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Responses of laboratory exposed catfish (*Clarias gariepinus*) to environmentally relevant concentrations of p,p′-DDT

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ABSTRACT

Technical grade DDT is annually sprayed for malaria control in many under developed countries world wide. Despite the controversy surrounding the use of DDT, minimal research concerning the effects on indigenous fish species in these areas has been conducted. In this study, the objectives were to identify some of the effects of sprayed p,p′-DDT on the common African sharptooth catfish species (*Clarias gariepinus*) under laboratory conditions. The effects were assessed by exposing specimens to three environmentally relevant concentrations of p,p′-DDT (0.66, 1.36 and 2.72 μg/l) for 21 days and analysing a suite of biomarkers in the plasma, gonads and body morphometrics. The biomarkers were specifically selected based on their practicality in developing countries, which could potentially be utilised for continued monitoring, and included alkali-labile phosphate (ALP), calcium, magnesium and zinc as the indirect measures of vitellogenin, gonadosomatic index, gonad mass manipulated using analysis of covariance, and condition factor. The results showed no significant ($p < 0.05$) dose-dependent changes in the plasma, gonads and body condition of *C. gariepinus*, indicating that these species were not responsive to the p,p′-DDT concentrations when exposed sub-chronically. This lack of a response suggested that mature *C. gariepinus* are tolerant to 21 days exposure of low levels of p,p′-DDT.

1. Introduction

The broad spectrum insecticide, dichlorodiphenyltrichloroethane (DDT) is considered to be a persistent and toxic endocrine disrupting chemical (EDC), in the form of o,p′-DDT and p,p′-DDT and its associated metabolites DDE and DDD (ATSDR, 2002). As such, the use of DDT has been banned in many countries under the global, multi-lateral agreement of the Stockholm convention on persistent organic pollutants (POPs) (Bouwman, 2004). However, restricted use has been authorised in some countries as an effective vector control in the fight against malaria (Mabaso et al., 2004).

Technical grade DDT predominantly consists of the active ingredient p,p′-DDT (65–80%), and to a lesser extent o,p′-DDT (15–21%), and is most often sprayed in the huts of villages using indoor residual spraying (IRS) techniques (Bornman et al., 2009). The majority of these sprayed areas are underdeveloped and have relatively limited funding and resources available for research and management of contamination. Consequently, there is still a scarcity of data regarding DDT and its health effects, particularly on indigenous organisms

Abbreviations: IRS, indoor residual spraying; VTG, vitellogenin; ALP, alkali-labile phosphate; EDCs, endocrine disrupting chemicals; CF, condition factor.

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in receiving aquatic ecosystems. It was in this context that the research on the sub-lethal effects from sub-acute p,p′-DDT exposure was initiated in the present study using *Clarias gariepinus*. The selection of *C. gariepinus* was based on their relatively high frequency of occurrence and abundance within DDT IRS areas, easy handling and relatively sedentary nature (Skelton, 2001). Further advantages for their inclusion are their ability to accumulate large amounts of pollutants without mortality, and indicate DDT effects of organic pollutants (Heath and Claassen, 1999; Mdegela et al., 2010).

Based on previous studies, the exposures to DDT were expected to induce sub-lethal effects primarily associated with endocrine disruption (Ankley and Johnson, 2004; Iwaniuk et al., 2006; Kime, 1998; Vasseur and Cossu-Leguille, 2006). However, inconclusive evidence that DDT induces endocrine disrupting effects in *C. gariepinus* was shown in the field study by Brink et al. (2012). A range of biomarkers, selected based on their ability to identify the extent of EDC effects using minimal resources, showed no significant relationship with DDT. Therefore, the aim of this study was to attempt to quantify the biomarker responses to DDT exposure in *C. gariepinus* under more controlled aquarium conditions and to determine the reproductive effects of p,p′-DDT exposure at environmentally relevant concentrations without *in situ* environmental influences.

