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Poecilia reticulata as a valuable biological indicator of endocrine disruption

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Abstract. Natural and man-made compounds that interfere with the endocrine system activity are termed endocrine disruptors (ED). Endocrine disruptors may induce either hormone agonist or hormone antagonist effects in human and wildlife by interference with the biosynthesis and biological action of endogenous hormones. The biological response of an organism exposed to an ED which is able to determine quantifiable alterations at the molecular, cellular or physiological level, represents a potential biomarker of exposure to ED. In vertebrates, the mechanisms and metabolic pathways modulated by hormones are genetically conserved. Therefore, endocrine disruption induced by a chemical compound in a taxon will, presumably, result in similar effects in other species. In view of the easy breeding and keeping facilities, their rapid reproduction rate, the short time interval between generations and their ecological relevance, a series of fish species are studied as biological indicators. Several toxicological tests are elaborated in these species, testing for endocrine-disrupting effects of chemical compounds. Some of these fish are members of the family *Poeciliidae* and particularly important is *Poecilia reticulata*.
Keywords: *Poecilia reticulata*, endocrine disruption, indicator biologic, biomarker.

Rezumat. Compuşii naturali şi sintetici care interferă cu activitatea sistemului endocrin, generand efecte agoniste sau antagoniste hormonale la om sau animale, efecte realizate prin interferenţa cu biosinteza sau acţiunea hormonilor endogeni, sunt denumiţi disruptori endocrini (DE). Reacţia sesizabilă în răspunsul biologic al unui organism expus la DE, tradus prin modificări cuantificabile la nivel molecular, celular ori fiziologic constituie un potenţial biomarker al expunerii la DE. La vertebrate, mecanismele şi căile metrabolice modulate de hormoni sunt conservate genetic. Prin urmare, dacă un compus chimic generează efecte de disrupţie endocrină la un taxon, este de aşteptat, ca efecte similare să fie generate şi la alte specii. Datorită condiţiilor facile de creştere şi întreţinere, a reproducerii uşoare, a intervalului între generaţii redus şi a relevanţei ecologice, o serie de specii de peşti au fost luate în studiu, elaborându-se teste toxicologice destinate testării funcţiei de DE a unor compuşi chimici. Printre speciile cercetate se numără şi binecunoscutele Poeciliidae, mai ales *Poecilia reticulata.*
Cuvinte cheie: *Poecilia reticulata*, disruptor endocrin, indicator biologic, biomarker.

Introduction. Researches on *Poecilia reticulata* (guppy fish), one of the most popular freshwater fish species all over the world, in the field of endocrine disruption are driven by characteristic biological and growth features of this species, allowing the identification of developmental endpoints and objective biomarkers to test for endocrine disrupting chemicals (EDC). Guppies are ease to care for, they exhibit a marked sexual dimorphism, are viviparous and reproduce easily. In addition, the time interval between generations is short and sexing in the guppy fish is a rather facile process (Petrescu-Mag 2009). Moreover, *Poecilia reticulata* males present a reproductive organ called gonopodium, which is a modified anal fin located directly behind the ventral fin. It can be easily detected thus sperm collection in guppies is not difficult. Males exhibit an intens, unique coloration, and a characteristic courtship behaviour, both features tightly controlled by androgen hormones.

Endocrine disruptors (ED) interfere with endogenous hormones biological effects, either directly as endocrine receptor agonists or antagonists, or indirectly by affecting the activity of enzymes involved in hormones synthesis and biological action (Georgescu et al 2004; Georgescu et al 2005). Behaving as a ligand for hormone receptors, an EDC may act either as a hormone agonist or a hormone antagonist. In agonist-like effects, the xenobiotic compound stimulates the receptor activity resulting in effects that are identical, although of much weaker intesity in comparison to the natural endocrine ligand. The biological activity of a xenobiotic is directly related to its ability to bind and activate the endocrine receptor. In contrast, in antagonist-like effects, the xenobiotic compound blocks or inhibits the endocrine receptor and the biological activity of the endogenuous hormone. Receptor inhibition may be accomplished by a competitive mechanism when the endogenous hormone and the EDC bind to the same situs on the hormone receptor, or by a non-competitive mechanism that is the xenobiotic binds to the endocrine receptor in a place which is different from the situs that binds the endogenous hormone. Despite that, the xenobiotic exerts hormone antagonist effects.

