet	01.07.2012	10		a3400	Dr. Ehrenstorfer
11	Tetramethrin	01.07.2012	10		a3400	Dr. Ehrenstorfer
12	Trinexapac-ethyl	01.07.2012	10		a3400	Fluka
13	Captan	01.07.2012	10		a3400	[illegible]de-Haen
14	Chlorthalo[illegible]	01.07.2012	10		a3400	Mitsui C[illegible]cals, Inc.
15	Folpet	01.07.2012	10		a3400	Mitsui Chem[illegible]Inc.
16	Tetramethri[illegible]	01.07.2012	10		[illegible]3400	Mitsui Chemicals
17	Trinexapac-et[illegible]	02.10.2012	1000,0		[illegible]idually	Sigma/Aldrich
18	Prohexadione	02.10.2012	1000,0	as Ca-salt	[illegible]dually	Dr. Ehrenstorfer
19	Flo[illegible]				[illegible]e10	Sigma/Aldrich
20	F[illegible]				[illegible]e11	Sigma/Aldrich
21	P[illegible]				[illegible]e5	Sigma/Aldrich
22	P[illegible]				[illegible]e5	Sigma/Aldrich
23						
24						
25						



Either choose a compound from the drop downlist or enter the compound name into this column.

Enter a unique identifier for all those compounds that were stored in the same solution (-> mixture).

Template for Compound Stability Data: Properties of Stored Sln

This section describes the solvent that was used to store the compound.

A	B								
TESTED PESTICIDES		PROPERTIES OF STORED SOLUTION							
OBLIGATORY	OBLIGATORY	OBLIGATORY	OBLIGATORY	OBLIGATORY	(if matrix-extract used)	(if extraction method applied)	(if extraction method applied)	OBLIGATORY	OPTI
Compound Name	**Preparation Date of Stored Solution**	**Main Solvent(s)** of stored sln	**Water Content of stored solution**	Pesticide **solved in pure solvent or matrix extract?**	**Matrix** used to prepare matrix-extract	**Extraction Method** used to prepare matrix extract	Detailed **information** on the **Cleanup Procedure**	**Acid or Base** added to solution before storage	**pH** o solu mea
DROPDOWN	(dd.mm.yyyy hh:mm)	DROPDOWN	DROPDOWN	DROPDOWN	DROPDOWN	DROPDOWN	DROPDOWN	DROPDOWN	
Carbendazim	11.09.2010	Acetonitrile/DMF (1:1	Negligible	Pure solvent		None	None	No acid or base added	
2,4,5-T-Methylester	15.05.2010	Acetonitrile	Negligible	Pure solvent		None	None	No acid or base added	
Acrinathrin (sum isomers)	01.07.2012	Acetonitrile	Negligible	Pure solvent				Acidified moderately (e.g. pH 3-5)	
Captan	01.07.2012	Acetonitrile	Negligible	Pure solvent				Acidified moderately (e.g. pH 3-5)	
Chlorthalonil	01.07.2012	Acetonitrile	Negligible	Pure solvent				Acidified moderately (e.g. pH 3-5)	
Folpet	01.07.2012	Acetonitrile	Negligible	Pure solvent				Acidified moderately (e.g. pH 3-5)	
Tetramethrin	01.07.2012	Acetonitrile	Negligible	Pure solvent		None	None	Acidified moderately (e.g. pH 3-5)	
Trinexapac-ethyl	01.07.2012	Acetonitrile	Negligible	Pure solvent		None	None	Acidified moderately (e.g. pH 3-5)	
Captan	01.07.2012	Acetonitrile	Negligible	Pure solvent		None	None	Acidified moderately (e.g. pH 3-5)	
Chlorthalonil	01.07.2012	Acetonitrile	Negligible	Pure solvent		None	None	Acidified moderately (e.g. pH 3-5)	
Folpet	01.07.2012	Acetonitrile	Negligible	Pure solvent		None	None	Acidified moderately (e.g. pH 3-5)	
Tetramethrin	01.07.2012	Acetonitrile	Negligible	Pure solvent		None	None	Acidified moderately (e.g. pH 3-5)	
Trinexapac-ethyl	02.10.2012	Acetonitrile	Negligible	Pure solvent		None	None	Acidified moderately (e.g. pH 3-5)	
Prohexadione	02.10.2012	Water	100%	Pure solvent				zed moderately (e.g. pH 8-10)	
Flonicamid	03.09.2014	Acetonitrile	2-5%	Matrix extract	Apple	QuEChERS (citrate)	MgSO4/PSA (150/25 mg/mL)	No acid or base added	
Formothion	03.09.2014	Acetonitrile	2-5%	Matrix extract	Apple	QuEChERS (citrate)	MgSO4/PSA (150/25 mg/mL)	No acid or base added	
Promecarb	03.09.2014	Acetonitrile	2-5%	Matrix extract	Orange	QuEChERS (citrate)	MgSO4/PSA (150/25 mg/mL)	No acid or base added	
Fenarimol	03.09.2014	Acetonitrile	2-5%	rix extract	Orange	QuEChERS (citrate)	MgSO4/PSA (150/25 mg/mL)	No acid or base added	

Pure Solvent

If a **matrix extract** was used for the storage experiment, enter the matrix and the extraction as well as the cleanup procedure that were used to prepare the extract.

EURL-SRM Template for Compound Stability Data: Storage Conditions

Details on the storage conditions (e.g. storage temperature) are used to compare the stability results.

A	B	STORAGE CONDITIONS						
TESTED PESTICIDES								
OBLIGATORY	OBLIGATORY	OBLIGATORY	OBLIGATORY	OBLIGATORY		OBLIGATORY	OBLIGATORY	OPTION
Compound Name	Preparation Date of Stored Solution	Storage Temperature in °C	Stored in Darkness?	Vessel Material	Vessel-Cap Type	Vessel Cap Inner Surface Material (surface in contact w. solution)	Vessel Optical Properties	DETAI
DROPDOWN	(dd.mm.yyyy hh:mm)		DROPDOWN	DROPDOWN	DROPDOWN	DROPDOWN	DROPDOWN	
Carbendazim	11.09.2010	-20	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
2,4,5-T-Methylester	15.05.2010	-20	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Acrinathrin (sum isomers)	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Captan	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Chlorthalonil	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Folpet	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Tetramethrin	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Trinexapac-ethyl	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Captan	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Chlorthalonil	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Folpet	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Tetramethrin	01.07.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Trinexapac-ethyl	02.10.2012	-20	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Prohexadione	02.10.2012	4	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Flonicamid	03.09.2014	23	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Formothion	03.09.2014	23	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Promecarb	03.09.2014	23	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	
Fenarimol	03.09.2014	23	Yes	Glass	Metal crimp cap w. seal/septum	PTFE (Teflon)	Brown transparent	

EURL-SRM Template for Compound Stability Data: Measurement & Reference

The storage duration is computed by calculating the difference between the measurement and preparation date.

Difference of stored versus new solution.

