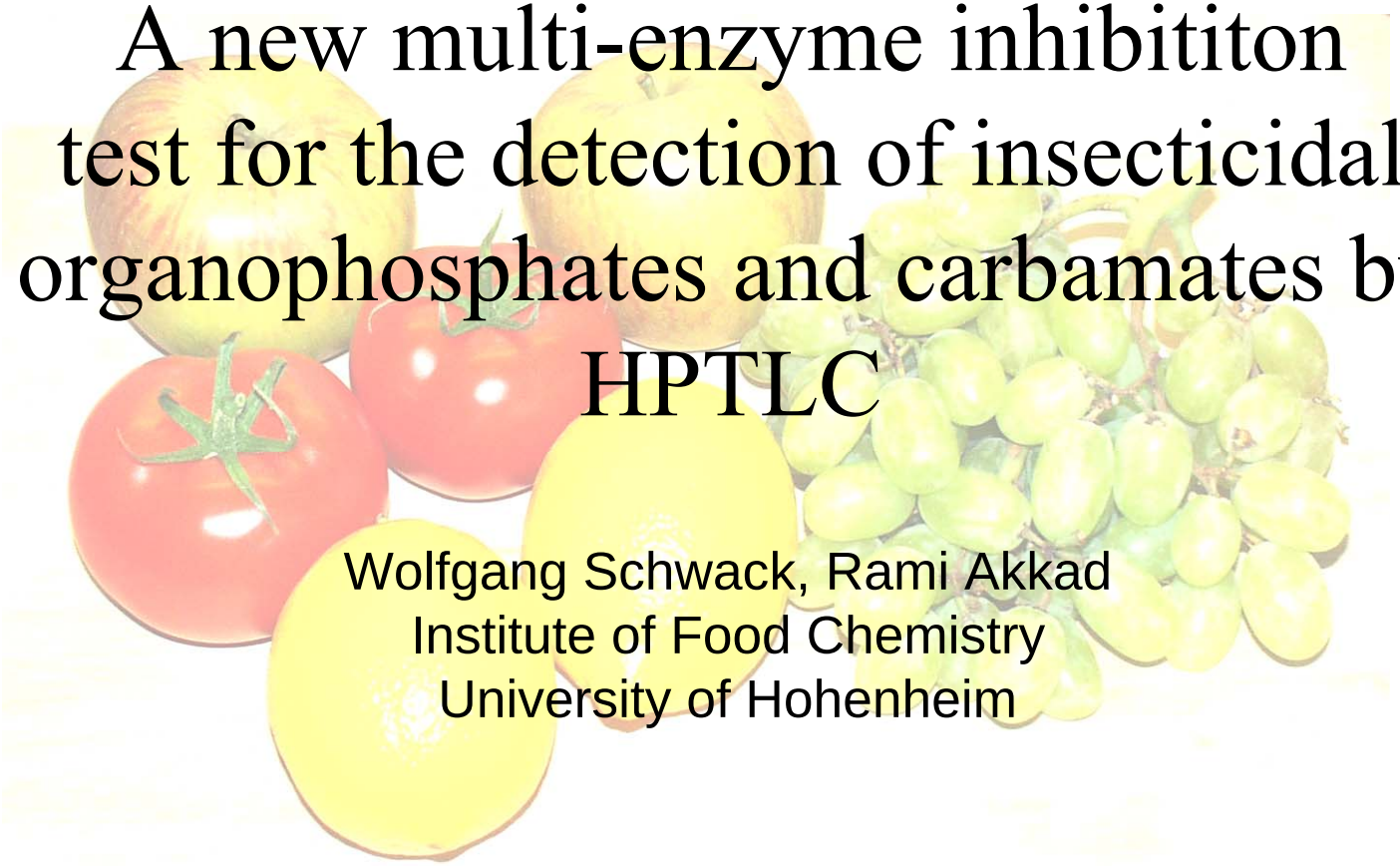
Universität Hohenheim
Institut für Lebensmittelchemie

A new multi-enzyme inhibititon test for the detection of insecticidal organophosphates and carbamates by HPTLC

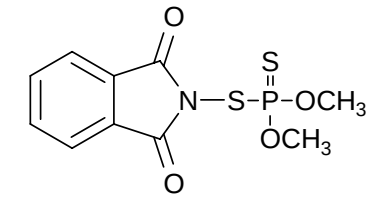


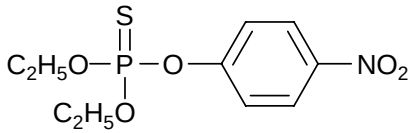
Wolfgang Schwack, Rami Akkad
Institute of Food Chemistry
University of Hohenheim

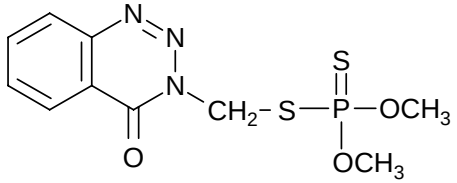
HPTLC 2008
June 11-13, 2008 (Helsinki)

