Environmental Toxicology

ADRENOCORTICOTROPHIN- AND CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE-STIMULATED CORTISOL SECRETION IN INTERRENAL TISSUE OF RAINBOW TROUT EXPOSED IN VITRO TO DDT COMPOUNDS

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Abstract—The effects of DDT compounds on the function and viability of interrenal corticosteroidogenic tissue were investigated in vitro in rainbow trout (*Oncorhynchus mykiss*) in a dose–response study. The *o,p*′-dichlorodiphenyldichloroethane (DDD) was the most potent of the tested chemicals in decreasing adrenocorticotropic hormone (ACTH)-stimulated cortisol secretion by head kidney fragments compared with controls. The dbcAMP-stimulated cortisol secretion was also impaired with doses of 50 and 100 mg/L *o,p*′-DDD but not 25 mg/L. Tissue viability, estimated by the release of lactate dehydrogenase (LDH), was similar to controls at 25 mg/L and was decreased significantly at 50 and 100 mg/L *o,p*′-DDD. These results suggest that *o,p*′-DDD is an adrenotoxic compound that may disrupt the 3′,5′-monophosphate (cAMP) generation step and, at higher doses, induce irreversible cytotoxic effects. The *p,p*′-DDT induced a significant inhibition in the secretory response to ACTH and dbcAMP only at 100 mg/L, while *p,p*′-DDD had no effect on the cortisol secretion. A significantly decreased viability was detected at the higher doses of *p,p*′-DDT and *p,p*′-DDD without a detectable disruption of cortisol synthesis. The steroidogenic interrenal cells may be less sensitive to the DDT compounds tested than other cell populations within the teleost head kidney.

Keywords—DDT   Dichlorodiphenyldichloroethane   Cortisol   Adrenocorticotropic hormone   Cyclic adenosine

INTRODUCTION

Numerous environmental xenobiotics have the capacity to interfere with the normal endocrine function of wildlife species causing physiological and developmental anomalies [1,2]. An adrenal dysfunction characterized by an impaired capacity of the interrenal tissue to secrete cortisol and to respond to adrenocorticotropic hormone (ACTH) has been recently diagnosed in fish chronically exposed to mixtures of environmental xenobiotics in the field [3–5]. However, a specific chemical has not been identified thus far as a causal agent.

There is now extensive evidence that the organochlorine DDT and its metabolites are adrenotoxic in mammals, birds, and fish [6–8; Benguira and Hontela, unpublished results]. Dichlorodiphenyltrichloroethane (DDT) was widely used as an insecticide from 1939 to 1970. Although banned in the USA, Canada, and Western Europe because of deleterious effects on fauna and environmental persistence, it is still used in many parts of the world and detectable levels are found in aquatic systems [9–11]. Technical grade DDT, which is the one used in crop spraying, is composed of diverse DDT compounds (*p,p*′-DDT, *o,p*′-DDT, *p,p*′-dichlorodiphenyldichloroethane [DDD], *o,p*′-DDD, *p,p*′-dichlorodiphenyldichloroethylene [DDE], and *o,p*′-DDE) and is more toxic than pure DDT [12]. Each of these (DDT, DDD, and DDE) have been measured in fish [9–11]. The total DDT residues measured in various species of juvenile fish in the St. Lawrence River, Canada, in 1988 were 0.008 to 0.050 mg/kg wet weight and up to 0.177 mg/kg wet weight in Lake Waterloo (ON, Canada) in 1986 [9,11]. Since fish occupy high trophic levels and ac-

cumulate environmental pollutants even when low doses are present, these xenobiotics can be released into blood circulation and induce an increased toxicity when fish are fasting or spawning [13].