A		Y	Z	AA	AB	AC	AD	AE	AF	AG		AI
TESTED PESTICIDES		DETAILS ON MEASUREMENTS AND REFERENCE STANDARD										
OBLIGATORY	OBLI	OBLIGATORY	OBLIGATORY	OPTIONAL	OPTIONAL	OPTIONAL	OBLIGATORY	OBLIGATORY	OBLIGATORY	OBLIGATORY	OBLIGATORY	OBLIGATORY
Compound Name	Preparation Date of Stored Solution	Measurement Date	asurement hnique	DETAILS on Measurement (e.g. degradation products observed/quantified ; any measurement particularities)	ISTD name	DETAILS on ISTD (e.g. at what stage was the ISTD added)	No of Replicate measurements of STORED sln	RSD STORED Sln (for >2 replicates) [%]	No of Replicate measurements of REFERENCE sln	RSD REFERENCE (for >2 replicates) [%]	Difference STORED vs. REFERENCE (REFERENCE =100%) [%]	Was REFERENCE solution FRESHLY prepared from certified standard?
DROPDOWN	(dd.mm.yyyy hh:mm)	.mm.yyyy hh:m	ROPDOWN		DROPDOWN							DROPDOWN
Carbendazim	11.09.2010	01.09.2014	C-MS/MS	Chlorpyrifos D10			5	10,0	5	1,6	10,7%	nly from certified std of SAME BA
2,4,5-T-Methylester	15.05.2010	01.09.2014	C-MS/MS	Chlorpyrifos D10			5	8,5	5	6,0	4%	nly from certified std of SAME BA
Acrinathrin (sum isomers)	01.07.2012	03.09.2013	C-MS/MS	ratio changed: from 1	PCB 138		5	3,0	5	6,0	9,0%	nly from certified std of SAME BA
Captan	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	1,5	5	3,5	6,7%	nly from certified std of SAME BA
Chlorthalonil	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	4,0	5	4,6	4,0%	nly from certified std of SAME BA
Folpet	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	1,6	5	1,2	13,2%	nly from certified std of SAME BA
Tetramethrin	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	6,0	5	3,5	-6,0%	nly from certified std of SAME BA
Trinexapac-ethyl	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	3,5	5	3,0	2,0%	nly from certified std of SAME BA
Captan	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	1,2	5	4,0	1,5%	nly from certified std of SAME BA
Chlorthalonil	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	3,5	5	1,6	4,5%	nly from certified std of SAME BA
Folpet	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	3,5	5	6,0	3,0%	nly from certified std of SAME BA
Tetramethrin	01.07.2012	03.09.2013	C-MS/MS		PCB 138		5	4,6	5	3,5	2,5%	nly from certified std of SAME BA
Trinexapac-ethyl	02.10.2012	03.09.2013	C-MS/MS		PCB 138		5	1,2	5	6,0	7,0%	nly from certified std of SAME BA
Prohexadione	02.10.2012	10.09.2013	C-MS/MS	Chlorpyrifos D10			5	4,5	5	2,6	8,0%	nly from certified std of SAME BA
Flonicamid	03.09.2014	08.09.2014	C-MS/MS	Chlorpyrifos D1	after solvent additio		5	3,5	5	3,5	-21,0%	nly from certified std of SAME BA
Formothion	03.09.2014	08.09.2014	C-MS/MS	Chlorpyrifos D1	after solvent additio		5	4,5	5	4,6	-26,0%	nly from certified std of SAME BA
Promecarb	03.09.2014	08.09.2014	C-MS/MS	Chlorpyrifos D1	after solvent additio		5	[illegible]	5	1,2	-8,0%	nly from certified std of SAME BA
Fenarimol	03.09.2014	08.09.2014	C-MS/MS	Chlorpyrifos D1	after solvent additio		5	[illegible],0	5	3,5	-5,0%	nly from certified std of SAME BA

Please consider the paragraphs F8-F11 of the AQC Guidelines for the measurement of the stored and reference solution.

Our results are available at www.eurl-pesticides-test.eu

EURL-DataPool

Home | Compound Data | myLab | EURL Network | Administration | Reference Labs | Tutorials

Compounds | Method Validation Data | MRL Residue Definitions

Compound Information

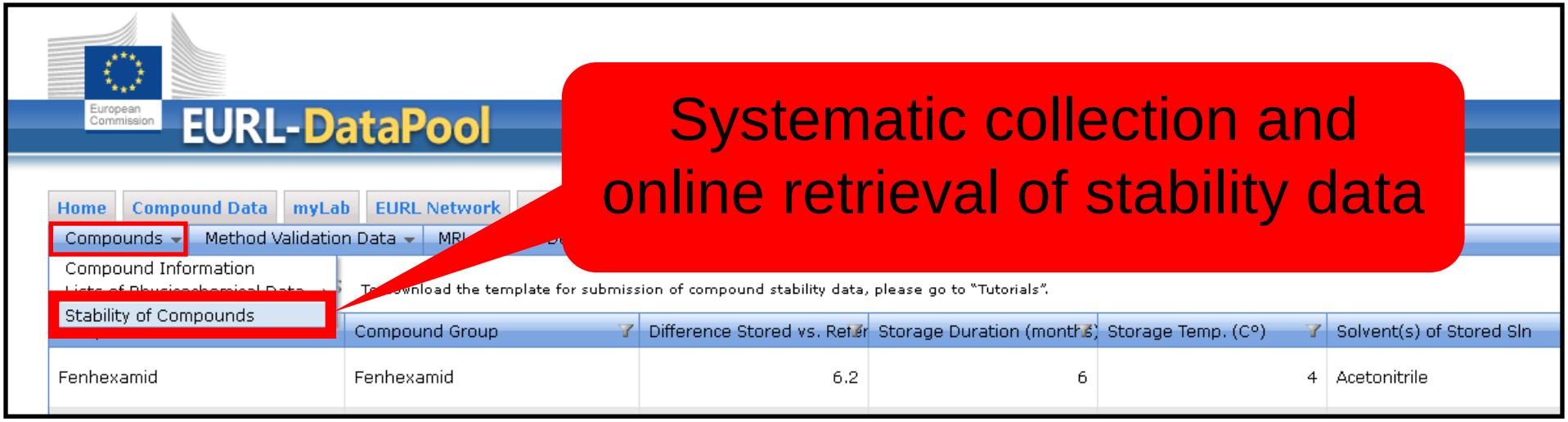
Stability of Compounds

To download the template for submission of compound stability data, please go to "Tutorials".