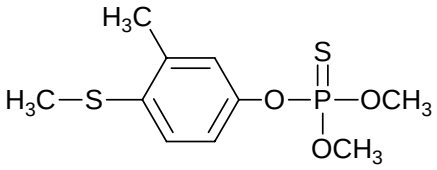
Universität Hohenheim
Institut für Lebensmittelchemie

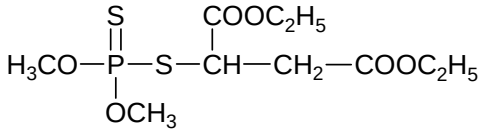
Inhibitors of cholinesterases (ChE)

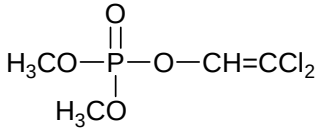


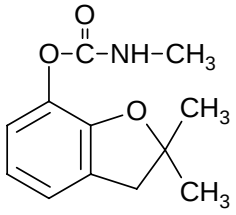


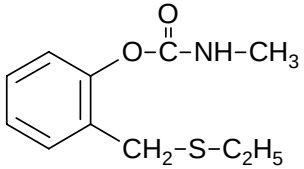


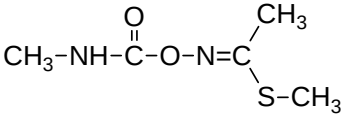


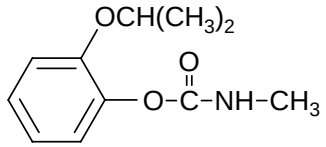


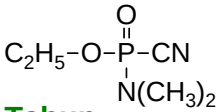




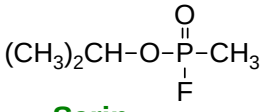




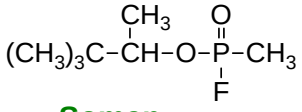




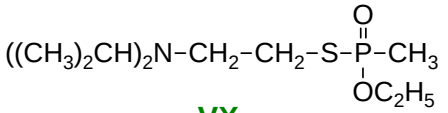
Tabun



Sarin



Soman

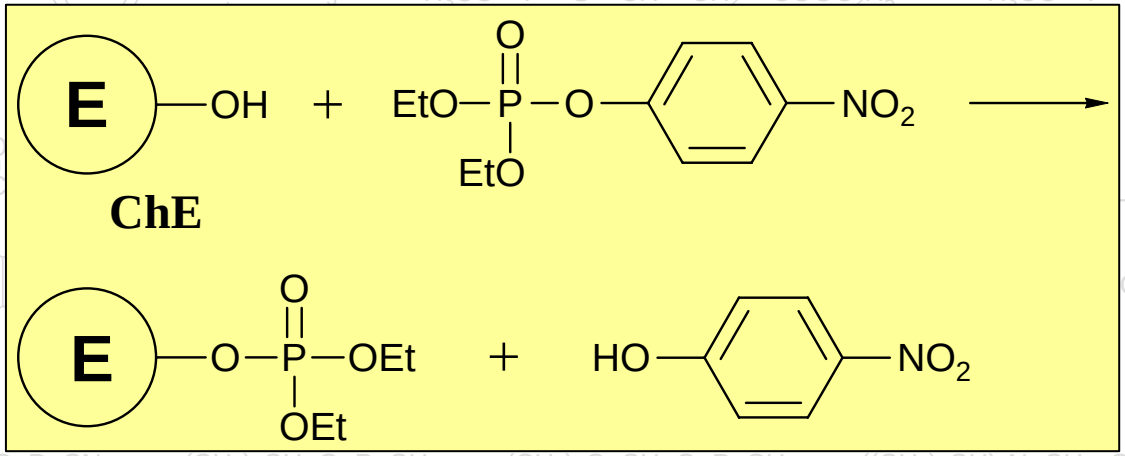


VX

Universität Hohenheim
Institut für Lebensmittelchemie

Inhibitors of cholinesterases (ChE)

- about 80 organophosphorus insecticides
- about 30 carbamate insecticides
- additionally metabolites (sulfur oxidation)



Tabun

=> endogenous acylcholine poisoning

Universität Hohenheim
Institut für Lebensmittelchemie

Screening ChE inhibitors

cuvette assays

- DIN 38415-1 (Ellman‘s reagent, photometry)
- BACHEM aCella^{TM} – AChE (luminescence)
- MOLECULAR PROBES Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (fluorescence)

