Supplementary Materials: Carbamate and organophosphate pesticides monitoring in food matrices using an affordable and simple spectroscopic acetylcholinesterase assay

Aristeidis S. Tsagkaris, Leos Uttl, Jana Pulkrabova, Jana Hajslova*

S1. Enzyme assays protocols

Ellman's assay

Thirty μL AChE (0.009 U well^{-1}) were incubated with 30 μL of an inhibitor for 15 min. Next, 30 μL of a 12.5 mM AThI: 1.5 mM DTNB (9:1) solution in PBS were added and the absorbance was measured at 412 nm after 2 min.

Indoxyl acetate assay

Fifty μL AChE (0.75 U well^{-1}) were incubated with 50 μL of an inhibitor for 15 min. Next, 10 μL of 10 mM IDA in EtOH was added and the absorbance was measured at 670 nm after 30 min.

Alpha-naphthyl acetate assay

Thirty μL AChE (1.1 U well^{-1}) were incubated with 30 μL of an inhibitor for 15 min. Next, 30 μL of 2 mM a-NAc: 0.66 mM FBBS (1 in EtOH: 5 in PBS) was added and the absorbance was measured at 525 nm after 2 min.

S2. AChE tolerance towards organic solvents

In the first phase of model experiments, the problem resulting from a limited solubility of some relatively non-polar OPs and CMs in aqueous solutions had to be figured out as both analytes solubilization and ChEs full activity and stability had to be preserved. In other words, the maximum amount of organic solvent in reaction medium still tolerated by enzyme and its minimal concentration dissolving of pesticides in medium were critical parameters to be searched. To achieve a compromise, AChE activity was measured using Ellman's assay in the presence of PBS-organic solvent solutions (ethanol, methanol, acetonitrile and dimethyl sulfoxide). To calculate residual AChE activity (%) the following equation was used,

Enzyme residual activity % = (ΔAsample – ΔAsaturated / ΔAblank – ΔAsaturated) x 100 where ΔAsample is the absorbance change of the sample, ΔAsaturated is the absorbance change of a saturated pesticide solution (pesticide concentration that totally inhibits the enzyme) and ΔAblank is the absorbance change of the control.

Rather surprisingly, AChE remained almost unaffected in the presence of up to 40% (v/v) EtOH, with residual activity ranging from 83-85% (Fig. S1). Regarding MeOH, almost 70% of enzyme activity was achieved in most cases when its concentration was up to 40% (v/v). It is important to notice that a high substrate concentration (12.5 mM AThI in this study) is necessary to retain AChE activity in the presence of EtOH and MeOH [1]. However, a high tolerance of solvent in medium was not the case when using DMSO and ACN, which strongly inhibited the enzyme when their concentration exceeded 20% v/v. To achieve the best conditions, PBS-EtOH mixture (7:3, v/v) solutions were used for the preparation of the tested pesticides in the rest of the experiments.

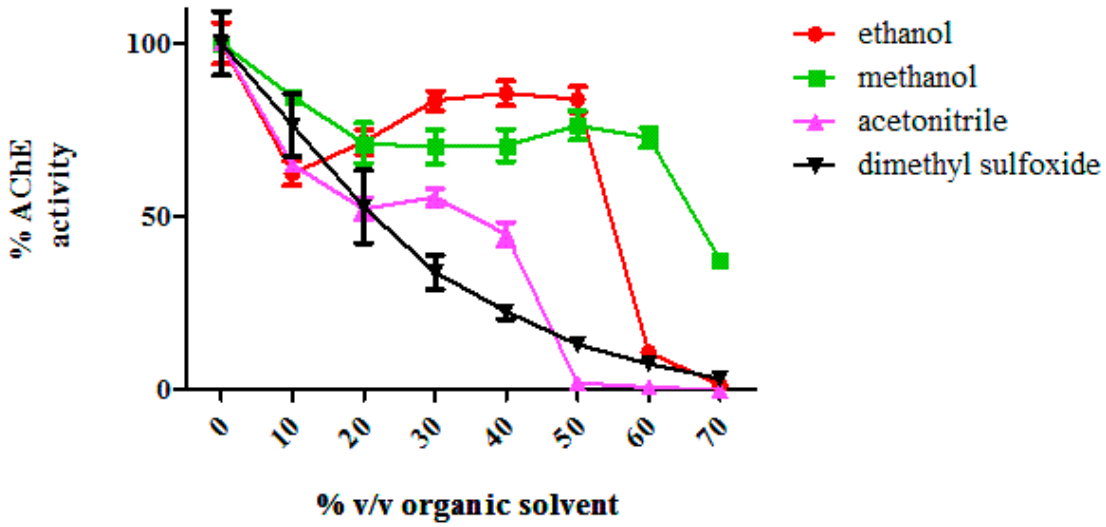

Figure S1. AChE tolerance towards various organic solvents after 30 min of incubation with ethanol, methanol, acetonitrile and dimethyl sulfoxide (*n*=3). The 100% AChE activity was set for 0% organic solvent in PBS.

S3. Calibration ranges for inhibitory effect investigation

Table S1. Calibration ranges for each pesticide investigated for potential cross-reaction with carbofuran. (*n*=7 calibration points and one blank sample).

class	pesticide	calibration range
CMs	aldicarb	0.52 – 262 µM
	carbofuran	4.5 - 2256 nM
	carbofuran-3-hydroxy	0.042 – 10 µM
	carbaryl	0.50 – 248 µM
phosphoryl OPs	chlorpyrifos-oxon	0.029 – 7.4 µM
	dichlorvos	0.045 - 11 µM
	malaoxon	0.031 – 7.9 µM
	paraoxon	0.18 – 36 µM
thiophosphoryl OPs	chlorpyrifos	2.8 – 1816 µM
	malathion	15 – 3100 µM
	parathion	18 – 3600 µM

S4. Carbofuran-3-hydroxy and dichlorvos validation and benchmarking

Table S1. Carbofuran-3-hydroxy and dichlorvos validation results in strawberry and lettuce for AChE assay.

analyte	spiking level	lettuce	strawberry

	(mg kg $^{-1}$)	R %	RSD %	LOD (mg kg $^{-1}$)	R %	RSD %	LOD (mg kg $^{-1}$)
carbofuran-3-hydroxy	0.31	80	20	0.16	88	17	0.19
	1.25	95	16		91	14	
dichlorvos	0.31	107	6	0.2	102	5.5	0.18
	1.25	95	10		87	12	

Table S3. Carbofuran-3-hydroxy and dichlorvos validation results in strawberry and lettuce for LC-MS/MS method.

analyte	spiking level	lettuce			strawberry		
	(mg kg $^{-1}$)	R %	RSD %	LOD (mg kg $^{-1}$)	R %	RSD %	LOD (mg kg $^{-1}$)
carbofuran-3-hydroxy	0.020	75	6.3	0.0017	84	3.1	0.0015
	0.100	77	3.2		83	2.4	
dichlorvos	0.020	78	4.7	0.0015	91	2.3	0.0012
	0.100	79	3.9		92	3.4	

S5. References

1. Fekonja, O.; Zorec-Karlovsek, M.; El Kharbili, M.; Fournier, D.; Stojan, J. Inhibition and protection of cholinesterases by methanol and ethanol. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 407–415.