



Development of enzymatic biosensors to detect biocides disinfectants to strengthen self-monitoring in food industry

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Introduction

A wide variety of biocides disinfectants are used on a daily basis throughout the food chain to limit the development of undesirable microorganisms present in the environment or on surfaces in contact with food intended for human or animal consumption. Following these treatments, residues of biocidal products may persist on the surfaces, in particular if these residues are not completely eliminated during the rinsing operations. It has also been proven that their transfer to food is possible.

The development of biosensors for the detection of quaternary ammonium compounds (QACs) in particular, represents a promising way to explore for self-monitoring in the field, but most of this research remains limited.

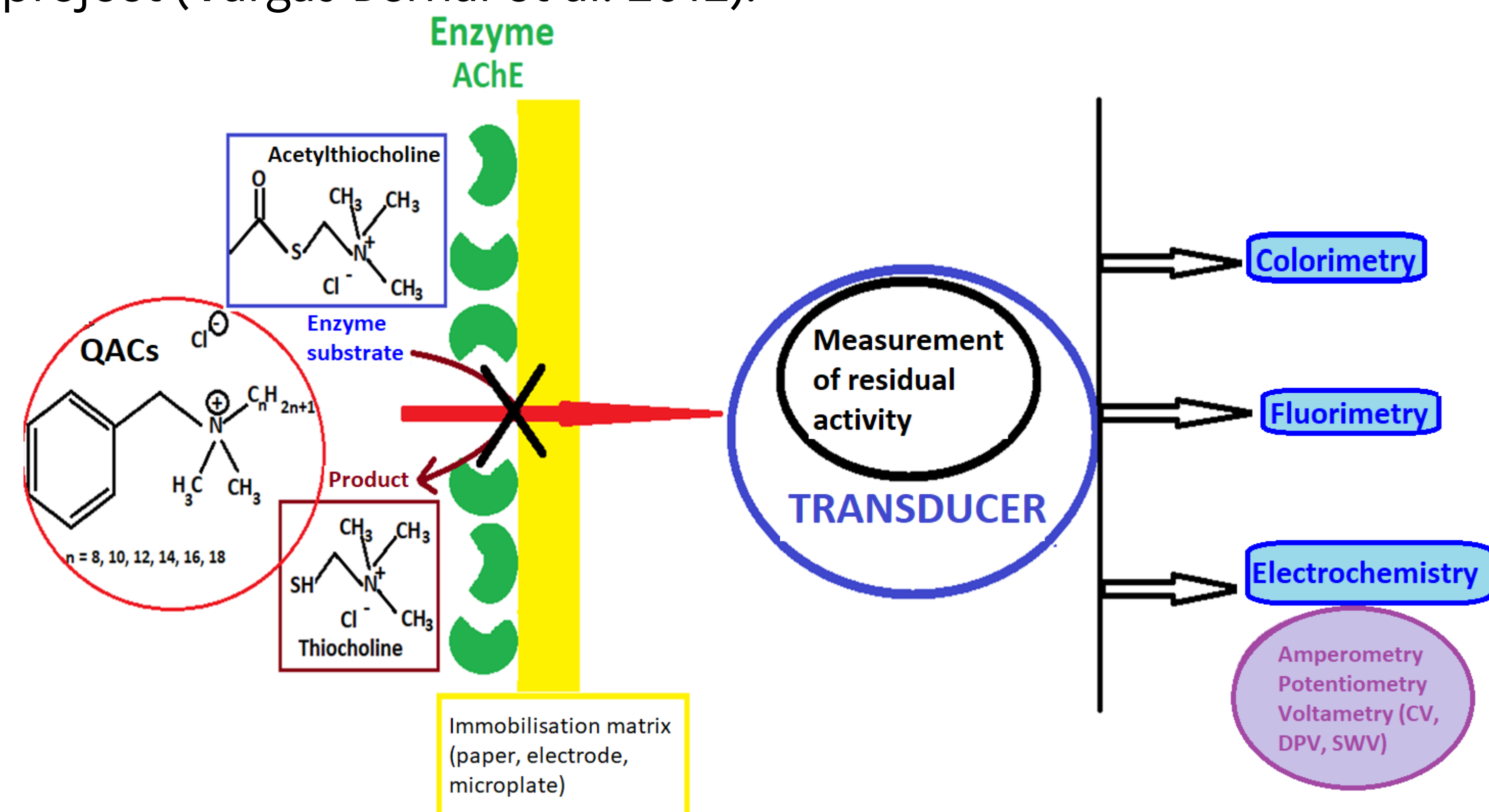
Inventory of kits

Self-checking in IAA is very limited to date and uses insensitive methods. The performances announced by the manufacturers are not subject to any evaluation or official validation. An inventory of test kits for the detection of residues of biocides disinfectants enabled us to show the few commercial methods available for self-monitoring. In the context of collaborative projects, we have established that the detection limits are high, in the order of 0.5 to more than 2000 mg / kg, i.e. 1000 times less sensitive than methods based on mass spectrometry (Soumet et al. 2020). In addition, these methods are not very selective (eg. pH, conductivity) or too specific (eg. ELISA).

Despite the fact that their sensitivity thresholds are high, strip tests are the most used by IAA. This is why it is necessary to develop innovative tools that are sensitive, fast, inexpensive, portable, even online, to set up more efficient self-monitoring, and thus better protect consumer health.

Enzymatic biosensors

Biosensors have been developed for organophosphate pesticides (OPPs) in different environmental and food matrices, based on the inhibition of enzymes (eg. Acetylcholinesterase (AChE), tyrosinase (Tyr)) in the presence of inhibitors. Due to their structural resemblance to the substrate (ie. Acetylcholine) of acetylcholinesterase (AChE), OPPs cause a reversible inhibition of this enzyme since they take the place of the substrate at the level of the enzyme active site (Vargas-Bernal et al. 2012). The results of the work on pesticides make it possible to reach detection limits of the order of $\mu\text{g}/\text{kg}$, ie 1000 times more sensitive, during this project (Vargas-Bernal et al. 2012).

