

Aqueous suspensions of fungicide dithiocarbamates for analytical purposes

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Introduction

The AQC guideline [1] recommends that validated methods should be used for all analytes included in a pesticide residue definition in order to comply with the EU maximum residue level (EU MRL). For fungicide dithiocarbamates (DTCs), the EU MRL residue definition is given as "Dithiocarbamates (dithiocarbamates expressed as CS₂, including maneb, mancozeb, metiram, propineb, thiram and ziram)" (Reg. (EU) 2017/171). This implies that pesticide residue laboratories testing food samples for compliance with EU MRLs should have validation data in place for at least the analytes listed in the DTC residue definition. In 2022 the French NRL for Pesticide Residues and the EURL-SRM conducted an online survey to find out - among others - for which DTCs of the EU MRL DTC residue definition the participating laboratories had validation data fulfilling the AQC guideline criteria. Approx. 90 % of the laboratories analyzing for DTCs had NO validation data for alkylene-bis-DTCs (e.g. metiram, propineb) and N,N-dimethyl-DTCs (ziram, ferbam). This is likely due to the low solubility of these substances in organic solvents and water, which makes it challenging to conduct validation experiments in which matrices are spiked with aliquots of appropriate standard solutions.

Aim of study

The aim of this study was to find a 'solvent' which preferably preserves the complex and polymeric structure of alkylene-bis-DTCs and N,N-dimethyl-DTCs so that these two aspects can be considered during method development and method validation experiments.

Preparation of DTC-suspensions – video tutorial

Several thickening agents were tested for the preparation of DTC-suspensions, with a mixture of water/acetonitrile/xanthan gum (95/5/0.2 v/v/w) (xanthan gum-mixture) found to be suitable for the preparation of reproducible stock and working DTC-suspensions. A video demonstrating the preparation of the xanthan gum-mixture and DTC-suspensions is available at [youtube.com/@eurl-srm](https://www.youtube.com/@eurl-srm), playlist "Lab Coat Practice":



Analytical method

Spiked samples were analyzed via the common moiety approach involving cleavage of DTCs with HCl/SnCl₂ (80°C for 3 h), partitioning of the evolved carbon disulfide (CS₂) into isoctane and measurement by GC-MS/MS [2].

Results

The lowest sedimentation rate was found for the metiram stock-suspension prepared with xanthan gum-mixture (Fig. 1). The impact of the tested suspensions on standard calibration curves of metiram revealed further differences. Here, the calibration curve based on suspensions prepared with xanthan gum-mixture showed the best correlation with the theoretical calibration curve as regards R² and slope (Fig. 2). Good recoveries were obtained for each calibration point (Fig. 2) and for zineb, mancozeb and propineb at 0.15 mg/kg (Fig. 3). Metiram-suspensions prepared with NaOH/EDTA/cysteine got an almost clear appearance very rapidly (Fig. 1). It can be assumed that the presence of NaOH and EDTA leads to partial de-complexation of DTCs, thus making the de-complexed DTC-anions more easily accessible to subsequent reactions, e.g. degradation (Fig. 3). Mancozeb, metiram, propineb and ziram stored in xanthan gum-stock suspensions showed acceptable stabilities within the periods tested (Tab. 1). Stability experiments of DTCs spiked on cucumber homogenates revealed a high degradation rate for thiram, whereas ziram, mancozeb showed only minor and metiram, zineb, propineb showed no degradation (Fig. 4).

Which 'solvent' is suitable?