	Compound Group	Difference Stored vs. Ref	Storage Duration (months)	Storage Temp. (C°)	Solvent(s) of Stored Sln
Fenhexamid	Fenhexamid	6.2	6	4	Acetonitrile
Prochloraz	Prochloraz	3.5	6	-20	Acetonitrile
Fenobucarb	Fenobucarb	-2.2	6	4	Acetonitrile
Fenothiocarb	Fenothiocarb	3.2	6	4	Acetonitrile

- Stability data for 321 pesticides submitted by 3 labs

EURL-SRM Compound Stability Database



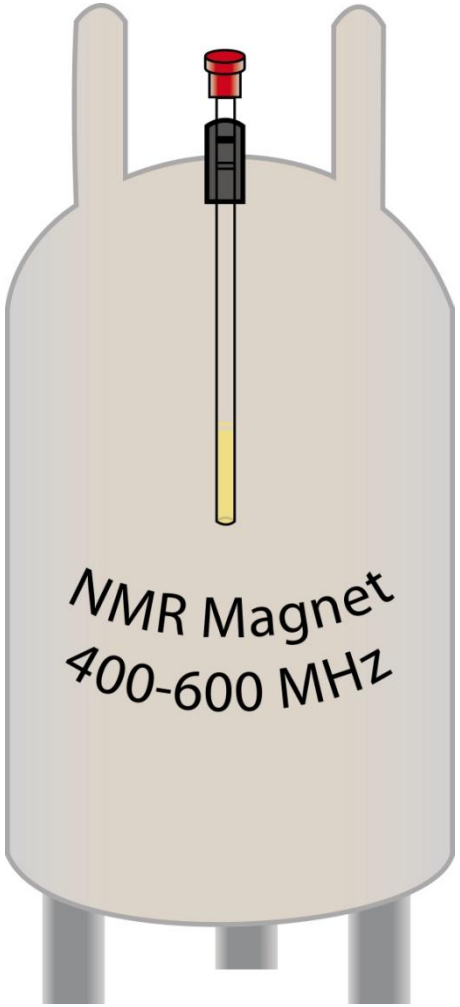
- Storage of data about the stability of pesticide standards in
  - **stock solutions**
  - **working solutions** (-> mixtures)
  - **sample extracts**
  - (pure standards)
- Template for Submission of Stability Data = standardized data format

- Comparison of compound stability
  - in different **solvents**
  - at different **storage conditions**
- Could be used to derive compound stability criteria

Stability tests = high costs and lots of labor

⇒ many laboratories are unable to conduct these experiments!

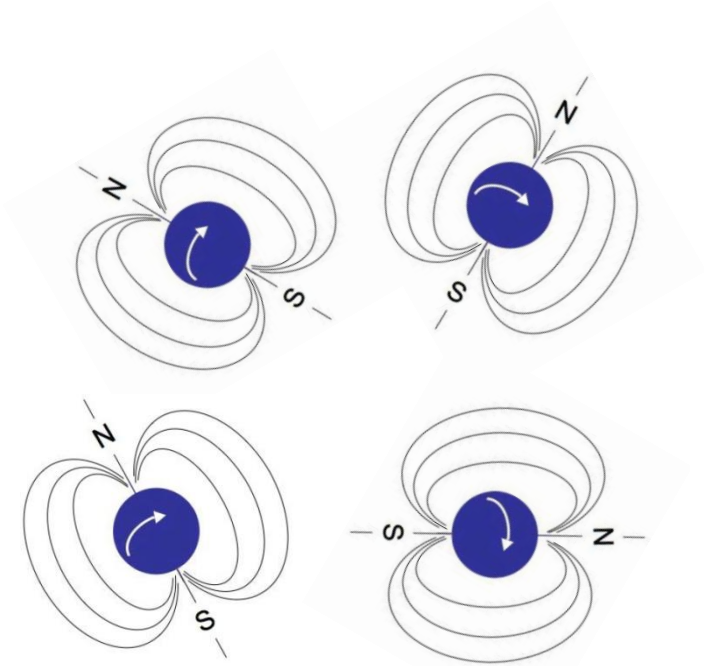
Cooperation and coordination by the NRLs may help?