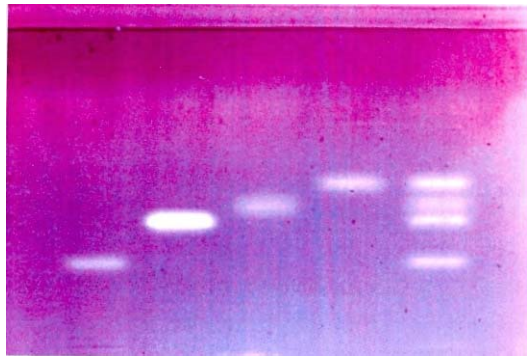
biosensor assays

=> ‚sum‘ of inhibitors – mixed mode inhibition
(expressed as paraoxon equivalents)

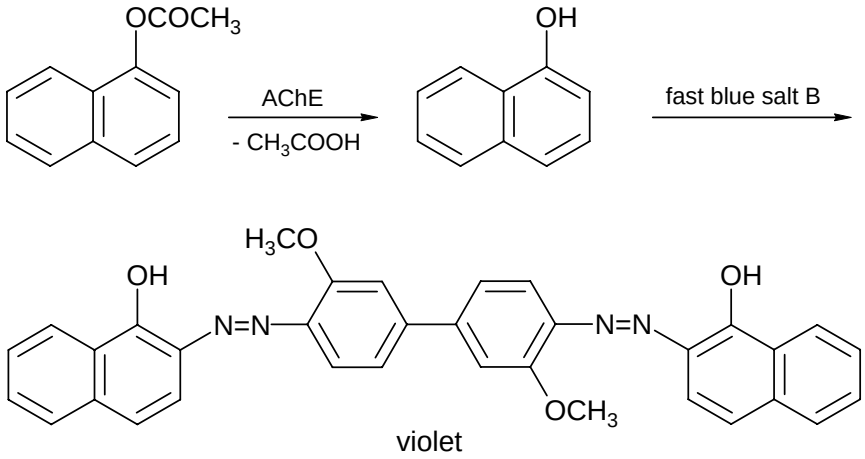
Universität Hohenheim
Institut für Lebensmittelchemie

Screening ChE inhibitors

HPTLC/ChE inhibition assay [1,2)]



from PhD thesis Christel Weins (2006)



violet

=> identification: Rf
=> detection limits: ~ 10 pg/band (paraoxon)
=> only oxons are inhibitors of ChE (oxidation)

1) Weins C, Jork H (1996) J Chromatogr A 750: 403-407
2) Marston A, Kissling J, Hostettmann K (2002) Phytochem Anal 13: 51-54

Universität Hohenheim
Institut für Lebensmittelchemie

Multienzyme inhibition assay

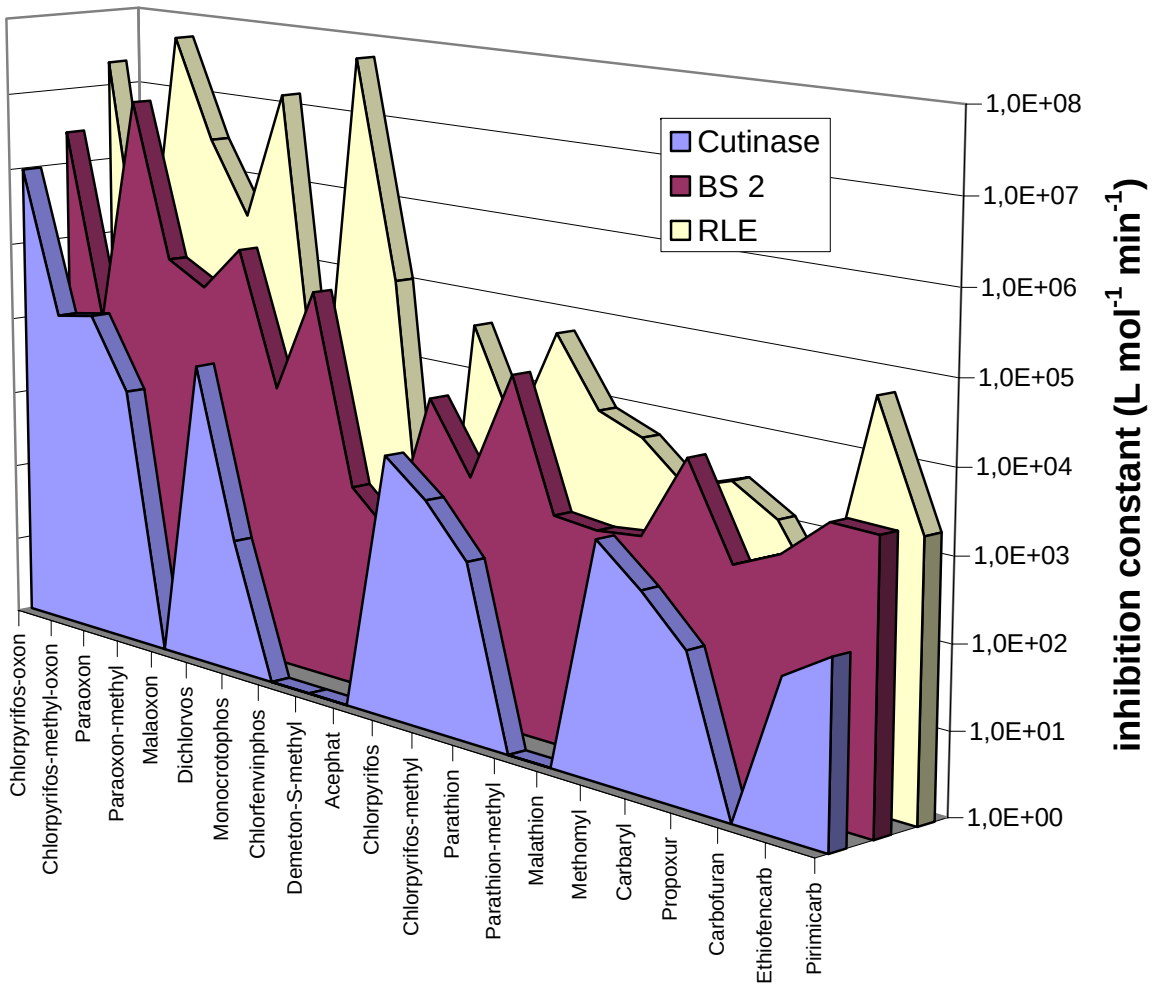
microtiter-plate assay [1)]