Indirect effects of EDC with the endocrine system have been acknowledged. Some xenobiotics inhibit the activity of several enzymes involved in hormones biosynthesis. One of these enzymes is aromatase, able to convert androgen into estrogen. Interference of EDC with aromatase activity will result in low estrogen synthesis and unbalanced estrogen-androgen activity with masculinization of female animals.

One additional mechanism of action of EDC is the interference with the activity of cytochrom P450 enzymes, which are key players of the biosynthesis and liver metabolization of steroid hormones.

Natural hormones are released by endocrine glands into the bloodstream and transported bound to albumin or binding globulins. Some EDC have the capacity to bind transport proteins thus increasing the free fraction of the hormone which is at the same time the active fraction, able to bind to the endocrine receptor. Eventually, another mechanism of action of EDC consists of downregulation of endocrine receptors in peripheral cells and tissues (Georgescu et al 2006).

The major contributing effect of several endocrine-regulated metabolic pathways in growth, development and reproduction are well-recognized; therefore, these may be considered as endpoints of adverse effects generated by exposure to a xenobiotic with endocrine-disrupting effects. Moreover, exposure biomarkers have been identified in indicator species to attest for changes in endocrine pathways involved in the process of endocrine disruption. One biological indicator species is *Poecilia reticulata* (Petrescu-Mag et al 2011), and several biomarkers of EDC exposure have been validated in guppy fish: the gonadosomatic index, the gonads histology, vitellogenin and plasmatic steroids, secondary sexual characteristics and the male courthship behaviour.

The gonadosomatic index represents a valuable biomarker of exposure to xenobiotics exhibiting antiandrogenic effects. It implies the measurement of total body weight and testicular weight, the ratio between the two parameters being called the gonadosomatic index (GSI). A study in *Poecilia reticulata* males revealed a significantly lower GSI in fish exposed to EDC (*i.e.* p,p'-DDE, vinclozolin and flutamide) in which the GSI ranged between 1.7 and 2.0% in comparison to fish from the control group in which the testes made up about 2.8% of the body weight (Baatrup et al 2001). In another experiment, a time-dependent tendency towards a lower GSI was observed in male guppies exposed to 17β-estradiol and 4-nonylphenol but no signifcant correlation with the concentration of the xenobiotic was reported (Li et al 2005). The organophosphorus pesticide monocrotofos (dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate) induced a significant decrease in testosterone levels in *Poecilia reticulata* with correspondent inhibition of testes development and sperm numbers (Tian et al 2012).

The gonadal histology is a relevant biomarker in *Poecilia reticulata* since particular microscopic alterations may suggest intervention of EDC. In a fish exposed to a xenobiotic, markedly altered, hypoplastic or even absent Sertoli cells or lack of development of the gonadal duct represent changes associated with altered reproductive

performances in that animal, and infertility which are effects frequently observed in exposure to EDC. Pathological aspects in the gonadal histology were observed in guppy fish subsequently to exposure to 17α-etinilestradiol (Volkova et al 2012). Male guppies exposed to environmental relevant levels of tributyltin (11.2–22.3 ng L^{-1}) and bisphenol A (274–549 µg L^{-1}) developed after 21 days of exposure 40-75% lower sperm production, although no changes in gonadal histology were present nor changes of sperm form or sperm size were detected (Haubruge et al 2000).

Vitellogenin (VTG), the precursor molecule of major egg yolk proteins, is a circulating glycolipoprotein in female *Poecilia reticulata*, with an estrogen-dependent synthesis. VTG represents a sensitive biomarker of estrogen stimulation, with a rapidly-induced, important response that can be detected within hours after exposure. In male, the VTG gene is physiologically suppressed, but exposure to xenoestrogens will stimulate its expression. Therefore, measurement of the VTG protein levels by immunoassays or VTG gene expression using the qPCR technique in the plasma, liver or in tissue homogenates have emerged as valuable detection methods of both estrogen agonist and estrogen antagonist exposure effects. Besides VTG assessment, the concentration of plasma sex steroids 17β-estradiol, testosterone, 11-ketotestoste-rone may be measured.