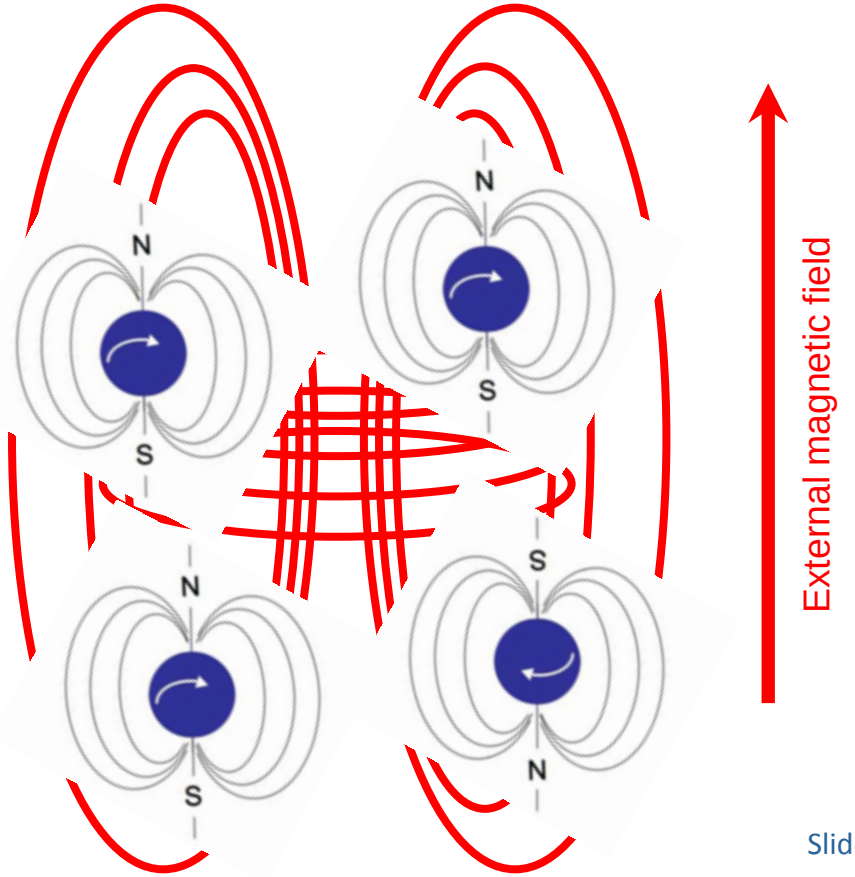


Testing the Stability of Pesticides in Stock Solutions by Quantitative NMR

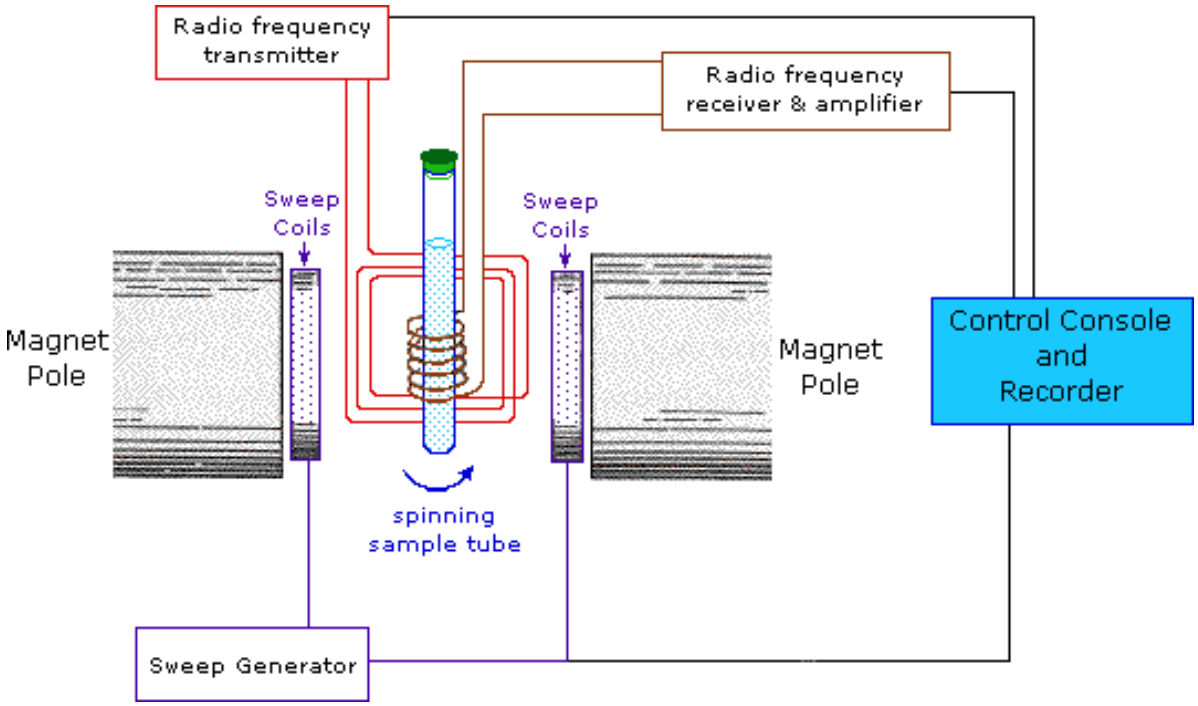
Spin Quantum Number I

- Atomic nuclei have a *spin quantum number I*
- If *I* differs from zero, the nucleus possesses a *magnetic moment (μ)* that may interact with an external magnetic field

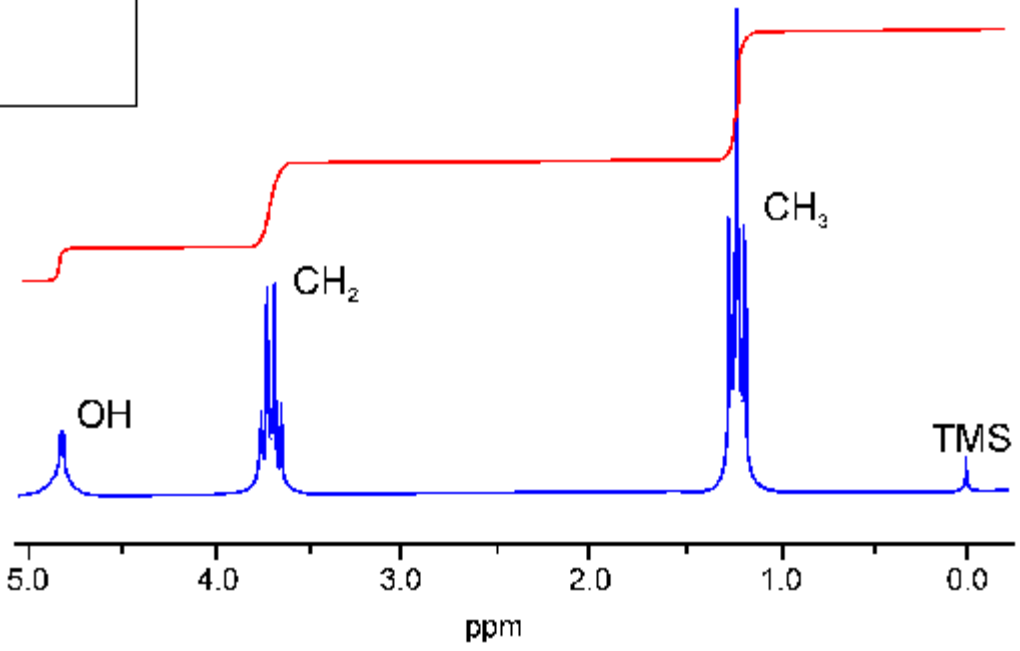




Principles of Nuclear Magnetic Resonance



1H spectrum of Ethanol:
3 types of protons
CH_3, CH_2, OH



- NMR is generally used for compound identification and structural elucidation

- Singal intensity in NMR-spectrum is directly proportional to the number of nuclei responsible for that specific resonance*

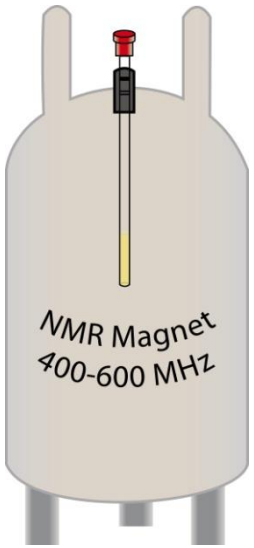
  => simultaneous access to both qualitative and quantitative information

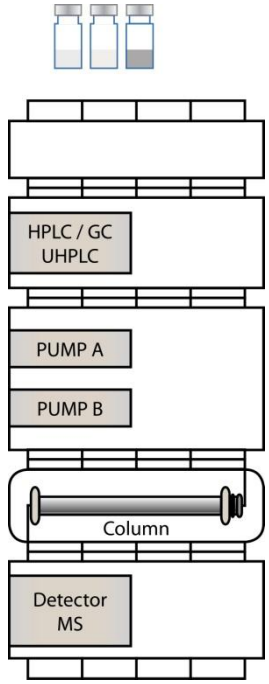
- Quantitative inaccuracy of qNMR has been reported to be less than 2.0%**

* S. Kumar Bharti, R. Roy, Quantitative 1H NMR spectroscopy, Trends in Analytical Chemistry, Vol. 35, p. 5-26, 2012
** F. Malz, H. Jancke, J. Pharm. Biomed. Anal. 35 (2005) 813

