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Disposable Biosensor Test for Organophosphate and Carbamate Insecticides in Milk

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A highly sensitive and rapid biosensor test based on disposable screen-printed thick-film electrodes was developed, which is suitable for monitoring organophosphate and carbamate residues in foods of animal origin with increased fat contents such as milk. The wild-type enzyme was combined with three engineered variants of *Nippostrongylus brasiliensis* acetylcholinesterase (NbAChE), to obtain enhanced sensitivity. The sample pretreatment could be reduced to a minimum. There was no extraction or fat removal necessary. With the biosensor test paraoxon concentrations down to 1 μg/L could be detected in milk. The detection limit for carbaryl was 20 μg/L. Recovery rates for paraoxon and carbaryl in milk samples lay between 89 and 107%. Ten milk samples from local markets were tested both with the biosensor test and with standard chromatographic multiresidue methods. Two milk samples caused AChE inhibition rates of >50%. Accordingly, 4 μg/L tebufenpyrad, 4 μg/L tetraconazole, and 2 μg/L bifenthrin were detected in one of these milk samples. The other milk sample contained 2 μg/L tebufenpyrad.

KEYWORDS: Acetylcholinesterase biosensor; organophosphate; carbamate; milk analysis

INTRODUCTION

Milk is a widely consumed commodity. Although organophosphorus (OP) and carbamate insecticides have a relatively low persistence in the environment, there are a number of ways in which insecticides can reach milk. These insecticides may originate from foodstuffs from plant materials that have been treated with insecticides or the use of insecticides directly on the animal or as hygienic treatments against insects in milk-processing factories (*1*). There are several reports of their presence in milk (*2*−*5*). Indeed, maximum residue limits (MRL) and acceptable daily intake (ADI) values for parent insecticides have been set by several organizations such as the Food and Agriculture Organization of the World Health Organization (FAO/WHO) (*6*) or the European Union (EU) (*7*), thus requiring adequate methodology for enforcement. There is a certain demand for fast screening tests especially for neurotoxic insecticides in food items that are consumed to a great extent by vulnerable groups such as young children and infants. Organophosphates and carbamates represent the main part of pesticides that are acutely toxic toward humans and for which ADI values have been set. Moreover, this toxicity is age-related in some cases; for example, chlorpyrifos and paraoxon are more toxic to the young as compared to adults (*8*, *9*). Standard analytical methods based on gas chromatography or high-performance liquid chromatography coupled with mass selective detectors are too time-consuming and too expensive to control sufficient amounts of milk samples before they are ingested by the consumer (*10*, *11*). A previously developed biosensor test proved to be appropriate for the control of the MRL of insecticides in infant food of plant origin, set by the European Union (*12*). In contrast to most other described acetylcholinesterase (AChE) biosensor methods, this test enabled accurate insecticide detection even at very low inhibitor concentrations without the need of any organic solvents. Del Carlo et al. recently described the analysis of milk and honey samples with an AChE biosensor but had to extract the insecticides with hexane and acetone and redissolve the dried extract in buffer (*13*). In our case matrix effects were eliminated by the introduction of an electrode treatment step with the detergent Tween-20 into the test protocol (*12*).

The aim of this study was to test the applicability of this biosensor test toward food of animal origin. Milk was chosen as test matrix because of its high fat content, which poses an analytical challenge in trace analysis (*14*−*17*). In general, most detection methods for pesticide residue in fatty foods consist of two key steps: extraction of target insecticides from milk and cleanup to remove interfering coextractives (*14*, *16*−*22*).

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Due to polarity variation of insecticides, a single class of extraction solvents cannot be used for efficient extraction of all target insecticides (*19*, *23*). The subsequent cleanup step may also be the reason for reduced recovery rates, even no recovery for some insecticides (*1*, *16*). In addition, it is still common to use several hundred milliliters of solvent for the treatment of one sample (*17*, *19*).