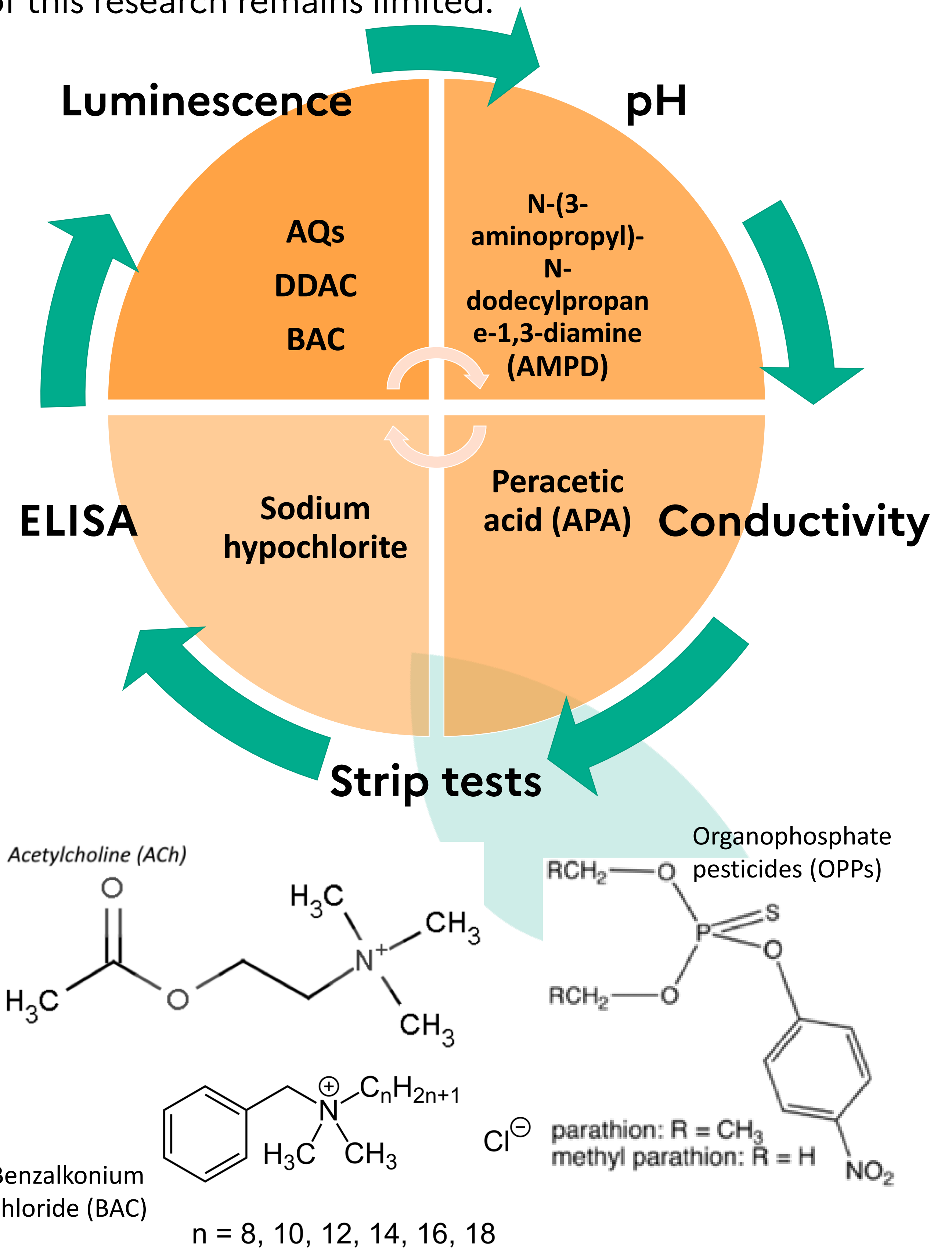


Conclusion

Very little feedback from the field on the levels of residues of disinfectant biocides remaining on surfaces in the dairy industry following cleaning and disinfection procedures exists due to a lack of simple, rapid and sensitive self-monitoring methods. We will then carry out a study of the contamination of the rinsing water through laboratory studies simulating the cleaning/disinfection protocols of the dairy industry. Finally, a preliminary consumer exposure study will be carried out through analyzes of samples of milk and dairy products.

References

- Soumet C, Valérie Gaudin V, Lancien P, Maris P, Slimani K, Rossi N, Hurtaux-Pessel D. 2020. Inventaire des méthodes et kits pour la détection des résidus de biocides désinfectants. RMT ACTIA CHLEAN. <https://www.actia-asso.eu/projets/chlean-2/>:1-6.
- Bergmann F, Shimoni A. 1952. Quaternary ammonium salts as inhibitors of acetylcholine esterase. *Biochim. Biophys. Acta.* 8:520-525.
- Vargas-Bernal R, Rodríguez-Miranda E, Herrera-Pérez G, Evolution and Expectations of Enzymatic Biosensors for Pesticides. In 2012; pp 329-356.
- Kucherenko IS, Soldatkin OO, Arkhypova VM, Dzyadevych SV, Soldatkin AP. 2012. A novel biosensor method for surfactant determination based on acetylcholinesterase inhibition. *Meas. Sci. Technol.* 23:065801.



QACs have a chemical structure similar to acetylcholine, like OPPs. The reversible inhibitory effect of certain QACs (tetraethylammonium ions) on AChE (electric eel) activity was demonstrated in 1952 by a manometric method (Bergmann et Shimoni, 1952). Since then, only Kucherenko's work in 2012 has focused on developing an electrochemical (conductimetric) biosensor based on AChE for the detection of two QACs (benzalkonium chloride (BAC), hexadecylpyridinium bromide) in aqueous solution (Kucherenko et al. 2012), while hundreds of articles have been published on the detection of pesticides in this way. So the strategies to be investigated for the detection of QACs by enzymatic biosensors are numerous (enzymes, substrates, transducers) and the recent development of nanotechnologies (i.e. Nanomaterials) makes it possible to predict a sensitivity in line with our expectations.