Main characteristics of qNMR and chromatographic techniques*

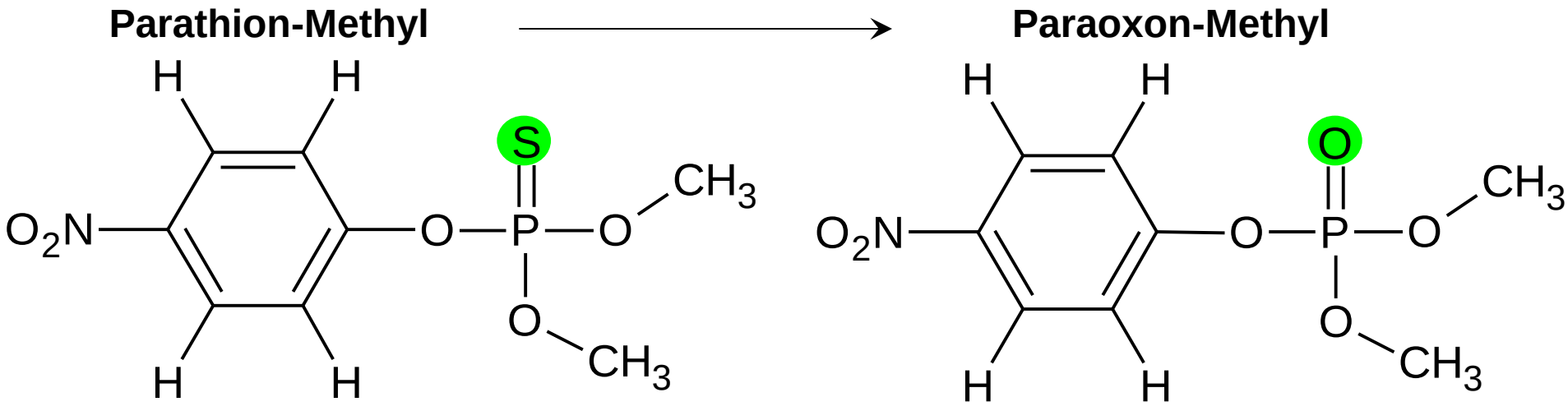
Quantitative NMR		LC-MS/MS GC-MS(/MS)
Weight/dilution Non-destructive analysis	**Sample preparation**	Weight/dilutions destructive analysis
Structural properties (restriction: e.g. ^{1}H)	**Detection**	Physical properties (restriction: e.g. ionization)
Certified reference material (one universal calibrant)	**Calibrant Quantitation**	Structurally identical reference needed
Internal/external standard (with/without calibration curve)		Internal/external standard (with/without calibration curve)
Low µM	**Sensitivity**	Low nM - pM
Resonance overlapping (with signals of solvent, metabolites, ...)	**Selectivity & Specifity**	Chromatographic separation = better specifity
Instrument independent	**Reproducibility**	Instrument-dependent
Possible	**Identification & Quantification of Impurites**	Difficult





* For details see: Simmler Ch, Napolitano JG, McAlpine JB; Universal quantitative NMR analysis of complex natural samples; Current Opinion in Biotechnology 2014, 25:51-59

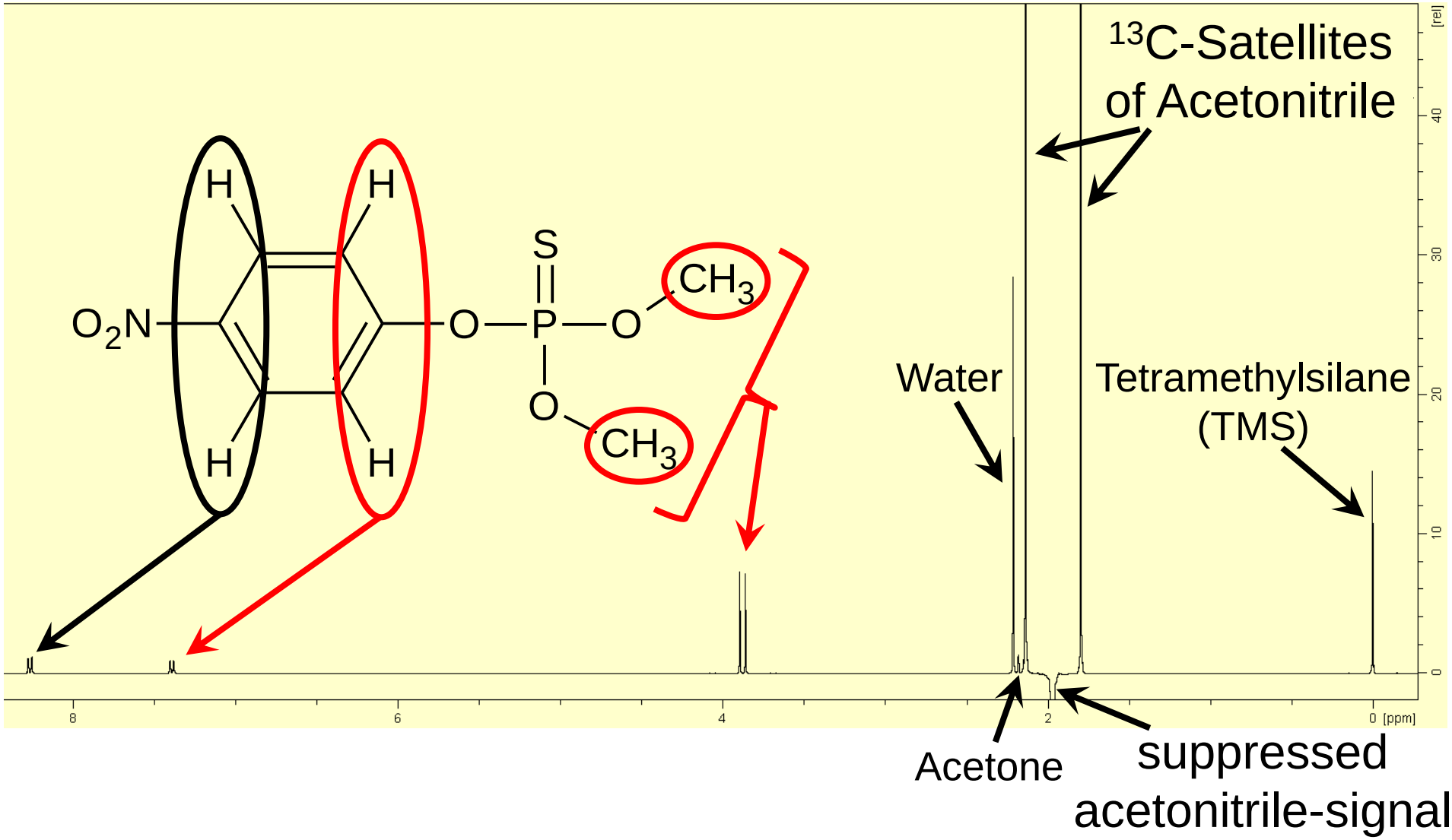
1st NMR Experiments: Mixtures of Model Compounds