In the present study a multienzyme biosensor array was applied to ensure the sensitive detection of all relevant neurotoxic insecticides. Most biosensor studies were conducted until now with commercially available electric eel AChE, but this enzyme turned out to be not sensitive enough for trace analysis (*12*, *24*). Therefore, we created a set of sensitive mutants by site-directed mutagenesis of *Nippostrongylus brasiliensis* AChE (NbAChE) (*25*). Three sensitive mutants were combined with the wild-type enzyme on a multienzyme array. An important prerequisite for a practical application and an advantage of this enzyme compared to AChEs from other origins is the high room temperature storage stability and the fact that NbAChE can be produced heterologously in the yeast *Pichia pastoris* at high yields. The applicability of the multienzyme biosensor for the analysis of foods of animal origin was tested using paraoxon and carbaryl as representatives for organophosphates and carbamates, respectively. The biosensor test was validated against standard analytical methods performed at the Chemical and Veterinary Official Laboratory (CVUA), Stuttgart, Germany (Chemisches und Veterinäruntersuchungsamt Stuttgart).

MATERIALS AND METHODS

Reagents and Samples. Tween-20 and pesticide standards were purchased from Riedel-de Haën (Seelze, Germany). Insecticide stock solutions were prepared in ethanol. All other reagents were of analytical grade as supplied by Sigma-Aldrich (Deisenhofen, Germany) or Fluka (Neu-Ulm, Germany). Milk samples used for the analysis were obtained from local stores in Stuttgart (Germany) between September 2003 and January 2004.

Expression of NbAChE in *P. pastoris*. Wild-type and mutant NbAChE were expressed in *P. pastoris* X33 strain (Invitrogen, Karlsruhe, Germany) transformed with a pPICZαB vector (Invitrogen) bearing the NbAChE B gene, as described in a former publication (*26*). The generation of the AChE mutants is described elsewhere (*25*). For AChE expression *P. pastoris* X33 was cultivated in complex medium, containing 2% (w/v) peptone, 1% (w/v) yeast extract, 1% glycerol (w/v), and 100 mM potassium phosphate buffer (1 M, pH 7.5). After 24 h of incubation at 30 °C and 200 Upm, the medium was exchanged by 200 mL of medium without glycerol. Induction was started by the addition of 0.5% (v/v) methanol at $OD_{550} = 1$. Every 24 h 0.5% (v/v) methanol was added. After 9 days, protein expression cultures were centrifuged at 5000*g* for 20 min, and the supernatant was used as the source of the enzyme.

Multielectrode Biosensor Fabrication. Multielectrode biosensors ("multisensors") were printed as described previously (*27*) on flexible polyvinyl chloride sheets from SKK (Denzlingen, Germany) using a DEK 249 screen printer (DEK Ltd., Weymouth, U.K.) and polyester screens (45°, 100T) that were purchased from Steinmann GmbH (Stuttgart, Germany). Screen-printing inks (ElectrodagPF-4l0, Electrodag 423 SS, and Electrodag 6037SS) were obtained from Acheson (Scheemda, The Netherlands). Ink for working electrodes contained 3% (w/w) hydroxyethyl cellulose (HEC) and 15% (w/w) 7,7,8,8-tetracyanoquinodimethane (TCNQ)−graphite [2.5% (w/w) TCNQ in T15 graphite from Lonza (Basel, Switzerland)]. Marastar SR 057 purchased from Marabu (Tamm, Germany) was used as insulation ink. The electrodes were cured for 30 min at 90 °C after each printing step before enzyme immobilization. Each enzyme was printed separately. The printing ink for the AChE multisensor contained 5% (w/w) bovine serum albumin (BSA) from Sigma (Deisenhofen, Germany) and 1 unit/mL AChE in 1% (w/w) HEC aqueous solution. After completion of the printing process, cross-linking was performed in glutaraldehyde vapor for 15 min at room temperature. NbAChE was directly used from the culture supernatant without further purification steps. The distribution of the AChE variants on the sensor from positions 1−4 was as follows: WT, M301A, M301A/W303A, and F345A.

Biosensor Measurement. All sensor experiments were carried out in a stirred buffer solution [0.01 M potassium phosphate buffer (PBS), 0.05 M NaCl, pH 7.5] at room temperature. Enzyme activity was determined by monitoring thiocholine formed by enzymatic hydrolysis of acetylthiocholine chloride (1 mM). Thiocholine was determined by anodic oxidation at 100 mV versus Ag/AgCl. For use in the inhibition experiments, the biosensor was incubated in a sample for 30 min at room temperature in a nonstirred solution, and percentage of inhibition was calculated after the measurement of residual activity, according to the formula $[A_0 - A_i/A_0] \times 100$, with A_0 = initial activity (current output before sample incubation) and A_i = remaining activity (current output after incubation). Current outputs ranged in the lower nanoampere range (data not shown).

