

**DISRUPTION OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS
IN TELEOST FISH AND AMPHIBIANS :
TOXICOLOGY AND COMPARATIVE PHYSIOLOGY MEET**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

There is substantial evidence that some of the chemicals used in industrial processes, pulp and paper production, waste water treatment and agriculture become bioavailable to aquatic species and interfere with their normal endocrine function. Exposures to metals or organic pollutants such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls impair the capacity of fish to secrete cortisol in response to adrenocorticotrophic hormone (ACTH) or to a physical confinement (Hontela 1997). To establish mechanism-based links between exposure to environmental pollutants and health effects in wildlife species exposed chronically in the environment, our research group has developed complementary experimental approaches, in the field as well as in the laboratory. Ecotoxicological field studies together with mechanistic toxicological laboratory experiments are used to identify chemicals with the capacity to disrupt the hypothalamo-pituitary-adrenal (HPA) axis, compare the adrenotoxic potential of these chemicals, and elucidate their mechanisms of action (Benguira and Hontela 2000; Benguira et al. 2002; Bisson and Hontela 2002). The objectives of this study were to quantitatively assess the impact of pesticides on adrenal steroidogenesis in fish and amphibians, and to identify the intracellular sites of action.

Materials and methods

Adrenal cells were isolated from rainbow trout, *Oncorhynchus mykiss*, yellow perch, *Perca flavescens*, and two amphibian species, the south african clawed frog, *Xenopus laevis*, and the bullfrog, *Rana catesbeiana*. Cells were incubated *in vitro* in presence of various test chemicals (cadmium and a series of pesticides, edosulfan, diazinon, mancozeb, atrazine, *o,p'*-DDD) and the secretory capacity of the cells was subsequently tested with ACTH, dbcAMP and pregnenolone). The cell viability as well as antioxydant defense capacity (GSH, catalase, lipid peroxydation) were also measured.

Results and discussion

Concentration-dependant effects on cell viability and the secretory capacity of the adrenal cells were observed for all the test pollutants, except for atrazine in *Xenopus* and the trout. The toxicological characteristics of the test chemicals were determined by the LC50 (lethal concentration that kills 50% of the adrenal cells) and EC50 (effective concentration that inhibits cortisol secretion by 50%). Toxicants with a strong capacity to disrupt cortisol secretion without causing cell death (high ratio LC50/EC50) were identified (ex. cadmium) as well as nonspecific toxicants that are highly cytotoxic (ex. diazinon, ratio LC50/EC50 about 1). Important differences in sensitivity to the toxicants between rainbow trout, a model teleost (Table 1) and other animal species investigated were also revealed. Disruption of the signalling pathways leading to cortisol as well as an imbalance in the prooxidant-antioxidant balance was observed, in a concentration-dependant pattern, in cells exposed to selected test pesticides. Endosulfan disrupted cortisol synthesis following acute *in vitro* exposures and while catalase activity was elevated at non-cytotoxic doses of endosulfan, activity of GPx was decreased, GST did not change and glutathione (GSH) levels were depleted. An increase in lipid peroxidation (LPO) was also detected. To investigate the effects of pesticides on the signalling pathways, *in vitro* experiments with *o,p'*-DDD and atrazine were completed. Exposure to *o,p'*-DDD induced a dose-dependant loss of the capacity to secrete cortisol. Stimulation with dbcAMP restored cortisol secretion while the actions of forskolin on cortisol secretion and cAMP production were blocked by *o,p'*-DDD but not atrazine. NaF-induced increase in cAMP production was also blocked by *o,p'*-DDD. These results suggest that adenylate cyclase is a target in *o,p'*-DDD mediated disruption of steroidogenesis and that atrazine is not an adrenotoxic chemical in teleost corticosteroidogenic cells. Our results increase the understanding of the mechanisms of action of environmental pollutants

within the HPA axis, and provide a mechanistic link between exposure and physiological effects, both important aspects Environmental Risk Assessment.

References

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Table 1. Adrenotoxicity (LC50, EC50 and LC50/EC50) of chemicals tested in adrenal cells of rainbow trout

Toxicant	Viability LC50 (μM)	ACTH-stimulated cortisol production EC50 (μM)	LC50/EC50
Atrazine	>50000	>50000	—
CdCl ₂	10800	168	64.29
ZnCl ₂	22800	355	64.22
Mancozeb	>5000	312	>16.04
Endosulfan	405	38	10.66
CH ₃ HgCl	1140	116	9.83
HgCl ₂	199	22.3	8.92
<i>o,p'</i> -DDD	385	130	2.96
Diazinon	305	233	1.31