The DDT and its metabolites are adrenolytic compounds that impair corticosteroid secretion and that have been extensively studied in mammals and birds both in vivo and in vitro. Cortisol, the major corticosteroid in teleost fish, is secreted following stimulation of the hypothalamo-hypophyso-interrenal axis by physical and/or chemical stressors [14,15]. Its synthesis is triggered by the release of CRF from the hypothalamus and subsequently the pituitary secretion of the ACTH [16,17], the major hormone regulating the production of corticosteroids by interrenal tissue. The ACTH binds to the membrane receptors of the interrenal steroidogenic cells and activates adenylate cyclase to generate cyclic adenosine 3′,5′-monophosphate (cAMP) from ATP. In turn, this secondary intracellular messenger triggers the enzymatic cascade leading to cortisol production [18–21]. The secretion of ACTH-stimulated cortisol was inhibited in dog adrenal slices pretreated with *o,p*′-DDD and in perfused adrenals [22]. The biochemical site of action of *o,p*′-DDD in this species has been located at a step following the generation of cAMP [23], specifically the conversion of cholesterol into pregnenolone and the 11β-hydroxylation of 11-deoxycortisol into cortisol, two intramitochondrial steps [24]. More recently, *o,p*′-DDD and other DDT compounds such as *p,p*′-DDD, *p,p*′-DDT, and MeSO₂-DDE have been studied in other mammals (guinea pig, mink, otter, gray seal) and in birds (chicken, domestic duck, and common eider) [6,7,25,26]. These studies demonstrated the covalent binding of *o,p*′-DDD, *p,p*′-DDD, and *p,p*′-DDT to the adrenal cortical cells and the subsequent activation of the compound

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into a reactive intermediate that binds and inhibits the 11β-hydroxylase [6,26]. Degeneration of the adrenal cortex and mitochondria were observed in the zona fasciculata and reticularis; however, species differences exist in the DDT compounds and the type of effects generated by these organochlorinated chemicals [6,7,25,27–29].

In contrast, the effects of DDT and its metabolites on the interrenal tissue of fish are not well documented. The response to ACTH was inhibited in superfused head kidneys of tilapia, *Sarotherodon aureus,* treated with 0.1 to 50 mg/L *o,p*′-DDD and 50 to 150 mg/L *p,p*′-DDE in the perfusion medium but not with *p,p*′-DDT (50 mg/L) [30]. Since the effects of *o,p*′-DDD on ACTH-induced cortisol secretion were reversed when cAMP was used to stimulate cortisol secretion, the authors suggested that the organochlorine interfered with the generation of cAMP in the interrenal cells of tilapia. The effects of *o,p*′-DDD and other DDT compounds on the interrenal function have not been tested in other fish species, and no further studies have investigated the effects on ACTH- and cAMP-stimulated cortisol secretion.

The objectives of the present study were to test in vitro the functional integrity and to elucidate the mechanism(s) of action of DDT and its metabolites in interrenals of rainbow trout (*Oncorhynchus mykiss*). The effects of *o,p*′-DDD, *p,p*′-DDD, and *p,p*′-DDT on ACTH- and cAMP-stimulated cortisol secretion by interrenal tissue fragments were assessed in a dose–response study. The viability of the tissue following the in vitro exposure to the toxicants was also determined by measuring the release of lactate dehydrogenase (LDH) into the incubation medium.

MATERIALS AND METHODS

Fish and maintenance

Juvenile rainbow trout, *O. mykiss,* (avg. body weight 100 ± 20 g, body length 7–8 inches) were purchased from a commercial supplier (Ferme Piscicole Des Bobines, Hereford, PQ, Canada) and maintained in 600-L flow-through tanks (3.8 L/min) of filtered and oxygenated water at 15 ± 1.5°C, 12L:12D, pH 7.2, and water hardness of 70 mg/L CaCO$_3$ for 2 weeks prior to the experiments. Fish were fed daily 10 g/kg of commercial trout food.

Incubation of interrenal fragments

An average of 8 to 12 fish per experiment were removed from the tank and sacrificed with MS 222 (0.05 g/L of fresh water; ICN Biomedicals, CA, USA). Fish were weighed and the pronephros (interrenal tissue) was dissected. The interrenals were placed in complete medium (14.2 g/L minimum essential medium), 5 g/L bovine serum albumin, 2.2 g/L NaHCO$_3$ (Sigma Chemical, St. Louis, MO, USA) and cut into fragments of about 1 mm^3 (coarse homogenate).