- cutinase (*Fusarium solani pisi*)
- rabbit liver esterase (RLE)
- BS2 esterase (*Bacillus subtilis*)

1) Walz I, Schwack W (2007) J Agric Food Chem 55: 10563-10571

Universität Hohenheim
Institut für Lebensmittelchemie

Multienzyme inhibition assay

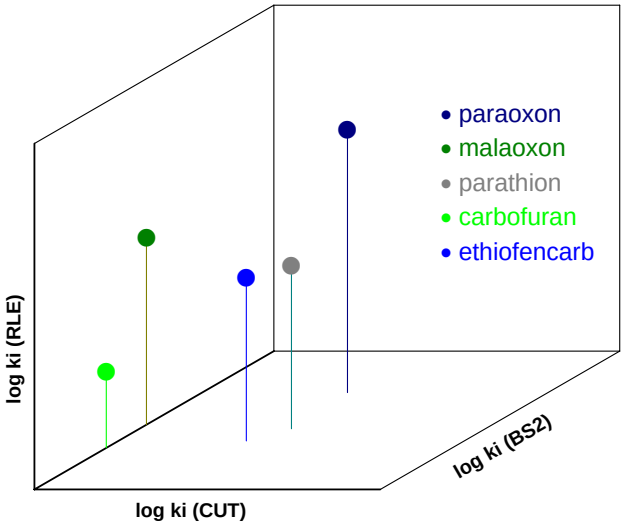


Universität Hohenheim
Institut für Lebensmittelchemie

Multienzyme inhibition assay

microtiter-plate assay [1)]

- cutinase (*Fusarium solani pisi*)
- rabbit liver esterase (RLE)
- BS2 esterase (*Bacillus subtilis*)



=> thions are inhibitors, too

=> highly sensitive (RLE): µg/L to ng/L

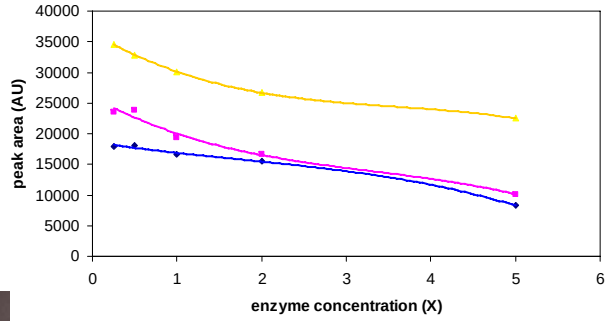
=> identification: inhibition pattern (single residue)

1) Walz I, Schwack W (2007) J Agric Food Chem 55: 10563-10571

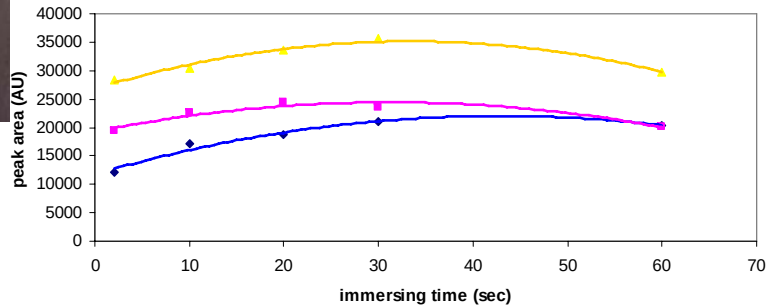
Universität Hohenheim
Institut für Lebensmittelchemie