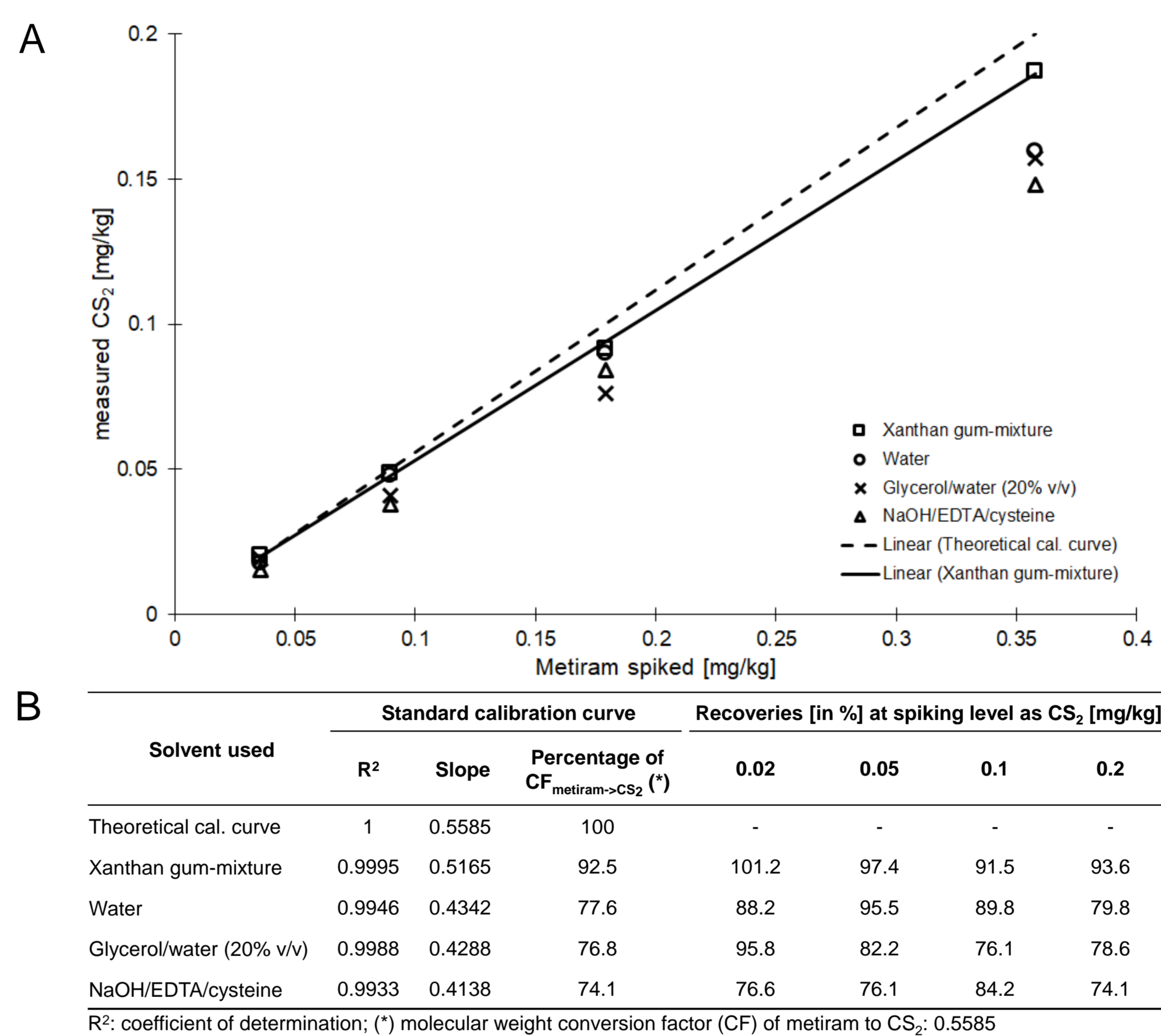


Fig. 2: Effect of different suspensions on standard calibration curves of metiram. Metiram suspensions were prepared in different solvents, spiked on frozen tomato and analyzed for CS₂.

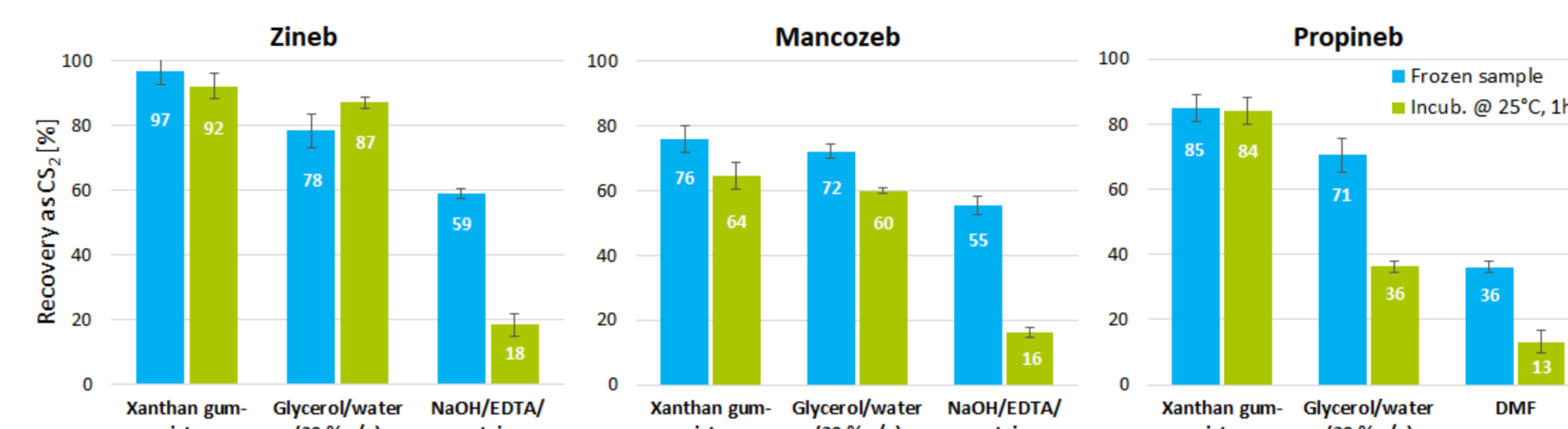


Fig. 3: Effect of different suspensions on recoveries of zineb, mancozeb and propineb. Tomato homogenates were spiked at 0.15 mg/kg (calc. as CS₂) and analyzed for CS₂ (n=3).