Reactivation of AChE Activity. To determine the AChE activity reactivation rate, 1 mM pyridine-2-aldoxime methochloride (2-PAM) in PBS was used as reactivating agent. The biosensor was incubated for 30 min in a stirred reactivation solution at room temperature. The final AChE activity was then measured in buffer solution. The percentage of reactivation was calculated according to the formula $[(A_r - A_i)/(A_0 - A_i)] \times 100$, with A_0 = initial activity (current output before sample incubation), A_i = remaining activity (current output after sample incubation), and A_r = reactivated activity (current output after reactivation with 2-PAM solution).

Biosensor Milk Test. One milk sample (10 mL) was mixed with 10 mL of 1 M potassium phosphate buffer (pH 7.5) to ensure a sufficient amount of water at a neutral pH value. The AChE biosensor was incubated in the milk sample for 30 min. Before the activity of the enzyme in the buffer solution was measured, the electrodes were placed in potassium phosphate buffer (10 mM, pH 7.5) containing 1 vol % Tween-20 for 15 min and then washed with pure potassium phosphate buffer before the initial AChE activity was measured and after incubation in the milk sample before the residual AChE activity was measured, following a method described previously (*12*).

Chromatography−Mass Spectrometry. Sample preparation was carried out according to the procedure published by Anastassiades et al. (*28*), with slight modifications: 10 mL of milk sample was used for extraction. One hundred microliters of internal standard solution consisting of 50 μg/mL PCB 138 and 200 μg/mL triphenyl phosphate dissolved in acetonitrile was added. For the subsequent dispersive cleanup step after acetonitrile extraction, 8 mL of extract was carried over to a tube containing 200 mg of PSA and 1.2 mg of anhydrous magnesium sulfate. After shaking, centrifuging, and decanting, fat was frozen out by leaving the sample in a refrigerator at −20 °C for 1 h. Rapid filtration was followed by transfer of 0.5 mL aliquots to GC/LC autosampler vials.

Paraoxon was measured by GC-MS employing an Agilent 6890 GC equipped with a PTV injector, an electronic pressure control (EPC), and a 7673A autosampler coupled to an Agilent 5973 mass selective detector (MSD) running a selected ion monitoring (SIM) method in EI mode. Chemstation software was used for instrument control and data analysis. The column was an HP-5MS 30 m × 0.25 mm, 25 μm film thickness. Helium was used as carrier gas in constant flow mode with a flow of 1 mL/min. Injection in solvent vent mode was performed with the following conditions: initial temperature, 40 °C; initial time, 0.8 min; temperature program, 1720 °C/min−300 °C (5 min), 2720 °C/min−260 °C (10 min); injection volume, 3 μL. Total run time was 49.7 min: oven temperature program, 40 °C (2 min)−15 °C/min−200 °C−5 °C/min−240 °C (10 min)−5 °C/min−260 °C (15 min). Transfer line temperature was set to 260 °C and solvent delay to 9.5 min. SIM masses for paraoxon were *m*/*z* 230 (target ion), 247, and 200.

Carbaryl was analyzed by LC-MS using an Agilent 1100 HPLC with degasser, binary pump, autosampler, and column thermostat coupled to an Agilent 1100 series MSD operating in SIM mode. Chemstation software was used for instrument control and data analysis. For separation a Zorbax Eclipse XDB C18 column, 150 mm × 2.1 mm, 3.5 μm particle size, was used. Gradient elution was performed with a