Experimental treatment

The coarse homogenate was distributed (0.03–0.05 g wet weight/well) with a digital microdispensor between wells of a microplate (24 flat bottom wells) containing 1 ml complete medium and 0, 25, 50, or 100 mg/L of *o,p*′-DDD (1-[*o*-chlorophenyl]-1-[*p*-chlorophenyl]-2,2-dichloroethane; Aldrich, Milwaukee, WI, USA) dissolved in 1% dimethysulfoxide (DMSO; Sigma Chemical), *p,p*′-DDT (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane; Sigma), or *p,p*′-DDD (2,2-Bis(4-chlorophenyl)-1,1-dichloroethane; Aldrich) dissolved in 5% DMSO (Fig. 1). *p,p*′-DDD and *p,p*′-DDT were the least hy-
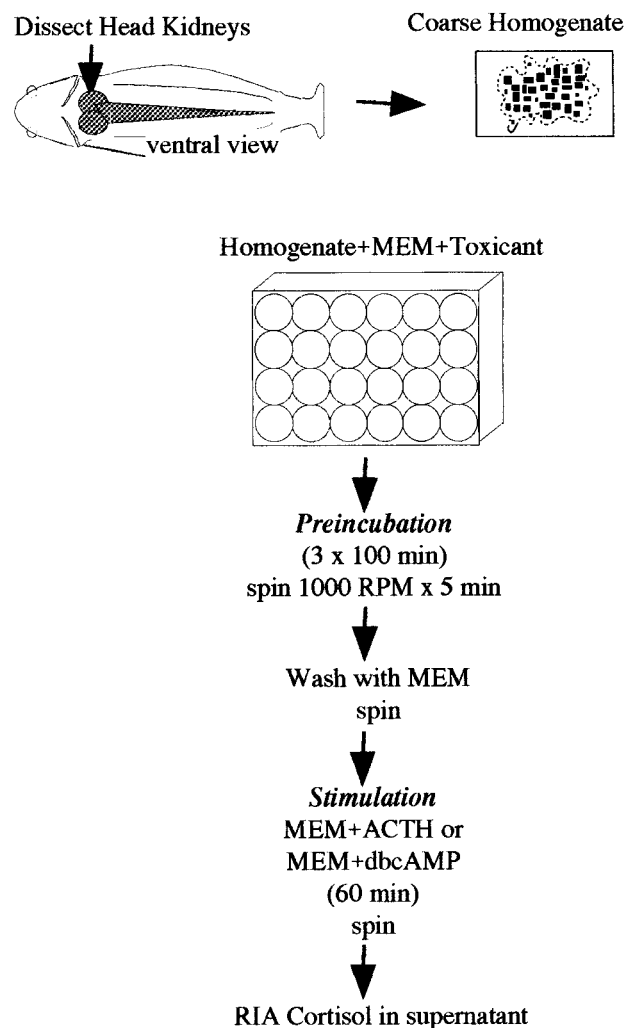

Fig. 1. Procedure for in vitro exposures of fish interrenal tissue to toxicants.

drosoluble and 5% DMSO was necessary to dissolve those two compounds, compared with 1% DMSO for *o,p*′-DDD. Blanks containing DMSO (with or without ACTH or dbcAMP) were also used. No effect on viability of interrenal cells was detected in up to 5% DMSO, as reported previously [31].

The method using interrenal fragments in vitro has been used previously with yellow perch, *Perca flavescens* (Henley et al., unpublished data). To determine the optimal exposure time with the toxicant in a pilot experiment with rainbow trout, fragments were incubated in 1 ml medium containing 50 mg/L *o,p*′-DDD for 120 min, the microplate was then centrifuged at 1,000 rpm for 5 min, the supernatant was collected, and fresh medium containing porcine ACTH (1 IU/ml; Sigma) was added to stimulate cortisol secretion for 60 min. Since no effect of *o,p*′-DDD could be detected in this pilot study using exposures to toxicants of 120 min (data not shown), the exposure time was prolonged to 300 min in the subsequent experiments (Fig. 1). The microplate was centrifuged at 1,000 rpm for 5 min every 100 min to maintain constant exposures to the toxicant and to collect the supernatant in which cortisol secretion would be determined (basal levels without stimulation by ACTH or dbcAMP). After the exposure to the organochlorine, following the last centrifugation where the supernatant was retrieved, the interrenal fragments were washed twice with 1 ml of fresh

medium without organochlorines. The coarse homogenate was then stimulated for 60 min with either 1 IU/ml ACTH or 2 mM dbcAMP, optimal doses determined in a pilot study (Henley et al., unpublished data), in fresh medium without toxicants to compare the maximum secretory response following incubation for 5 h with the toxicant. At the end of the experiment, the plates were centrifuged, supernatants were collected for cortisol analyses, and the fragments from each well were weighed to the nearest 0.1 mg.