The overall suite of biomarkers used was indicative of various levels of effects (Phillips and Rainbow, 1993). Specifically, the levels of alkali-labile phosphate (ALP), calcium (Ca), zinc (Zn) and magnesium (Mg) were selected to indicate subcellular changes. According to Bjornsson and Haux (1985) and Lv et al. (2006), these biomarkers can be used as indirect measures of vitellogenin (VTG) concentrations. VTG is an essential protein in the female fish reproductive cycle and can be activated in males exposed to oestrogen mimicking compounds, such as DDT (Cheek et al., 2001; Lomax et al., 1998; Overdorster and Cheek, 2001). Such stimulation of VTG has been induced in male *C. gariepinus* in the presence of EDCs (Mdegela et al., 2010). However, measurements of VTG are often laborious and expensive, which proves difficult in many developing countries (Marin and Matozzo, 2004; Verslycke et al., 2002). Thus, the specific use of ALP, Ca, Zn and Mg, instead of VTG, was incorporated into this study in order to determine the feasibility of using these relatively simple and cost-effective endpoints to identify DDT induced responses.

In terms of identifying higher level responses, the changes in the catfish gonad and body were assessed, using the gonad somatic index (GSI), analysis of co-variance (ANCOVA) adjusted gonads and condition factor (CF) (Fiest et al., 2005; Sepuveda et al., 2003; Sheahan et al., 2002). The former two are both indicative of gonad condition. Although GSI is the most commonly used index to monitor reproductive cycles (Schweer, 2002), Packard and Boardman (1999) suggested the gonad data rather be adjusted with ANCOVA, than using GSI, in order to remove the obscuring effects of fish mass on gonad mass. Consequently both approaches were incorporated and compared in this study. Whilst the CF was selected as a general biomarker of fish health, which correlates fish body mass to its length using a predicted standardised mass for *C. gariepinus* (Hagenaars et al., 2008). It is based on the assumption that fish mass will decrease due to the depletion in energy resources that are used to cope with the increased stresses posed by a toxicant (Stevenson and Woods, 2006).

Overall, the objectives of this study were to evaluate these biomarkers and to quantify the responses of the indigenous fish species, *C. gariepinus*, exposed to p,p′-DDT under laboratory conditions. The results of these analyses were utilised to identify if any biomarkers are suitable in evaluating the effects of environmentally relevant concentrations of p,p′-DDT that are probable within a DDT sprayed area.

2. Materials and methods

2.1. Fish

Healthy, mature *C. gariepinus* males (maturity based on body and urogenital papilla size) were obtained from a broodstock of fish bred at Bushveld catfish aquaculture farm near Pretoria, South Africa (De Graaf and Janssen, 1996). The fish were transported to the aquarium facility at the University of Johannesburg and left to acclimate to normal laboratory conditions for 12 weeks. The aquaria received continuous flow of dechlorinated water at 27 °C and were maintained on a 12 h light/dark cycle. The fish were fed daily with trout pellets. After the acclimation period, 8 specimens for each exposure set were selected based on body size, health and maturity (Ankley et al., 2001). Each of these fish were transferred into separate 100 l glass test chambers, where the specimens where further acclimated for an additional six weeks. Water characteristics were monitored daily using a Cyberscan DO100 meter, pHScan meter and cyberscan Con400 and the mean (±SD) were as follows: temperature 26.23 ± 4.56 °C; pH 7.35 ± 5.10; conductivity 271 ± 90.03 μS/cm; oxygen saturation 79.94 ± 11.94%; and oxygen levels 7.2 ± 10.9 mg/l.

2.2. Exposures

The mature catfish were exposed for 21 days to sub-lethal, environmentally relevant concentrations of 0.659 μg/l, 1.36 μg/l and 2.724 μg/l p,p′-DDT. According to Ankley and Johnson (2004), 21 days were a suitable exposure period in which to identify endocrine disruptive effects in fish. For each exposure concentration, fish were divided into 2 groups consisting of a solvent control and a p,p′-DDT exposure. Each DDT exposure group contained a separate control group, as solvent levels differed for each exposure group (discussed below). The exposure setup consisted of a flow through system from stock tanks pumped into the 100 l glass test chambers and maintained by refreshing the 0.659 μg/l, 1.36 μg/l and 2.724 μg/l exposure tanks with stock of 0.013 μg/l/h, 0.028 μg/l/h, and 0.056 μg/l/h, respectively. In the p,p′-DDT stock tanks, the relevant concentrations were obtained by dissolving a nominal quantity of 50 mg/l p,p′-DDT powder (1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane, Sigma) in grade ethanol solvent (Sigma), which did not exceed the 20 μg/l solvent, as recommended by Hutchinson et al. (2006). This solution was then added into the stock tanks containing 900 l of dechlorinated water in volumes that would make up the required exposure concentrations. Grade ethanol was added to the control tanks in the same volumes that it was added as solvent to the respective