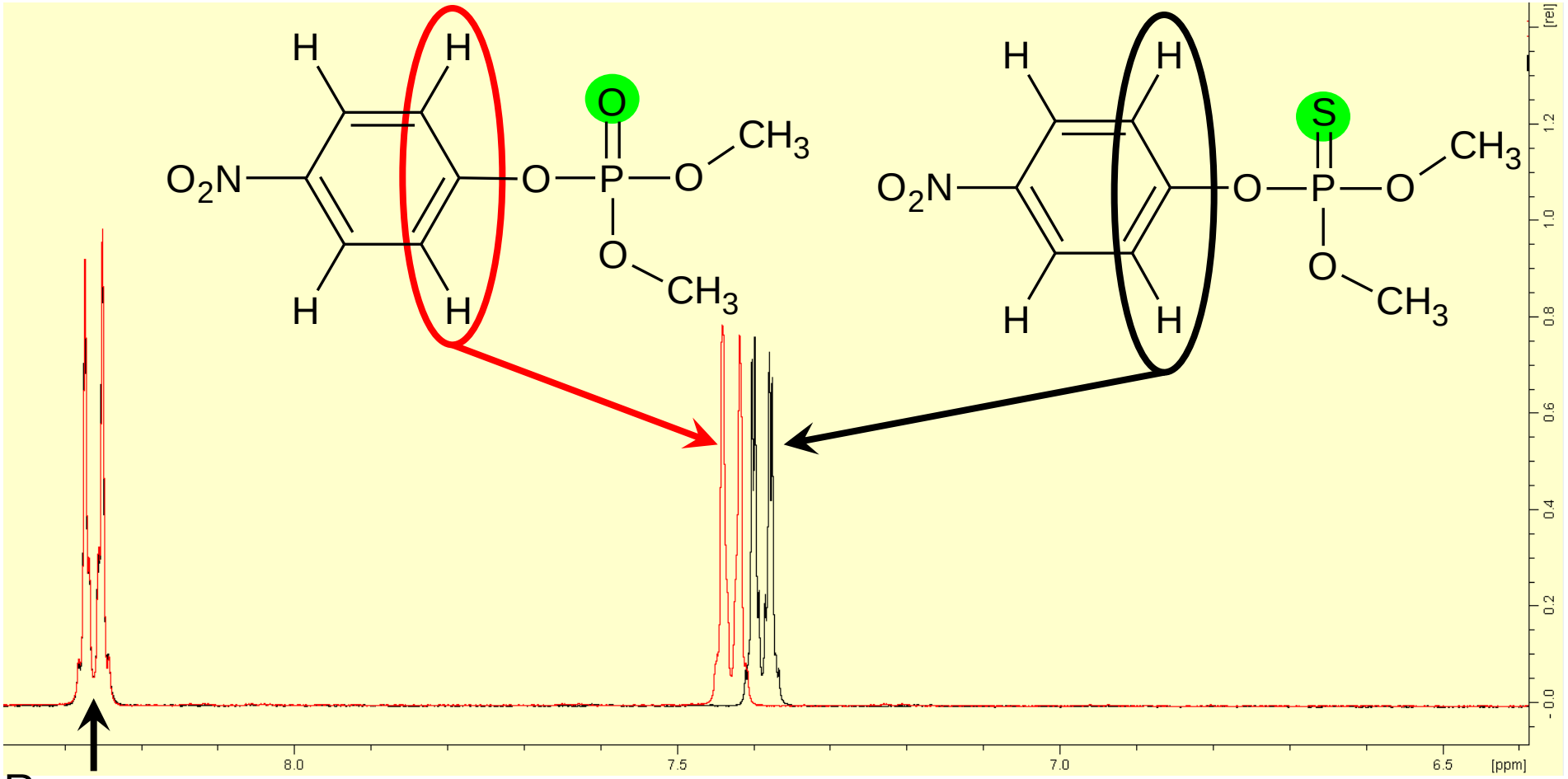
- **Simulation of the degradation of parathion-methyl to paraoxon-methyl:** mixtures of compounds prepared at different concentrations (1 mg/ml -> 0 mg/ml)
- ^{1}H-NMR spectra at 400 MHz (Bruker Avance 400)
- **Solvent suppression technique**: suppress acetonitrile-signal
- **External calibrant**: 1,2,4,5-tetrachloro-3-nitrobenzene