HPTLC/multienzyme inhibition assay

enzyme concentration

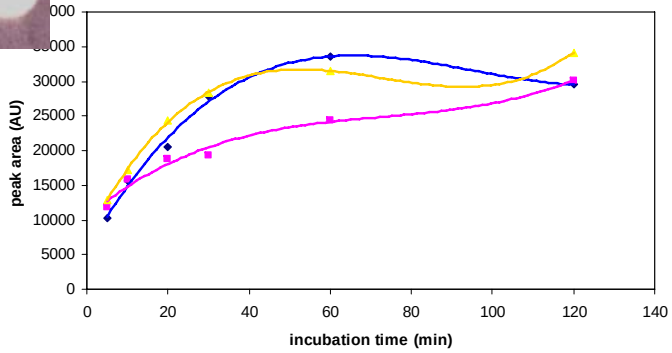


substrate dipping time



esterase incubation time

20 min 30 min 60 min

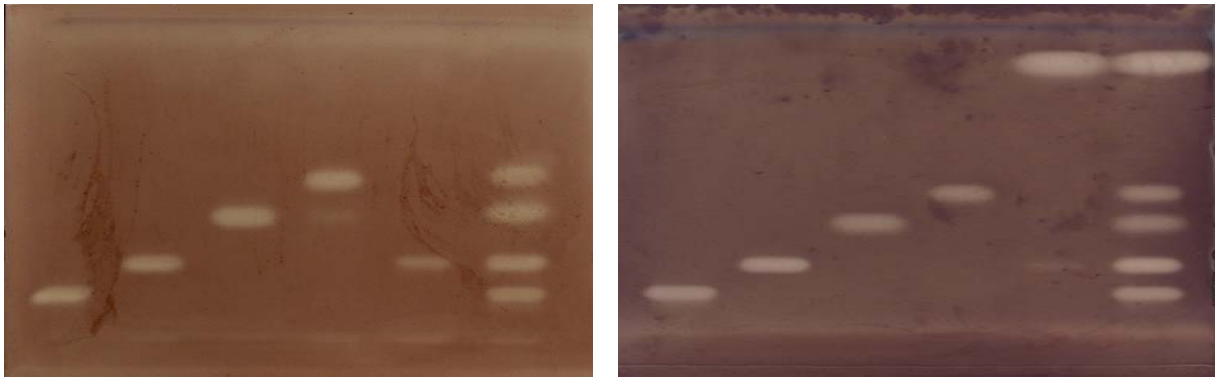


Universität Hohenheim
Institut für Lebensmittelchemie

HPTLC/multienzyme inhibition assay

HPTLC (silica gel 60 F_{254}) – ADC2 [1)]
dipping into esterase solution (2 s)
incubation (37°C, humid chamber, 60 min)
dipping into substrate solution (30 s) [2)]
reaction time: 3 min
heating the plate at 50°C (5 min)
scan at 530 nm

