Supplementary Materials

Planarian cholinesterase: *in vitro* characterization of an evolutionarily ancient enzyme to study organophosphorus pesticide toxicity and reactivation

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Supplemental Tables.

Table S1. Calculated K_d ranges converted from IC_{50} values for reversible inhibitors

	Substrate			
Inhibitor	*0.1 mM ATCh*	*1 mM ATCh*	*0.1 mM BTCh*	*1 mM BTCh*
Ethopropazine	5.1×10^{-5}	1.4×10^{-4}	3.4×10^{-5}	1.2×10^{-4}
BW284c51	1.1×10^{-5}	3.5×10^{-6}	8.6×10^{-6}	5.0×10^{-6}
Donepezil[b]	8.9×10^{-5}	6.9×10^{-5}	ND[a]	ND[a]
Edrophonium	1.7×10^{-5}	1.7×10^{-5}	ND[a]	ND[a]
Tacrine	3.4×10^{-5}	1.6×10^{-4}	ND[a]	ND[a]

[a]ND (not determined)
[b]Since inhibition was not found to be reversible, $K_d = IC_{50}$

Table S2. Reported IC_{50} (M) values of reversible inhibitors for human AChE

Inhibitor	**IC_{50} (M)**	**References**
Ethopropazine	$1.8\text{-}2.6 \times 10^{-4}$	(Atack et al. 1989)
BW284c51	$1.8\text{-}2.8 \times 10^{-8}$	(Giacobini 2000; Giacobini 2001)
Donepezil[b]	5.7×10^{-9}	(Giacobini 2000)
Edrophonium	$2.5\text{-}5.7 \times 10^{-6}$	(Atack et al. 1989)
Tacrine	$1.8\text{-}2.4 \times 10^{-7}$	(Giacobini 2000; Giacobini 2001)
Propidium[a]	3.0×10^{-7}	(Taylor and Lappi 1975)

[a]K_d value for *Torpedo californica* AChE

Supplemental Figures

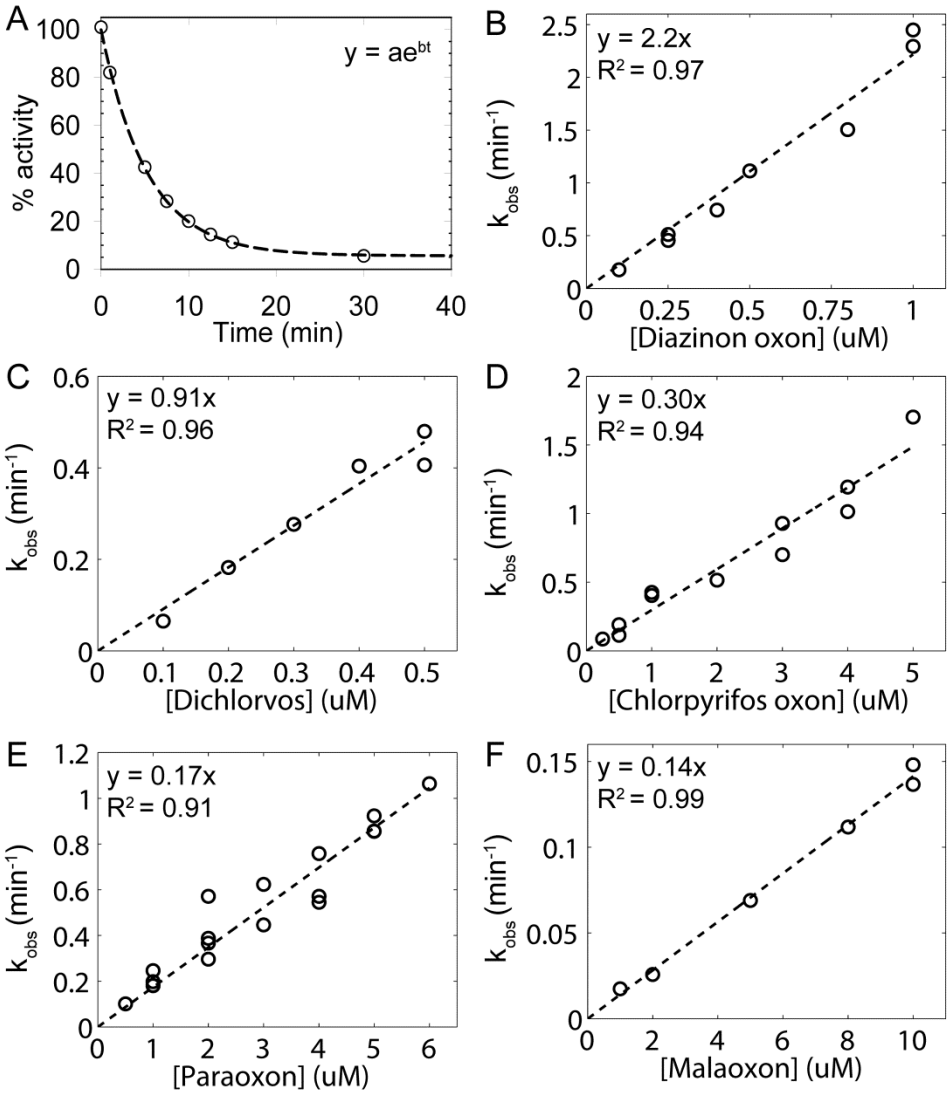

Fig. S1 Rates of inhibition by OPs. **a** Raw inhibition values obtained over time from inhibition with 1 μM chlorpyrifos oxon shown as an example. Percent activity values over time were fit by an exponential decay regression, equation shown, and the parameters a and b (k_{obs}) were determined using SigmaPlot software. This was repeated for each experiment on the varying concentrations of inhibitors. **b-f** For each OP (diazinon oxon (**b**), dichlorvos (**c**), chlorpyrifos oxon (**d**), paraoxon (**e**), and malaoxon (**f**)), k_{obs} vs concentration was plotted and the bimolecular rate constant (k_r) was determined as the slope of the linear regression. Regression equations and R^2 values for each fit are shown