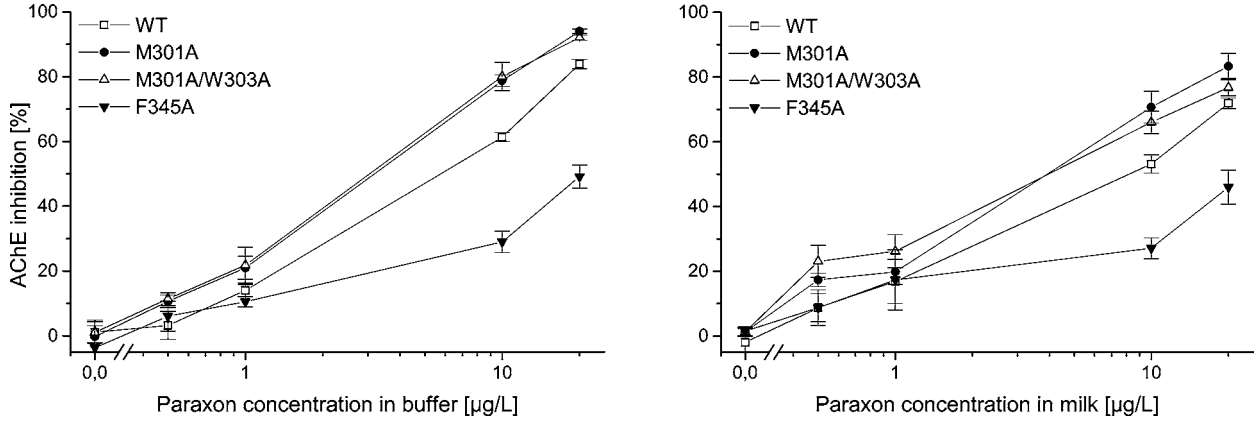

Figure 1. Paraoxon calibration curves for the multienzyme biosensor: incubation in buffer solution (*25*) and diluted (1:2) milk samples spiked with different paraoxon concentrations ($n \geq 3$).

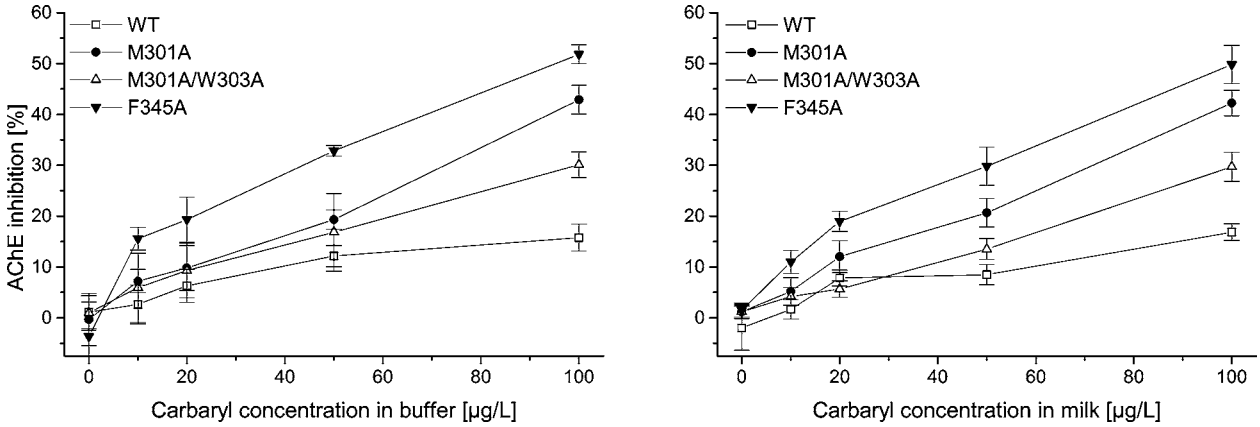

Figure 2. Carbaryl calibration curves for the multienzyme biosensor: incubation in buffer solution and diluted (1:2) milk samples spiked with different carbaryl concentrations ($n \geq 3$).

5 mM aqueous solution of ammonia acetate adjusted to pH 3 with acetic acid (A) and an acetonitrile/water mixture (97:3, v/v) containing 5 mM ammonia acetate (B): A/B = 75/25 (0 min)−100/0 (9 min). Flow was set to 0.4 mL/min and ramped to 0.5 mL/min after 9 min. Column temperature was kept at 40 °C, and the injection volume was 5 µL. The MSD worked in positive API-ES mode monitoring the following ions (*m*/*z*): 145 (target ion; fragmentor voltage, 90 V) and 202 (fragmentor voltage, 50 V). Spray chamber settings were as follows: gas temperature, 350 °C; drying gas, 10.0 L/min; nebulizer pressure, 40 psig; capillary voltage, 3000 V.

RESULTS

Test Performance in Milk. The applicability of the multienzyme biosensor system on milk was tested in organic milk samples, containing 3.5% fat, which were spiked with different amounts of paraoxon or carbaryl. Inhibition values of the wild-type (WT) and mutant NbAChEs after incubation in milk were compared with the inhibition values obtained in buffer solutions containing equivalent paraoxon or carbaryl concentrations. Recovery rates were calculated from the difference of these two inhibition rates. The inhibition by OPs or carbamates was confirmed by chemical reactivation of the AChE activity by the strong nucleophil 2-PAM.