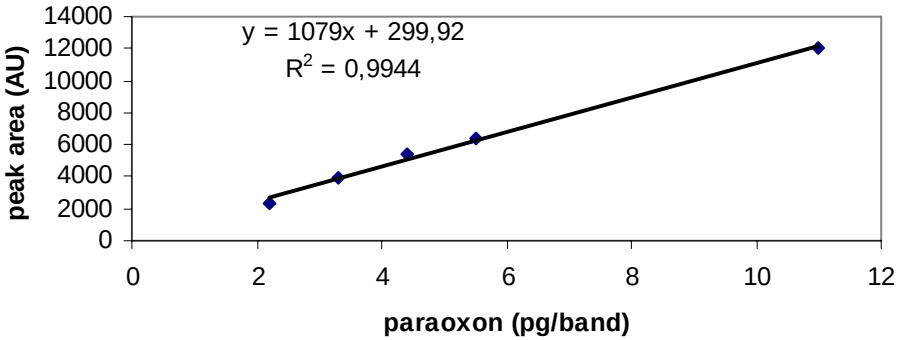
cholinesterase **BS2 esterase**

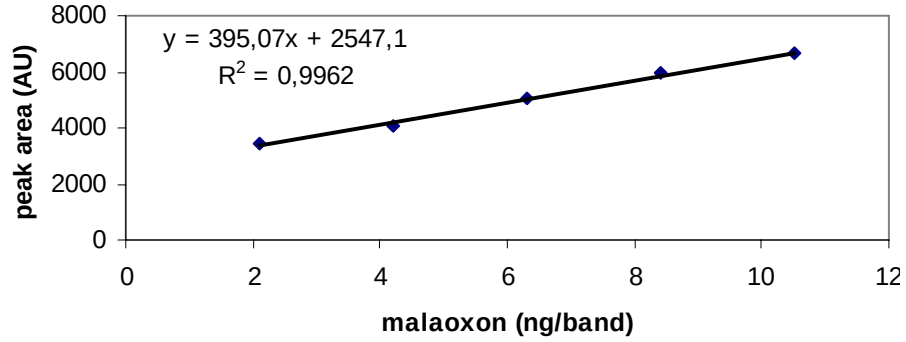


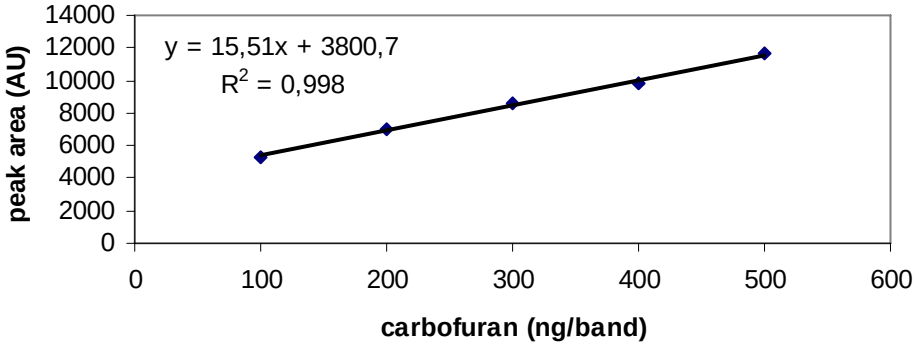
1) dichloromethane/ethyl acetate/n-hexane (15/20/65)
2) α-naphthyl acetate/fast blue salt B

