Ecotoxicological consequences of pharmaceutical facility discharges on wild fish : a french case of study

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1. Introduction

Discharges of wastewater treatment plants receiving effluents from pharmaceutical factories represent a non-negligible source of biologically active chemicals in surface waters able to induce ecological/ecotoxicological effects on wildlife and populations. Recently, ecotoxicological effects on fish [1][2][3] and tadpoles [2] exposed to dilutions of Indian bulk drug manufactures effluents have been concluded, suggesting that deleterious biological effects could be induced. Furthermore, an *in situ* study has demonstrated a high proportion of intersex in gudgeons living downstream from a French bulk drug manufacture discharges which could be explained by the nature of pharmaceutical production [4]. The present work consists of an evaluation of long-term adverse effects induced by another French pharmaceutical manufacture discharges in wild fish using a multi biomarker approach.

2. Materials and methods

A pharmaceutical manufacture was selected due to unfavorable criteria (bulk drug production, high volume discharges, small size river, only on-site sewage treatments). During autumn 2012 & 2013, two samplings of 20 three-spined sticklebacks (*Gasterosteus aculeatus*) living in receiving waters, were caught by electrofishing, and were comparatively studied to sticklebacks living in a 'reference site' nearby.

A set of complementary biochemical and histological analysis was measured in all sampled fish. General health status was evaluated by Fulton's condition factor and splenic macrophages aggregates densities. Hepatotoxicity was studied by histopathological semi-quantitative evaluation, activities of biotransformation hepatic enzymes (EROD, CYP 3A and, GST), and oxidative stress consequencies (lipoperoxidation as TBARS). Endocrine disruption was evaluated by intersex frequency and plasmatic vitellogenin (VTG) concentration. Genotoxicity was investigated by analysis of DNA damages on erythrocytes using flow cytometry analysis, and neurotoxicity was estimated by acetylcholinesterase activities (AchE) in muscle.

3. Results and discussion

3.1. Histopathological results

The histopathological semi-quantitative results showed a marked hepatotoxic effect in sticklebacks living downstream pharmaceutical discharges (DPD) mainly characterized by fibrosis, subnormal lipidic vacuolization (steatosis and lipidic degeneration), and a high proportion of altered parenchyma. Splenic macrophage aggregate density (MMA) was highly increased in fish from DPD that suggest an alteration of general health status.

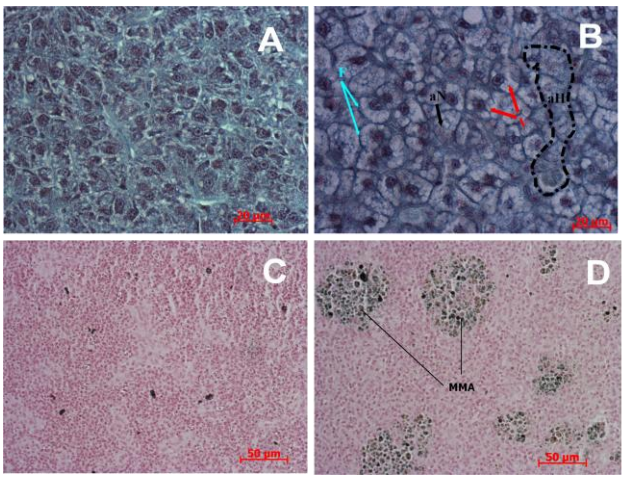

Figure 1 : Histological analysis of liver (A-B) and.spleen (C-D) in reference (A-C) and contaminated site (B-D).Liver: Fast Red & picro-indigo-carmin stain. Spleen: Perls' stain. (F) =fibrosis; (An)=altered nucleus; aH=altered hepatocytes ; (V)= lipid droplets; (MMA)=melanomacrophages aggregates.

	Reference site	DPD
Fulton's condition factor	**0.94 ± 0.07**	**1.01 ± 0.15**
Liver		
Vacuolisation score (0-10)	**2.5 ± 0.7**	**7.5 ± 0.8**
Fibrosis (0-3)	**0.5 ± 0.5**	**2.0 ± 0.3**
Altered parenchyma (0-10)	**0.43 ± 0.5**	**1.5 ± 0.7**
Nuclear alteration (0-20)	**13 ± 1.5**	**10.1 ± 1.6**
Spleen		
Surface MMA ratio %	**0.65 ± 1.4**	**7.06 ± 8.06**
Gonads		
Intersexe	**Ø**	**Ø**

Table 1: Histopathological semi-quantitative analysis. Values correspond to mean (n=20) ±SD. (DPD)=downstream pharmaceutical discharges.

3.2. Biochemical and DNA damages results

Inductions of hepatic biotransformation enzymes activities (EROD, GST, CYP 3A) indicate a significant elevation of liver functioning which could confirm histological lesions identified. However neither significant differences in TBARS levels (indicating lipid peroxidation in liver) nor in AchE activity in muscle were noticed. Finally, elevated levels of DNA damages in erythrocytes were identified in sticklebacks living in DPD site.

	Reference site	DPD
EROD (pmol/mg/min)	**0.84 ± 1.58**	**47.21 ± 27.30**
GST (U/g)	**1234 ± 620**	**2660 ± 648**
CYP 3A (U/mg)	**3.11 ± 2.10**	**11.50 ± 4.97**
TBARS (nmol/g)	**19 ± 16**	**10 ± 7**
AchE (U/g)	**58 ± 23**	**51 ± 19**
DNA damages (CV)	**0.17 ± 1.03**	**3.37 ± 0.43**

Table 2: Biomarkers responses and DNA damages on erythrocytes for G.aculeatus, collected in a reference site and downstream pharmaceutical discharges.

4. Conclusions

This work emphasizes the interest of a multi-biomarker approach to monitor ecotoxicological effects induced by industrial discharges. These complementary tools provide additional elements which highlight the possibility of deleterious impacts on fish health causing by drug production.

5. References

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