Histological analysis

Some of the interrenal fragments exposed to 0 and 100 mg/L *o,p'*-DDD for 300 min were fixed in Bouin solution immediately after exposure for a preliminary investigation of the histomorphological changes caused by *o,p'*-DDD. Fragments were then dehydrated and embedded in paraffin. The blocks were cut, colored by Trichrome stain [32], and slides were observed and analyzed by image analysis (National Institute of Health Image Program, Washington, DC, USA).

Assessment of viability

The medium collected at 0 to 300 min was pooled and LDH, an enzyme leaked from dead cells, was measured in medium from interrenal fragments exposed to *o,p'*-DDD, *p,p'*-DDT, *p,p'*-DDD, or controls immediately after preincubation (Fig. 1). The LDH was assayed by commercial kits (Boerhinger Mannheim, Montréal, PQ, Canada) using pyruvate and NADH. Viability was expressed as percent of maximum LDH levels released from interrenal tissue incubated in distilled water without toxicant (100% mortality). The percent of dead cells was calculated by dividing the amount of LDH leaked into the medium by maximum LDH. Cell viability (percent of viable cells) was obtained by subtracting percent of dead cells from 100% [33].

Biochemical and statistical analysis

The ACTH and dbcAMP-stimulated cortisol secretion was measured in medium by RIA (ICN Biomedicals, catalogue number 07221106). All the results are expressed as percentage of control representing nanograms of cortisol per milliliter of medium. Statistical analyses were done by an all pairwise multiple comparison test, Dunnett's method ($p < 0.05$).

RESULTS

Preincubation cortisol levels

Cortisol levels were below the detection limit of the assay (<5 ng/ml) following the first 100 min of incubation of interrenal fragments, as demonstrated previously (Henley et al., unpublished data). The levels of cortisol remained low throughout the additional 200 min of incubation in all the treatments (controls incubated without toxicants or tissues exposed to toxicants) and therefore the effects of exposure to toxicants could not be determined for basal secretion.

Effects of o,p'-DDD on ACTH- and dbcAMP-stimulated cortisol secretion in vitro

Cortisol secretion in response to ACTH or dbcAMP by fragments exposed for 5 h to the medium without the toxicant (controls, dose 0 mg/L *o,p*'-DDD in 1% DMSO) was 1,385.37 ± 128.73 ng/ml medium/g of interrenal tissue and 1,123.73 ± 131.73 ng/ml medium/g of interrenal tissue (mean ± SEM), respectively (Fig. 2). However, *o,p'*-DDD treated ACTH-stim-
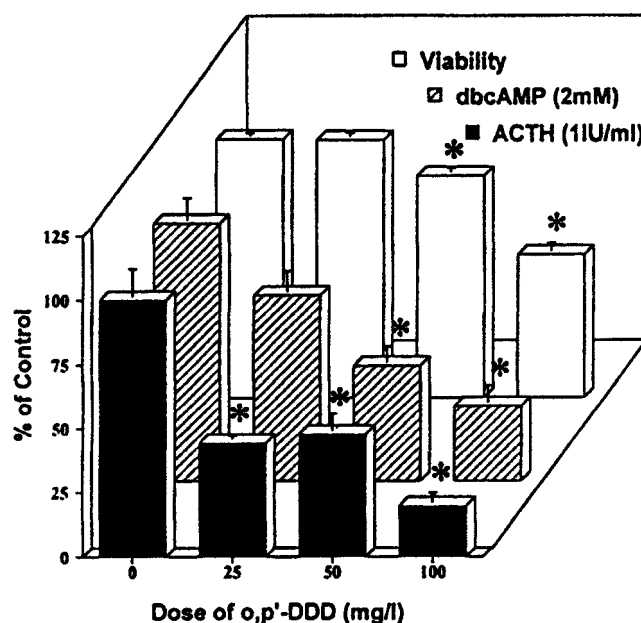