Universität Hohenheim
Institut für Lebensmittelchemie

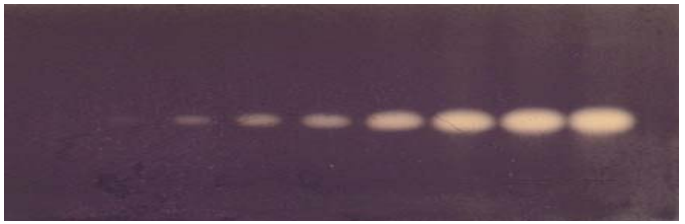
Calibration

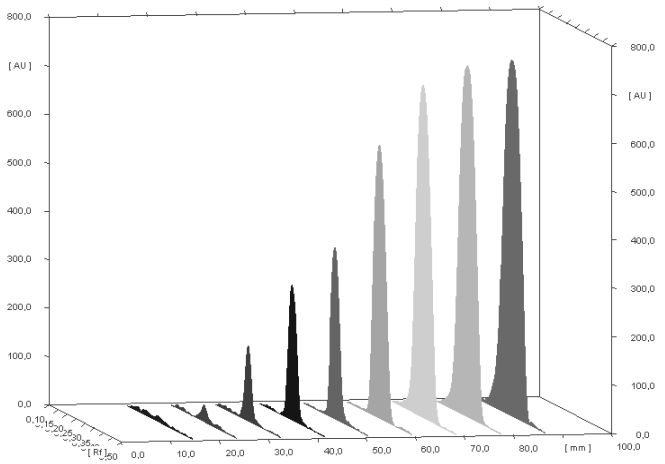






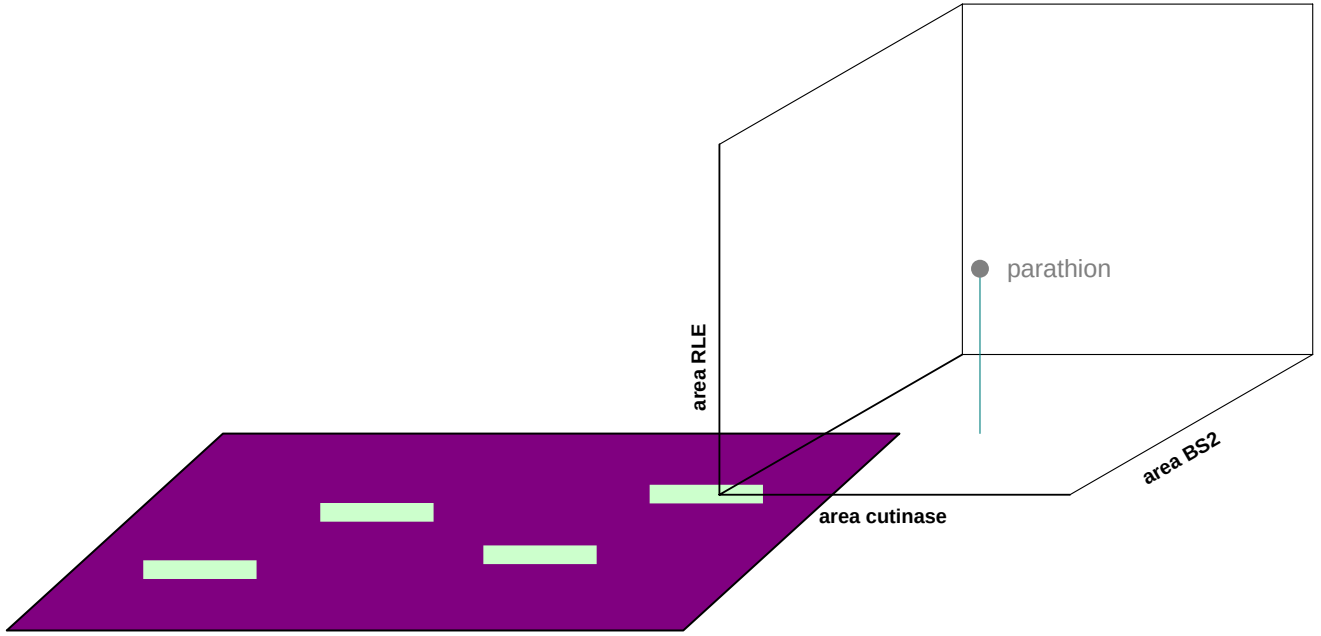
BS2 esterase





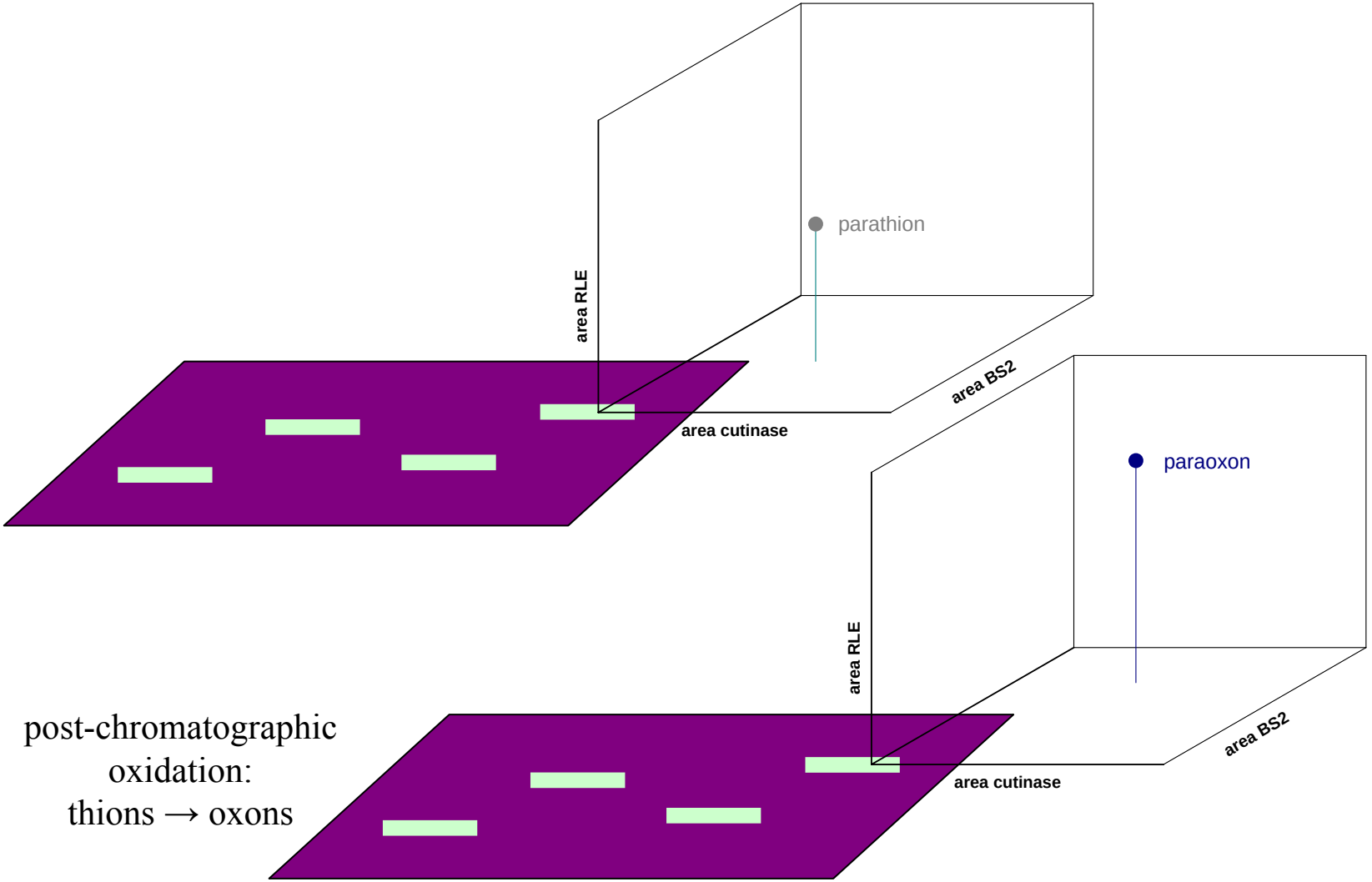
Universität Hohenheim
Institut für Lebensmittelchemie