Figures 1 and **2** show the calibration curves of paraoxon and carbaryl in milk and buffer, respectively. The inhibition values received in milk samples correlated well with the calibration curve in buffer solution. There was no unspecific inhibition caused by the milk sample itself. Mutant M301A showed 20% inhibition after incubating in milk with 2 µg/L paraoxon, corresponding to 21% inhibition in buffer spiked with 1 µg/L paraoxon. To obtain optimal enzyme conditions, the milk samples were diluted with equal amounts of phosphate buffer (1 M, pH 7.5), leading to the final measuring concentration of 1 µg/L paraoxon. The inhibitory power of an OP or carbamate toward a certain AChE is represented by its bimolecular rate constant (k_i) (*29*). A high k_i value describes a strong AChE inhibitor. The k_i value of the M301A mutant toward paraoxon was determined previously to be 1.5×10^6 M^{-1} min^{-1} (*25*). In the case of carbaryl, 20 µg/L carbaryl in buffer and 40 µg/L in milk both caused an equal inhibition of the most sensitive mutant F345A ($k_{i,F345A} = 1.2 \times 10^5$ M^{-1} min^{-1}) of 19%. Furthermore, the inhibition rates of the different AChE variants behaved as expected considering their k_i values for paraoxon and carbaryl. The coefficient of variation was on average 10% for the measurements in paraoxon-spiked milk and 15% for carbaryl-spiked milk compared to mean coefficients of variation of 10 and 11% for paraoxon- and carbaryl-spiked buffer samples, respectively.

The detection limits of the multienzyme biosensor (signal-to-noise ratio ≥ 3) were the same in buffer solution and in milk samples for the most sensitive mutants toward paraoxon and carbaryl in each case, considering the dilution factor in the analysis of milk. Therefore, for paraoxon the mutants M301A and M301A/W303A with the highest k_i values showed a detection limit of 0.5 µg/L (1.8 nmol/L) in the biosensor measurements. The detection limit for carbaryl was 10 µg/L (50 nmol/L) using the mutant F345A. Due to the 1:2 dilution of the milk with buffer the detection limits in milk samples

Table 1. Recovery Rates and Detection Limits of the NbACHE Variants on the Multisensor for Paraoxon in Organic Milk

AChE	recovery rate (%)	detection limit (μg/L)	k_i value (*25*) (M^{-1} min^{-1})
WT	97	2	1.1×10^6
M301A	102	1	1.5×10^6
M301A/W303A	95	1	1.8×10^6
F345A	93	20	3.4×10^5

Table 2. Recovery Rates and Detection Limits of the NbACHE Variants on the Multisensor for Carbaryl in Organic Milk

AChE	recovery rate (%)	detection limit (μg/L)	k_i value (*25*) (M^{-1} min^{-1})
WT	107	40	3.1×10^4
M301A	102	40	5.2×10^4
M301A/W303A	89	40	3.4×10^4
F345A	89	20	1.2×10^5

were determined to be 1 μg/L (3.6 nmol/L) paraoxon and 20 μg/L (100 nmol/L) carbaryl (see **Tables 1** and **2**).

Table 1 shows the recovery rates for paraoxon in milk samples to be in the range between 93 and 102%. Similar results were obtained for carbaryl with recovery rates in the range between 89 and 107% (**Table 2**). NbAChEs inhibited by paraoxon or by carbaryl could be clearly reactivated by 2-PAM, giving an average reactivation rate of 93%.

Validation against Standard Chromatographic Methods. Measurements with the multienzyme biosensor in milk were validated against standard analytical methods. Paraoxon- and carbaryl-spiked 3.5% fat organic milk samples were used for biosensor measurements, whereas one fraction of each sample was analyzed by GC/LC-MS investigations. The calculated insecticide concentrations fit very well to the added insecticide concentration in the milk sample (**Table 3**). In the milk sample spiked with 20 μg/L paraoxon, 19.1 and 17.7 μg/L paraoxon were detected with the sensitive mutants M301A and M301A/W303A, respectively. The average recovery rate in this case was 92%. With chromatographic analysis methods no paraoxon was found in this milk sample. The GC-MS analysis showed a detection limit of 30 μg/L (0.11 μmol/L) for paraoxon in milk. Therefore, higher insecticide concentrations were added. Spiking organic milk with 100 μg/L paraoxon led to a mean inhibition of 90% of the multienzyme biosensor. Such high inhibition values indicate an insecticide concentration of >40 μg/L in the milk sample as an inhibition of 83% corresponds to 40 μg/L paraoxon in the calibration curve (**Figure 1**). GC-MS investigations of this milk sample resulted in 92 μg/L paraoxon.