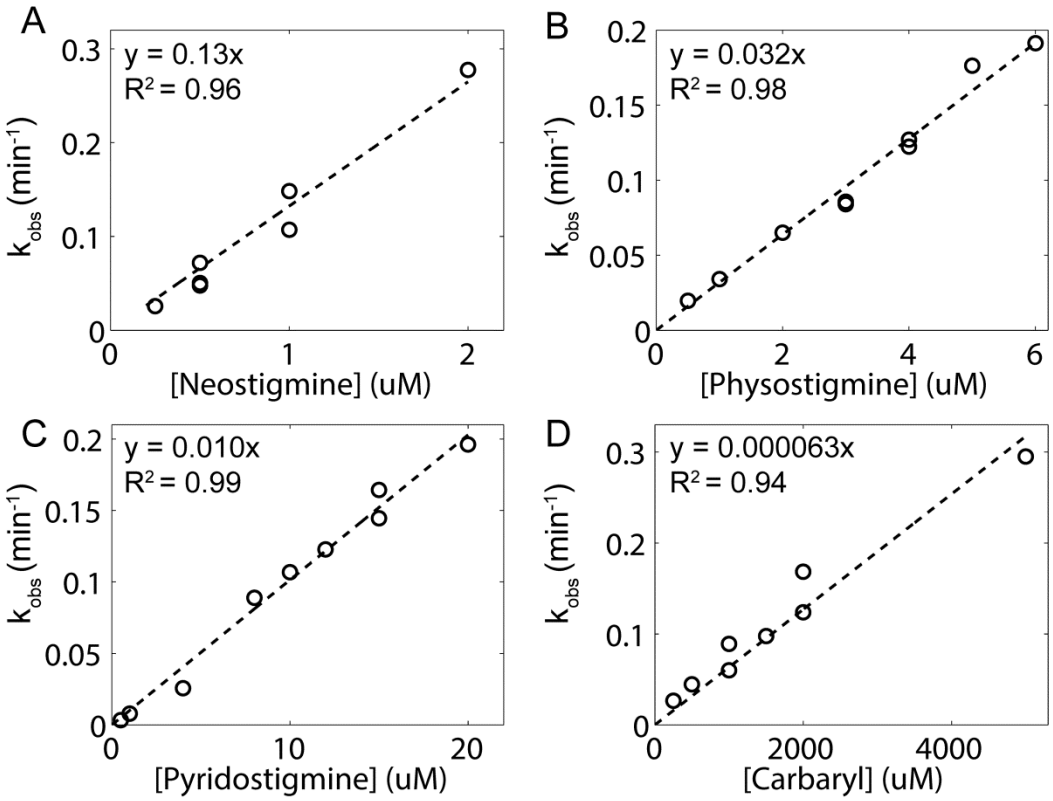

Fig. S2 Rates of inhibition by carbamylating agents. For the carbamates **a** neostigmine, **b** physostigmine, **c** pyridostigmine, and **d** carbaryl, k_{obs} (as calculated in Materials and Methods and Fig S1) was plotted as a function of concentration and the bimolecular rate constant (k_r) was determined as the slope of the linear regression. Regression equations and R^2 values for each fit are shown

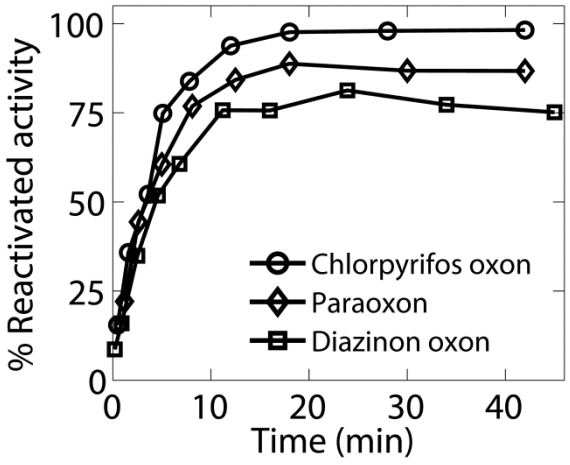

Fig S3 Reactivation rates are similar for OPs forming the same diethylphosphoryl DjChE conjugate. DjChE inhibited by either 1 μM chlorpyrifos oxon (circles), 5 μM paraoxon (diamonds), or 1 μM diazinon oxon (squares) was reactivated by 100 mM NaF. Percent reactivated activity is based on an uninhibited control measured several times over the course of reactivation. The k_{obs} for each OP were found to be similar at 0.25, 0.25 and 0.26 min^{-1} for chlorpyrifos oxon, paraoxon, and diazinon oxon, respectively