CVUA Karlsruhe

^{1}H-NMR-Spectrum of Parathion-Methyl

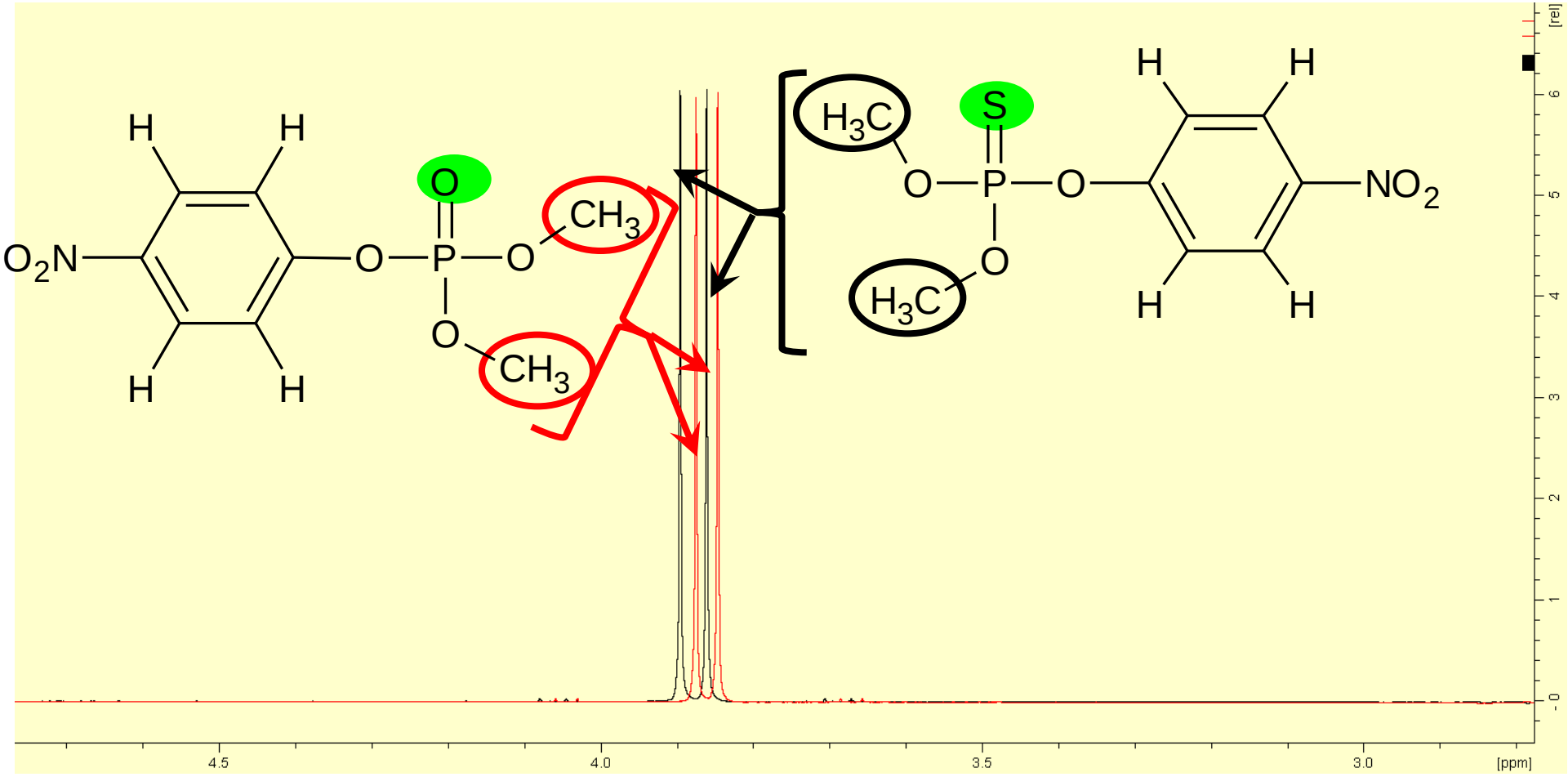


Mixtures of Parathion-Methyl and Paraoxon-Methyl

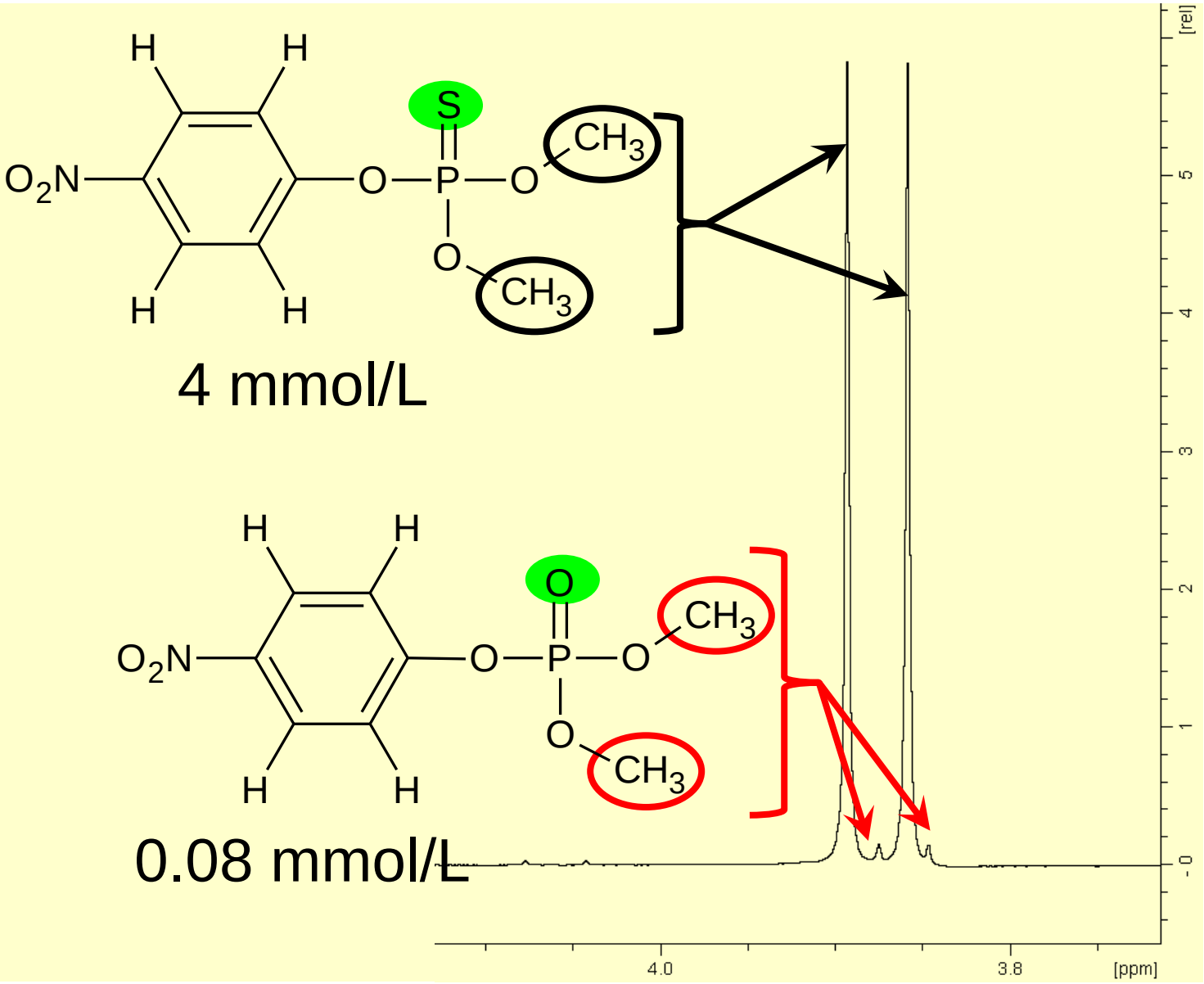


Resonance overlapping

Mixtures of Parathion-Methyl and Paraoxon-Methyl

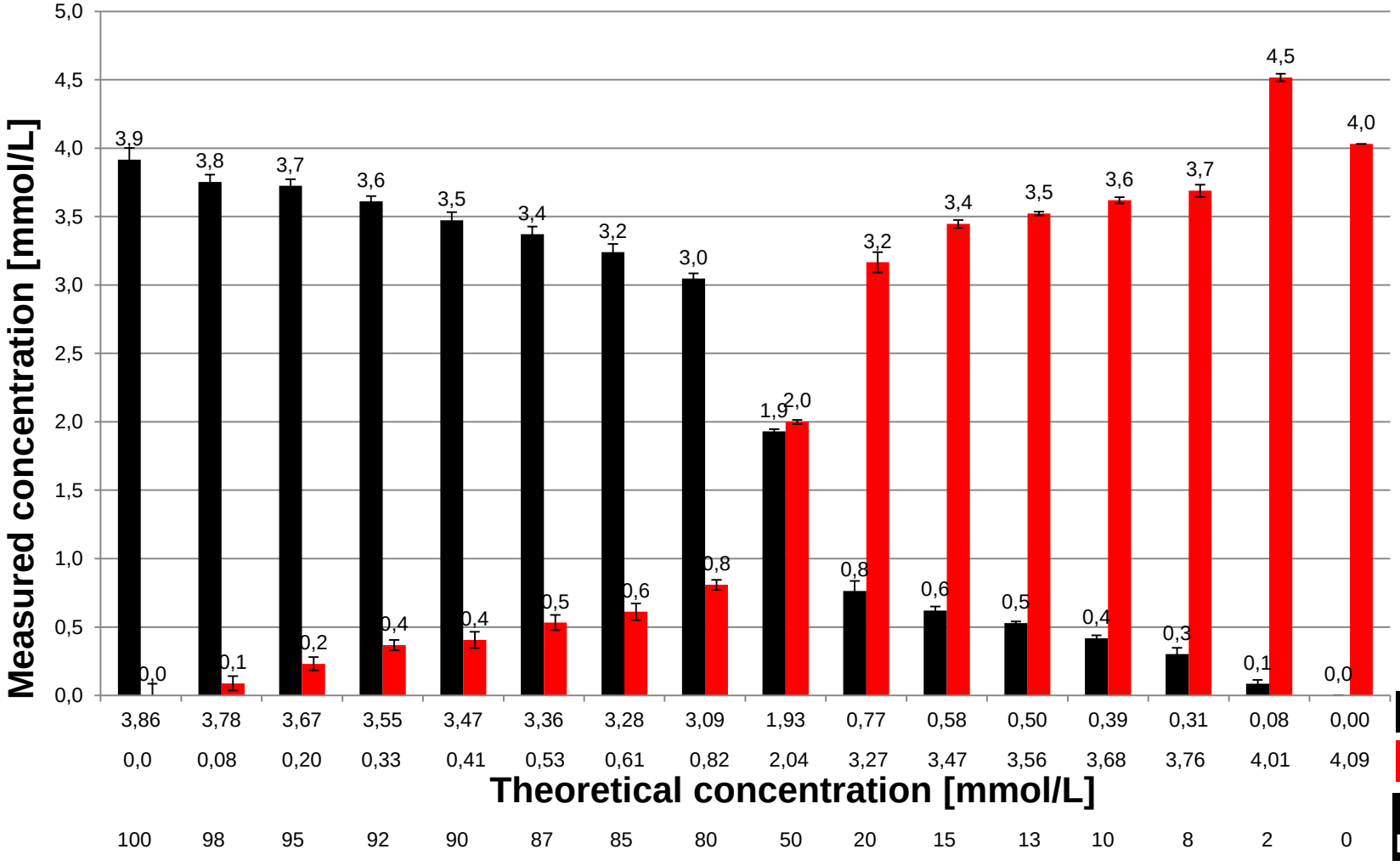


Mixtures of Parathion-Methyl and Paraoxon-Methyl



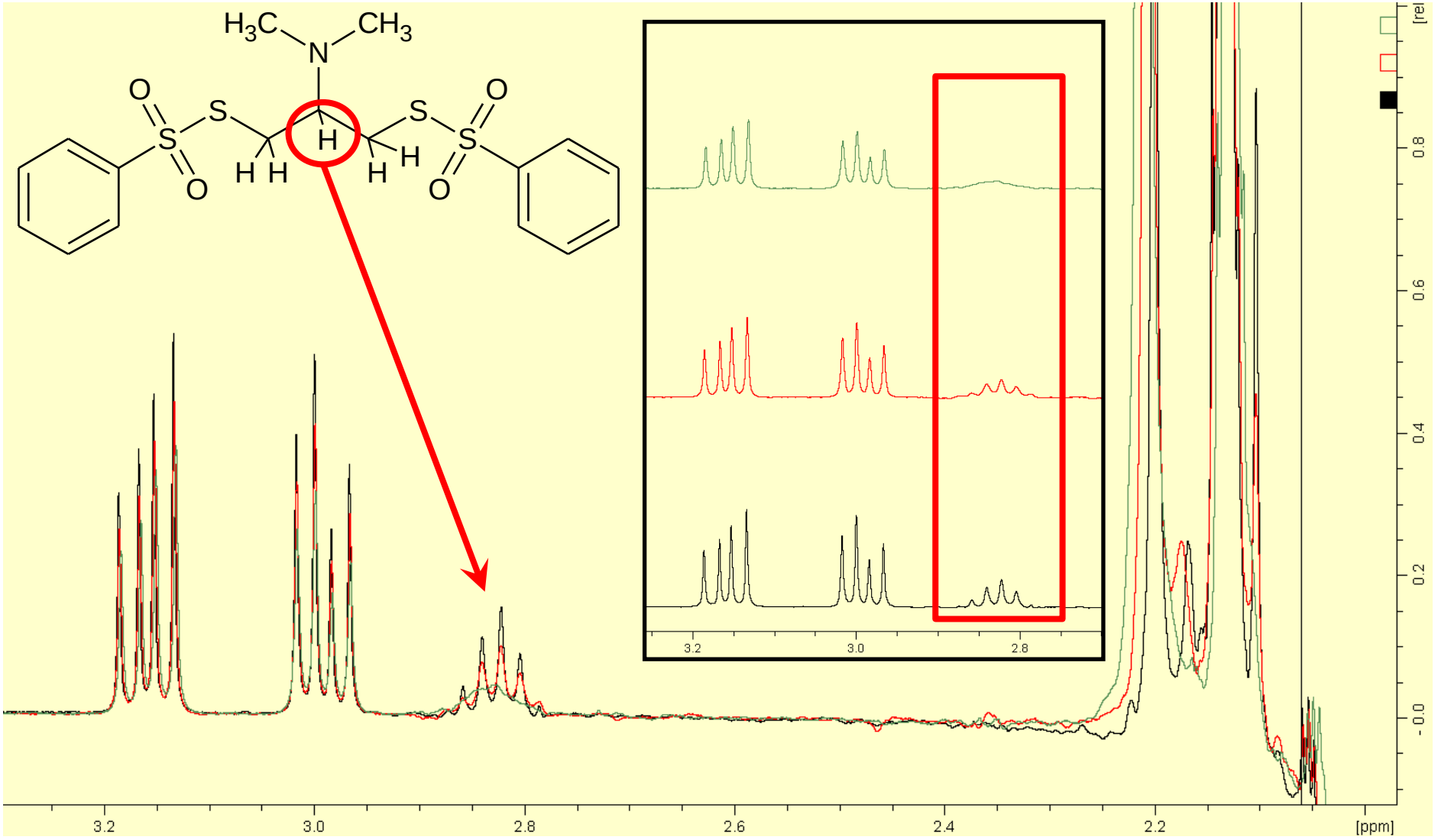
Simulation of Parathion-Methyl Degradation

Mixtures of Parathion-Methyl and Paraoxon-Methyl quantified by qNMR



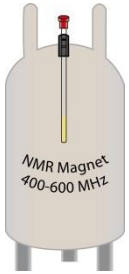