Stability experiments

Tab. 1: Stability of stock DTC-suspensions in xanthan gum-mixture. More details can be found at www.eurl-pesticides-datapool.eu.

DTC	Storage duration [d]	Diff. STORED vs. REFERENCE ^(a) [%] (reference = 100%)
Mancozeb	16	1.9
	23	3.8
	135	-11.6
Metiram	14	3.3
	21	3.6
	135	2.7
Propineb ^(b)	36	0.2
Propineb ^(c)	36	3.4
Ziram	36	-1.0

concentration: 1 mg/mL; storage temperature: -18°C; (a) reference suspension was freshly prepared from certified standard of the same batch; (b) provider 1; (c) provider 2

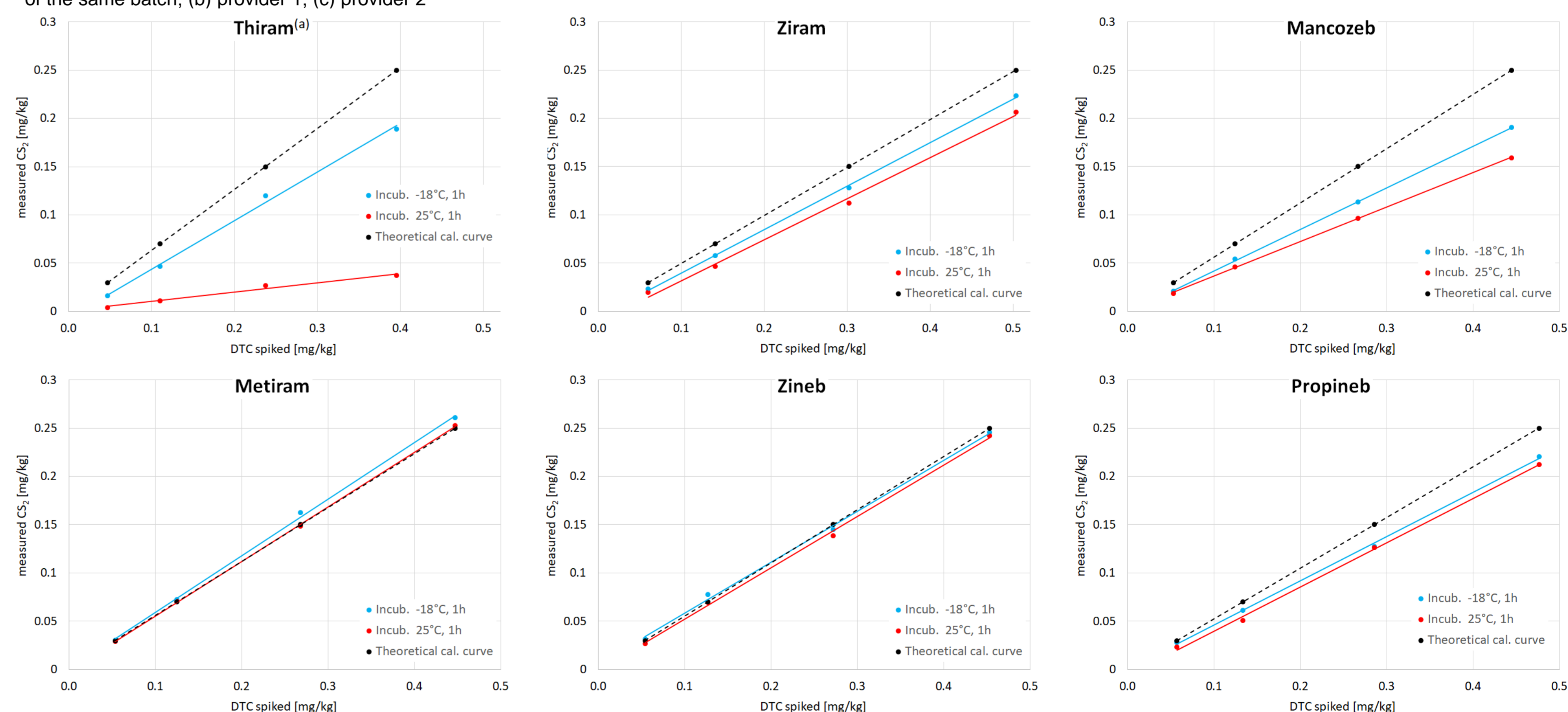


Fig. 4: Stability of DTCs in cucumber homogenates. DTC suspensions were prepared in xanthan gum-mixture (^(a) thiram-solutions were prepared in toluene), spiked either on frozen (-18°C) or thawed (RT) homogenates and incubated at the corresponding temperature for 1 h before CS₂-hydrolysis.