„Multidimensional“ analysis



Universität Hohenheim
Institut für Lebensmittelchemie

„Multidimensional“ analysis



post-chromatographic
oxidation:
thions → oxons

Universität Hohenheim
Institut für Lebensmittelchemie

LOD/LOQ

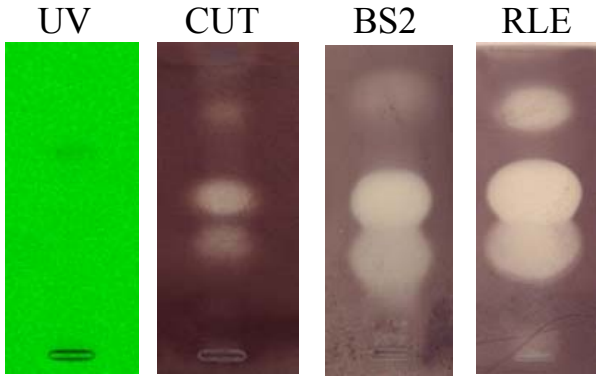
insecticide	enzyme	LOD	LOQ	k_i (L mol^{-1} min^{-1})	k_i AChE
paraoxon (pg/band)	BS2	2,4	3,5	$8,9 * 10^6$	**$4,7 * 10^5$**
	RLE	2,4	3,5	$5,5 * 10^7$	
	CUT	140	207	$1,6 * 10^4$	
malaoxon (ng/band)	BS2	2,1	3,0	$4,8 * 10^4$	**$2,7 * 10^5$**
	RLE	8,7	11,1	$3,5 * 10^5$	
	CUT	663	982	0	
carbofuran (ng/band)	BS2	72	107	$9,1 * 10^2$	**$9,0 * 10^5$**
	RLE	130	189	$1,18 * 10^2$	
	CUT	965	1415	0	

Universität Hohenheim
Institut für Lebensmittelchemie

LOD/LOQ
- practical working range -

insecticide	enzyme	LOD	LOQ	k_i (L mol^{-1} min^{-1})	k_i AChE
paraoxon (pg/band)	BS2	2,4	3,5	$8,9 * 10^6$	**$4,7 * 10^5$**
	RLE	2,4	3,5	$5,5 * 10^7$	
	CUT	140	207	$1,6 * 10^4$	
malaoxon (ng/band)	BS2	2,1	3,0	$4,8 * 10^4$	**$2,7 * 10^5$**
	RLE	8,7	11,1	$3,5 * 10^5$	
	CUT	663	982	0	
carbofuran (ng/band)	BS2	72	107	$9,1 * 10^2$	**$9,0 * 10^5$**
	RLE	130	189	$1,18 * 10^2$	
	CUT	965	1415	0	

apple extract (QuEChERS [1)]),
without clean-up,
spiked with malaoxon,
paraoxon, carbofuran



1) Anastassiades M et al. (2003) J AOAC Int 86: 412-431

Universität Hohenheim
Institut für Lebensmittelchemie

LOD/LOQ
- practical working range -

insecticide	enzyme	LOD	LOQ	k_i (L mol^{-1} min^{-1})	k_i AChE
paraoxon (pg/band)	BS2	2,4	3,5	$8,9 * 10^6$	**$4,7 * 10^5$**
	RLE	2,4	3,5	$5,5 * 10^7$	
	CUT	140	207	$1,6 * 10^4$	
malaoxon (ng/band)	BS2	2,1	3,0	$4,8 * 10^4$	**$2,7 * 10^5$**
	RLE	8,7	11,1	$3,5 * 10^5$	
	CUT	663	982	0	
carbofuran (ng/band)	BS2	72	107	$9,1 * 10^2$	**$9,0 * 10^5$**
	RLE	130	189	$1,18 * 10^2$	
	CUT	965	1415	0	

LOD: ~10 pg/band to ~100 ng/band

sample: 0.1 mg/kg
extraction: 10 g/10 mL (QuEChERS [1])

=> 1 µg/10 mL

1:100 → **10 pg/10 µL**

100:1 → **100 ng/10µL**

1) Anastassiades M et al. (2003) J AOAC Int 86: 412-431

Universität Hohenheim
Institut für Lebensmittelchemie

Conclusions

- effect-directed trace analysis of organophosphorus and carbamate insecticides (rapid screening)
- RLE and BS2 partly more sensitive than cholinesterases
- more selectivity (inhibition) than using ChE‘s
- up to 5 signals for identification and quantification
- protocol of practicability for rapid screening?
- low risks of matrix interferences (enzyme inhibition) as compared to microtiter-plate assay?