EURL-SRM Stability Test of a Bensultap-Stock Solution (1mg/ml) in Acetonitrile

NMR-spectra were recorded after 0 day (black) / 3 days (red) / 7 days (green) of storage

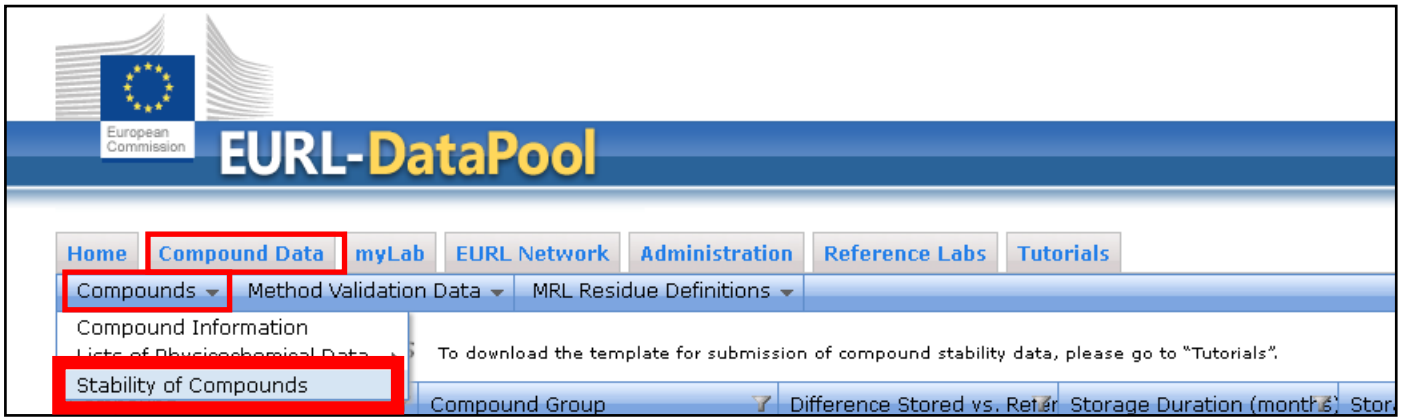


EURL-SRM Summary

- EURL-SRM: Stability of pesticides in stock solutions by qNMR for 50 compounds



- Results our stability experiments are available at www.eurl-pesticides-test.eu



- Any contribution to compound stability database is highly appreciated!

Thank You
for Your Attention

www.eurl-pesticides.eu