In the case of carbaryl, the most sensitive mutant F345A resulted in 90.3 μg/L carbaryl after incubation in organic milk spiked with 100 μg/L carbaryl. The investigation with LC-MS resulted in 99 μg/L carbaryl.

All four NbAChE variants inhibited by 20 μg/L paraoxon could be reactivated with 2-PAM with a mean reactivation rate of 101%. A mean reactivation rate of 88% was obtained in the case of the milk sample containing 100 μg/L carbaryl.

Milk Analysis. The multienzyme biosensor system was applied in real food analysis. For this purpose 10 different milk samples from local markets in Stuttgart (Germany) were tested and compared with the results obtained by standard analytical methods (GC-MS and LC-MS) at the CVUA Stuttgart (see **Table 4**).

All milk samples, except the organic milk with 3.5% fat, caused an inhibition of >10% of at least one AChE variant. Two milk samples of the same brand, which differed only in their fat content, caused inhibition rates of up to 82% with the mutant M301A. The double-mutant M301A/W303A was highly inhibited in both samples, too. All inhibited NbAChEs in these biosensor measurements could be reactivated with reactivation rates of at least >50%, which is a clear sign for the presence of OPs or carbamates. Another batch of the 1.5% fat milk sample from the same brand resulted in lower inhibition rates. In this case the mutant M301A and the double mutant showed inhibitions just above 10% that could be 100% reactivated. In the remaining seven milk samples the inhibition rates of the NbAChEs were lower and did not exceed 30%. NbAChE inhibitions of >10% were all well reactivatable, indicating AChE inhibitors in the milk samples in small amounts.

The investigations at the CVUA Stuttgart mainly confirmed the biosensor results. With GC-MS or LC-MS measurements no pesticides were detected in the organic milk sample in agreement with the biosensor test. In contrast, tebufenpyrad (4 and 2 μg/L, respectively), tetraconazole (4 μg/L), and bifenthrin (2 μg/L) were found in the two milk samples of the same brand with 3.5 and 1.5% fat. These samples also caused the highest inhibition with the biosensor tests. With standard analytical methods no pesticides were detected in the remaining samples.

DISCUSSION

In the present study a previously described multienzyme biosensor with high sensitivity toward a broad spectrum of OPs and carbamates was applied for insecticide detection in milk as a representative of food of animal origin. The biosensor enabled the detection of paraoxon and carbaryl in milk with high sensitivity and accuracy. The analysis of spiked organic milk samples showed a good reproducibility even at very low inhibitor concentrations. The recovery rates lay well in the required range between 70 and 110% regulated by law in the European Union in 1997 (*7*). The detection limits for paraoxon and carbaryl in milk behaved as expected according to the calibration curves in buffer. Diluting the samples with phosphate buffer and adding an additional washing step with Tween-20 eliminated matrix effects, and no unspecific inhibitions were observed.

Table 3. Validation of the Multienzyme Biosensor System in Milk against GC/LC-MS Methods: Inhibition of WT NbAChE and Mutant NbAChE M301A, M301A/W303A, and F345A after Incubation in Paraoxon/Carbaryl-Spiked Organic Milk[a]

	biosensor test								GC/LC-MS
	AChE inhibition (%)				calcd insecticide concn (μg/L)				insecticide concn (μg/L)
spiked milk sample	WT	M301A	M301A/ W303A	F345A	WT	M301A	M301A/ W303A	F345A	
20 μg/L paraoxon	52	68	61	32	19.5	19.1	17.7	25.2	nd
100 μg/L paraoxon	93	95	89	81	>40	>40	>40	>40	92
100 μg/L carbaryl	8	17	11	28	98.5	74.5	80.7	90.3	99

[a] The corresponding insecticide concentration was calculated using the calibration curve for paraoxon and carbaryl in milk (**Figures 1** and **2**). Paraoxon was detected by GC-MS, whereas carbaryl was detected by LC-MS. WT, wild-type NbAChE; nd, not detected.