Exposure of male guppies to a concentration of 1 µg/L 17β-estradiol and 150 µg/L 4-nonylphenol, respectively, indicated significant induction of VTG synthesis after 60 days of estradiol exposure and 7, 14, 21 days of 4-nonylphenol exposure (Li et al 2005). The effect was concentration-dependent since exposure to lower 4-nonlyphenol levels of only 10 µg/L was associated with induction of VTG synthesis after 21 days exposure time (Li et al 2005). In another study on male guppies exposed to the organophosphorus compound monocrotofos at nominal concentrations of 0.01, 0.10 and 1.00 mg/L for 90 days, from birth until adult life, a decrease of testosterone levels and significant increase of 17β-estradiol levels was obtained, with induction of VTG synthesis and feminization of male fish (Tian et al 2012).

Secondary sexual characteristics reflect reproductive parameters in fish. In *Poecilia reticulata*, males presenting large orange, intensely coloured spots develop an increased mating rate, being more easily accepted by females at courtship. Baatrup and Junge showed that the total area of orange spots and the intensity of the colour are significantly diminished after 30-days exposure to antiandrogens such as dicarboximide, vinclozolin, p,p'-DDE and flutamide (Baatrup et al 2001). Monocrotofos is another compound able to induce a decrease in the total area and intensity of orange spots in *Poecilia reticulata* males thus suggesting significant demasculinization, an effect reflected in a decreased mating rate of these specimens (Tian et al 2012).

The male courtship behaviour in *Poeciliidae* is well-known and was described in detail. The motric behaviour is easily quantified using computerized programs of image aquisition. In that sense, Baatrup developed a computerized vision system (DISPLAY) which identifies and determines complex behaviour patterns in fish as well as intraspecies interactions in guppies (Baatrup 2009). During courtship, the male will place himself in the visual field of the female fish, usually in front of her. It will bend its body in a C- or sigmoid-shape with short, quick movements of the body, trying to approach the female from the lateral side for mating. The orange colouration will intensify during the process thus increasing the males' attractiveness. Following their research, Baatrup and Junge defined this courtship behaviour as highly species characteristic and proposed it as a potential relevant biomarker for the evaluation of endocrine-disrupting effects (Baatrup et al 2001). Aquatic EDC may influence aromatase activity and thus the estrogen levels in the nervous system of the fish resulting in detrimental effects on the courtship behaviour of male guppies. In male fish, exposed for 2 weeks to 10 ng/L and 50 µg/L of 17α-ethinylestradiol (EE_2), it was evidenced that even short-term exposure may stimulate the aromatase activity in the brain. Likewise, in another study in male *Poecilia reticulata,* the reproductive behaviour was importantly affected after exposure to EE_2 20 ng/L, an effect associated to alterations in brain aromatase activity (Volkova et al 2012).

Therefore, it is concluded that the fish brain aromatase activity may be considered as a valuable biomarker for the identification of endocrine-disrupting activity of various xenobiotics (Hallgreen et al 2010). To be emphasized, exposure to EDC not only affects reproductive behaviour but also exerts other behavioral effects. Experiments indicate that exposure to EE_2 and xenoestrogen of zebrafish and guppies result in increased levels of cortisol and adrenocorticotropic hormone (ACTH) suggesting increased stress and enhanced anxiety with negative impact on growth, foraging and immune system functions (Hallgren et al 2011).

Secondary sexual characteristics and reproductive performances in a study group of male guppies (*Poecilia reticulata*) exposed to the antiandrogenic pesticide DDT and its active metabolite p, p′-DDE were compared to that of unexposed males. The same attributes were monitored in the descendents and it was noticed that none of the male characteristics, such as sperm numbers, colour or courtship behaviour changed significantly in either group. These data may suggest weak and highly heterogenous antiandrogenic effects for p,p'-DDE in different populations, even within the same species (Kristensen et al 2006).

Concluding remarks. The *Poecilia reticulata* species profiles as a biological indicator of key importance for the detection of endocrine-disrupting compounds, due to some particular attributes such as: an easily detectable gonopodium enabling collection of sperm, intense and specific body coloration and a particular male courtship behaviour which are under androgen hormones control. Guppies exhibit several biomarkers proved to be highly sensitive in other fish species, *e.g.* vitellogenin, the gonadosomatic index, aromatase activity and the gonadal histological pattern, all being easily assessed and quantified for comparison in *Poecilia reticulata.*

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