Summary and outlook

DTC-suspensions based on xanthan gum-mixture offer a good possibility to obtain reliable results in DTC-method validation and development experiments. Stability studies are ongoing with regard to storage time, standards of different providers and DTCs (e.g. zineb). Interlaboratory studies are in preparation.

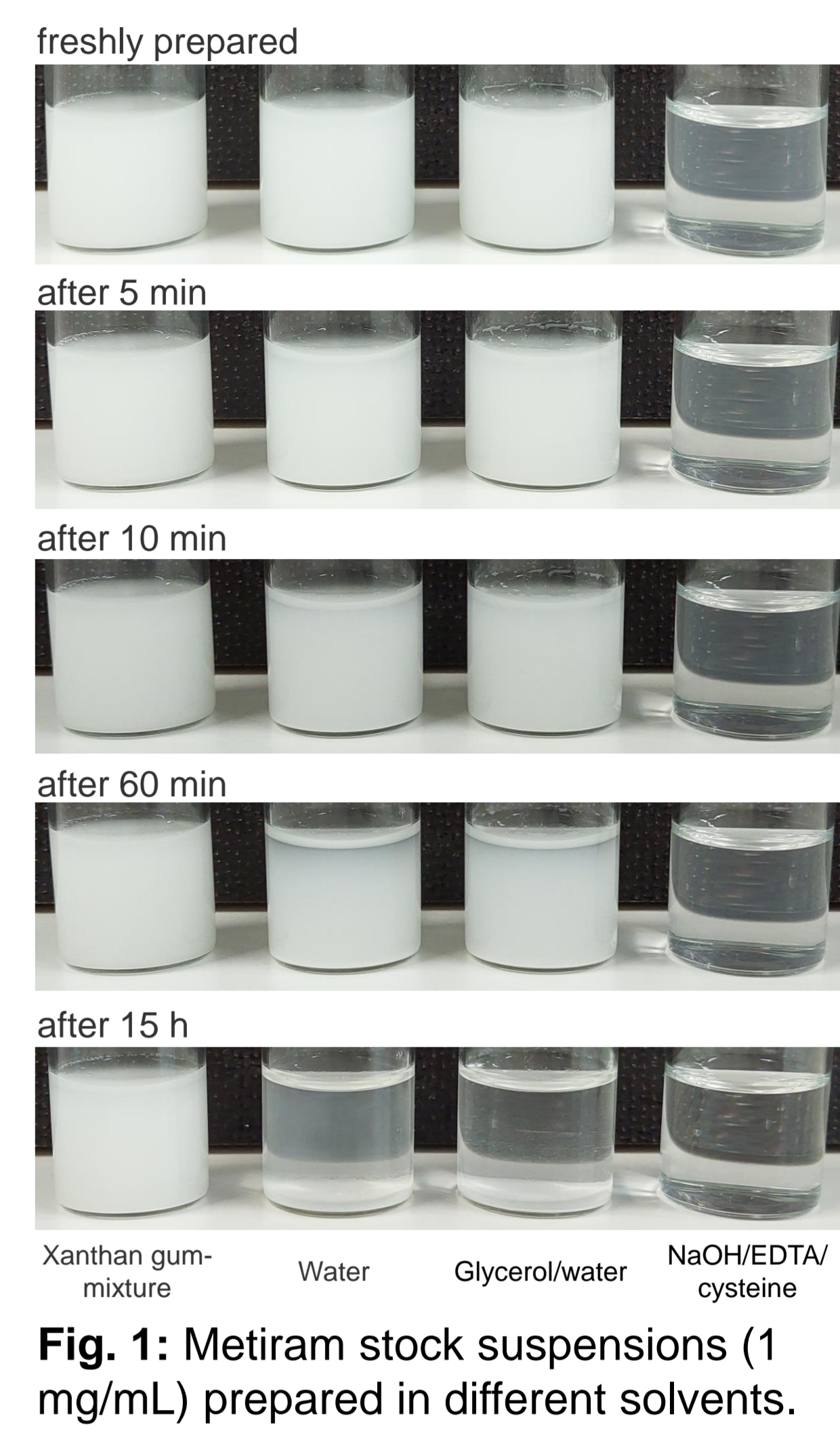


Fig. 1: Metiram stock suspensions (1 mg/mL) prepared in different solvents.

PD-29

EPRW 2024



Co-funded by
the European Union



Baden-Württemberg