Table 4. Analysis of Real Milk Samples with the AChE Biosensor Test and with GC/LC-MS[a]

	AChE inhibition (%)				AChE reactivation (%)				GC/LC-MS
milk sample	WT	M301A	M301A/W303A	F345A	WT	M301A	M301A/W303A	F345A	
organic milk 3.5% Heirler Cenovis (Radolfzell, Germany)	0	0	1	2	0	0	0	0	nd
Milfina 3.5% (Aug 2003), Aldi (Mühlheim, Germany)	63	82	78	40	79	89	61	54	4 μg/L tebufenpyrad 4 μg/L tetraconazole, 2 μg/L bifenthrin
Milfina 1.5% (Aug 2003), Aldi (Mühlheim, Germany)	66	82	80	37	67	82	86	57	2 μg/L tebufenpyrad
Milfina 1.5% (Nov 2003), Aldi (Mühlheim, Germany)	35	14	11	12	32	100	100	67	nd
Milchwerke Oder-Spree 3.5% (Beeskow, Germany)	7	9	15	7	20	100	100	0	nd
Alpenmilch 3.5% Weihenstephan (Freising, Germany)	29	20	15	5	100	56	100	0	nd
Bergbauern 1.5% Dairy Berchtesgadener Land (Piding, Germany)	19	18	18	7	100	50	71	0	nd
Kaufland Classic 3.5% Kaufland (Neckarsulm, Germany)	−3	16	24	3	0	100	100	0	nd
Hofgut 3.5% Hohenloher dairy (Schwaebisch Hall, Germany)	11	9	13	6	75	61	100	83	nd
Mibell 3.5% Edeka (Offenburg, Germany)	8	16	24	15	60	56	72	83	nd

[a] WT, wild-type NbAChE; nd, not detected.

Validation experiments against standard chromatographic methods confirmed the reliability of the biosensor test. The recovery rates of paraoxon and carbaryl in spiked milk samples were close to 100% both for the biosensor test and for the GC/LC-MS analysis. Furthermore, 20 μg/L paraoxon could be found with the biosensor, whereas GC-MS investigations could not detect paraoxon in such small concentrations.

The analysis of milk samples from local stores in Germany caused in 2 of 10 cases an almost quantitative inhibition of some AChE variants on the multienzyme biosensor. The inhibited enzymes were clearly reactivatable with 2-PAM, indicating an inhibition caused by OPs or carbamates (*30*−*32*). In these two samples small amounts of tebufenpyrad, tetraconazole, and bifenthrin were detected with GC/LC-MS. Tebufenpyrad and bifenthrin are used as insecticides, but they are not AChE inhibitors, whereas tetraconazole is applied as a fungicide. The detection of several insecticides in these samples indicates the presence of other insecticides in general. The drawback of the standard analytical methods is their restricted number of pesticides covered in one measurement and their lower sensitivity toward some insecticides compared to the biosensor test. Therefore, the presence of other not frequently used insecticides in these samples leading to high NbAChE inhibitions is likely. With standard analytical methods no pesticides were found in the remaining samples. This confirms the low inhibition rates obtained with the biosensor test, indicating small AChE inhibitor concentrations that cannot be detected by GC/LC-MS.

The biosensor has been shown to detect all AChE inhibitors in one measurement as a sum parameter, even with high sensitivities. This is a clear advantage over GC/LC-MS analysis for certain applications such as the screening for especially harmful neurotoxins in food that is consumed by vulnerable groups. On the other hand, it shows the limitations of such a biosensor test, which gives no quantitative or substance-specific information about the type of pesticides. Therefore, for legal applications the biosensor test has to be combined with standard analytical methods to get the required information about active ingredients.

The results obtained in the milk analysis are in agreement with former monitoring reports about the presence of organophosphates and carbamates in cow's milk (*3*−*5*). A study conducted in Mexico showed that ∼40% of the analyzed milk samples contained detectable levels of organophosphates (*1*). Eight of 96 analyzed samples contained residues exceeding established maximum residue limits, and 5 of these samples exceeded the ADI of children by up to 17 times. These and other findings are a clear argument for the establishment of a fast prescreening test for neurotoxic organophosphates and carbamates, as described in this work. This would enlarge the number of tested milk samples and increase consumer safety.

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