Effect of the succession of endosulfan exposure and bacterial challenge on DNA integrity and non specific immunity of the three-spine stickleback, Gasterosteus aculeatus

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Adrien Marchand[1], Jean-Marc Porcher[1], Cyril Turies[1], Edith Chadili[1], Stéphane Betoulle[2], Anne Bado-Nilles[1]

[1] Institut National de l'Environnement Industriel et des Risques (INERIS), UMR-I 02 SEBIO, BP 2, 60550 Verneuil-en-Halatte, France.
[2] Université de Reims Champagne-Ardenne (URCA), UMR-I 02 SEBIO, Moulin de la Housse, B.P. 1039, 51687 Reims, France.

E-mail contact: Anne.Bado-Nilles@ineris.fr

1. Introduction

Endosulfan ($C_9H_6Cl_6O_3S$) is an active substance of phytosanitary products, used as an insecticide on crops, fruits, cereal or tobacco (Antonious and Byers 1997) and found in surface water, groundwater, sediment and rain of many regions in the world and an important source of poisoning in many countries (Dar et al. 2015).

Endosulfan is known to have genotoxic and immunostimulating effects such as an increase of phagocytic capacity (Tellez-Bañuelos et al. 2011). In this way, fish weakened by chemical stress might be unable to resist again pathogen aggression. In fact, a connection has been established between the destabilization of the fish immune system caused by sub-lethal doses of contaminants and a defect in fish pathogen resistance (Shelley et al. 2012).

To test this hypothesis, we propose to evaluate the impact of the succession of two stresses, chemical (by endosulfan exposure) then biological (by injection of lypopolisaccharides, a bacterial endotoxin), on a freshwater model species, the three-spined stickleback, *Gasterosteus aculeatus* (Sanchez et al. 2007). Firstly, we tested a four days' endosulfan exposure in order to determine the effects of this pesticide on DNA integrity and innate immune parameters in our model species. Based on the outcome of first exposure the succession of stress was then performed.

2. Materials and methods

To perform endosulfan single exposure, 100 three-spined sticklebacks were randomly distributed in 10 tanks (10L) and left in semi-static acclimatization for seven days (daily water renewal). Then, fish were exposed to endosulfan dissolved in water at 14, 7, 3.5 and 1.75 $\mu g.L^{-1}$ during four days. Finally, fish were anaesthezized, sacrificed and blood and spleen samples were collected.

From the results of endosulfan single exposure, two concentrations were selected for the succession of stresses: 1.75 and 0.85 $\mu g.L^{-1}$. After seven days of acclimatization, 50 fish were exposed to each concentration or to DMSO for four days (10 fish per tank, daily water and contaminant renewal). Then, the endosulfan exposure was stopped and 10 fish of each concentration were anaesthetized and sacrificed. 20 fish of each concentration were injected with bacterial endotoxin (lipopolisaccharides, LPS) and 20 fish were injected with phosphate saline buffer (PBS, solvent control). Three days after injections, all the remaining fish were anaesthetized and sacrificed.

Non specific immune capacity of splenic leucocytes (i.e cell mortality, differential numeration of leucocyte sub-population and phagocytic activity), blood erythrocyte concentration and erythrocyte DNA breaks quantity were evaluated by flow cytometry.

3. Results and discussion

3.1. Single exposure to endosulfan

Blood samples of fish exposed to 1.75 and 3.5 $\mu g.L^{-1}$ of endosulfan shown a clear reduction of erythrocyte concentration. In addition, after four days exposure at 1.75 $\mu g.L^{-1}$ of endosulfan, an increase of erythrocyte DNA breaks quantity was observed. The results suggest concentration-dependent and non-linear time dependant manifestation in genetic damage expression.

Concerning non specific immunity, fish exposed to 1.75 µg.L^{-1} and 3.5 µg.L^{-1} shown an activation of the phagocytic capacity in both adhesion and ingestion components, compared to DMSO control. In addition, fish exposed to 1.75 µg.L^{-1} shown an inhibition of their reactive oxygen species production, but an increase in cell mortality by necrosis. These results show that endosulfan may have the ability to stimulate spleen leucocytes of three-spined stickleback. Endosulfan has endocrine disruptors properties and may mimic cytokine pro-inflammatory signal by fixation on oestrogenic receptors present on fish leucocyte membrane (Sekine et al. 2004, Iwanowicz et al. 2014). The reduction of ROS production and the absence of effect on respiratory burst may indicate a reduction of leucocyte oxydative stress. In contrary some literature studies have found an increase of oxydative stress in fish exposed to endosulfan (Dar et al. 2015).

3.2. Succession of endosulfan exposure and bacterial challenge

LPS injection, without pre-exposure to endosulfan, caused an enhancement in erythrocyte concentration, but no effect on DNA breaks quantity. But when fish were pre-exosed to endosulfan, an increase in DNA breaks quantity is observed, more important at the highest endosulfan pre-exposure concentration. These results suggest that single LPS injection couldn't induce DNA breaks. But, if the injection was preceded by four days of endosulfan exposure, an increase in DNA breaks was observed.

Concerning non-specific immune parameters, an important reduction of phagocytic capacity to engulf pathogen is observed in fish pre-exposed to 0.8 µ.L^{-1} of endosulfan and injected with LPS. The same thing is observed in fish pre-exposed to 1.75.µg.L^{-1} and injected both with PBS and LPS. In addition, an increase of cell mortality by apoptosis is observed at the same conditions. Indeed, endosulfan could mimic interleukine-6, a cytokine involved into the MAP-kinase apoptotic pathway (Sekine et al. 2004). The injection of LPS leads to the inflammatory process and release of pro-inflammatory cytokines. The combination of the two stresses may have provoked an activation of MAP-kinase apoptotic pathway by interleukin releases, leading to massive leucocytes apoptosis.

4. Conclusions

This study point out genotoxic and immunostimulating potential of endosulfan in three-spined stickleback. When chemical exposure was followed by a bacterial challenge, it is possible to observe a destabilization of non specific immunity with a drastic reduction of phagocytic capacity and an augmentation of splenic leucocyte mortality by apoptosis. In addition, genotoxic effect was observed after LPS injection only with endosulfan pre-exposure. This study points out the importance of stress on stress experiments to determine all potential effects of chemical exposure in fish.

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