Microfluidic biosensor for the continuous enzymatic detection of organophosphorus compounds

J. Lachaux[1]*, C. Mairaville[1], M. Ben Frej[1], H. Volland[1], S. Simon[1] and K. Perez Toralla[1]

[1] Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (MTS), Gif-sur-Yvette, 91191, France
*julie.lachaux@cea.fr

The use of pesticide organophosphate (OP) has serious consequences for the contamination of soil and ground waters. Because of their high toxicity, there is a negative impact on aquatic wildlife and potentially also for human health. In particular, these compounds can inhibit acetylcholinesterase (AChE), blocking neurotransmission in the central nervous system. As compared to other OP detection techniques, measuring the activity of AChE and its inhibition in the presence of OP can provide a more rapid, sensitive and specific response. Although the development of AChE-based microfluidic devices has made significant progress in the last few years [1], only few systems can continuously analyze the presence of OP in circulating water (most providing only one result per time-point for each sample). We demonstrate here an integrated AChE-based biosensor allowing the continuous, on-site detection of liquid OP compounds, which could be used for water environmental monitoring.

In this work, we implemented an AChE inhibition colorimetric test based on the use of acetylthiocholine (substrate) and Ellman's reagent [2] to determine the half-maximal inhibitory concentration (IC50) of commercial OP pesticides (malaoxon) in aqueous solutions. A specific experimental bench (Fig 1.A) has been set-up in order to implement the enzymatic inhibition test in droplets-based microfluidic. Two off-chip incubation steps of 30 min each (Figure 1.A, steps 1 and 2) are performed in a continuous flow. Droplets generated by the injection of an oil flow at the T-junction (Figure 1.A, step 3) are analyzed continuously by microscopy through an observation chamber (Figure 3.A, step 4). Three inhibition experiments were performed at IC50 ($3{,}5.10^{-9}$ M for the malaoxon) with an analysis of the yellow signal intensity. Graph shows a significant variation in signal intensity in the presence of OP, showing inhibition of enzyme activity (Figure 3.B). The variation of yellow signal intensity can also be visually observed in microscope images (Figure 3.B). Under these conditions, we estimate that the microfluidic system has a very good sensitivity of $3{,}5.10^{-9}$ M for malaoxon. More experiments will be carried out to test a larger pesticides panel and concentration range. It is therefore a promising miniaturized device in term of liquid organophosphorus detection while permitting a continuous flow analysis.

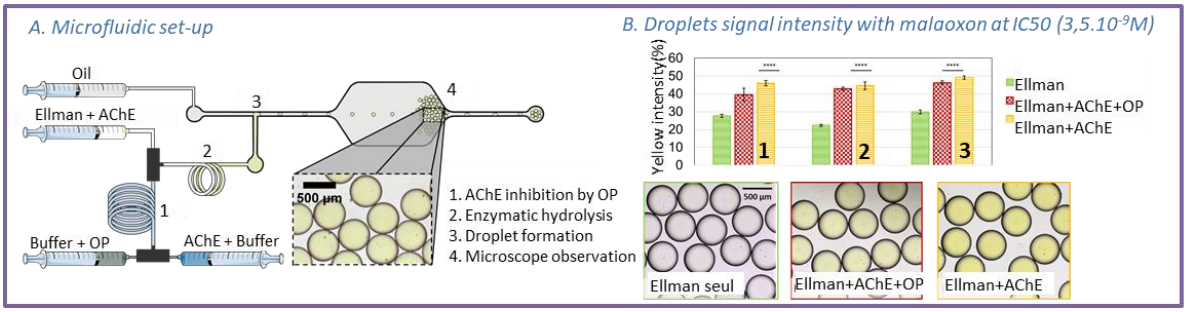

Figure 1: Experimental set-up of enzymatic inhibition in droplets-based microfluidic (A), and the results of yellow signal intensity for 3 experiments with malaoxon at IC50 (B).

References

[1] M. Bhattu, M. Verma, D. Kathuria: *Recent advancements in the detection of organophosphate pesticides: a review*. Anal. Methods **13**, 4390 (2021)

[2] G.L. Ellman, K.D. Courtney, V. Andres, R. M. Feather-Stone: *A new and rapid colorimetric determination of acetylcholinesterase activity.* Biochemical Pharmacology **7**, 88-90 (1961)