Fig. 2. Adrenocorticotropic hormone (ACTH)- and dbcAMP-stimulated cortisol secretion and viability (% control, mean ± SEM) following 5 h of exposure to *o,p'*-DDD. Statistical significance was evaluated by Dunnet's test ($p < 0.05$). Each control and viability bar represents the mean of 22 to 24 replicates and each *o,p'*-DDD bar represents 10 to 12 replicates.

ulated fragments increased cortisol levels significantly less ($p < 0.05$) than nonexposed ACTH-stimulated controls following the 5-h incubation. Cortisol output in the medium was significantly lower for the doses of 25, 50, and 100 mg/L *o,p'*-DDD (44, 48, and 20% of controls, respectively). Similar results were observed with dbcAMP-stimulated fragments exposed to 50 and 100 mg/L *o,p'*-DDD (45 and 29% of control, respectively). However, the cortisol production measured in the dbcAMP-stimulated group exposed to the dose of 25 mg/L *o,p'*-DDD was not significantly different from the control group (72% of controls).

To distinguish between cytotoxicity (decrease in cortisol secretion due to cell death) and endocrine toxicity (disruption of cortisol synthesis without cell death) due to *o,p'*-DDD, the viability of the tissue fragments was evaluated after 5 h of exposure to the toxicant by measuring the LDH levels. Viability of the fragments decreased in a dose-related manner (Fig. 2), and no mortality was detected at the dose of 25 mg/L *o,p'*-DDD (99.6% viability compared to controls). A significant decrease in viability is observed at the doses of 50 and 100 mg/L (85.9 and 55.3% of controls, respectively).

Effects of p,p'-DDT on ACTH- and dbcAMP-stimulated cortisol secretion in vitro

No significant effects were observed on cortisol secretion after ACTH- and dbcAMP-stimulation and on viability of tissue fragments at the dose of 25 mg/L *p,p'*-DDT (Fig. 3). At the dose of 50 mg/L of *p,p'*-DDT, a significant decrease in viability (69.2% of controls) was observed, without a significant effect on ACTH- or dbcAMP-stimulated cortisol secretion. Incubation with a dose of 100 mg/L *p,p'*-DDT significantly decreased cortisol output after both ACTH- and dbcAMP-stimulation (51.7 and 39.9% of controls, respectively). Moreover, the viability of interrenal fragments was significantly decreased at this dose (66.5% of controls) (Fig. 3).
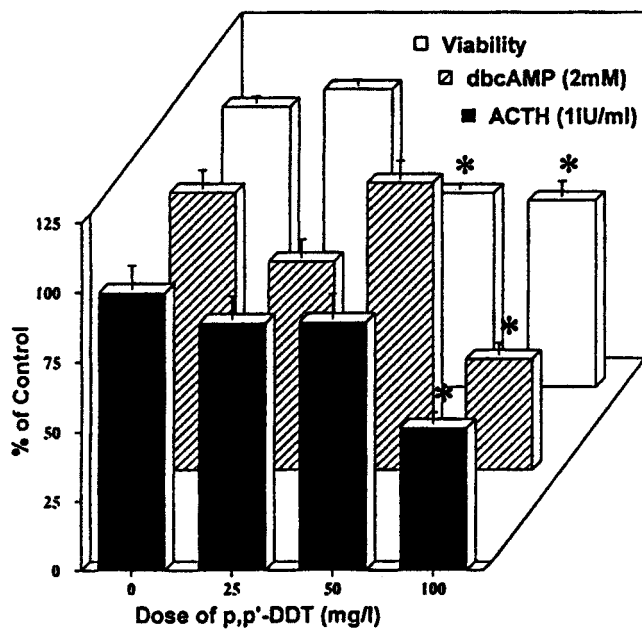
Fig. 3. Adrenocorticotropic hormone (ACTH)- and dbcAMP-stimulated cortisol secretion and viability (% control, mean ± SEM) following 5 h of exposure to *p,p*′-DDT. Statistical significance was evaluated by Dunnet's test (*p* < 0.05). Each control and viability bar represents the mean of 22 to 24 replicates and each *p,p*′-DDT bar represents 10 to 12 replicates.

Effects of p,p′*-DDD on ACTH- and dbcAMP-stimulated cortisol secretion in vitro*

After 5 h of in vitro exposure to *p,p*′-DDD, no effect on cortisol secretion following ACTH- and dbcAMP-stimulation was observed at any of the doses used (Fig. 4). However, as for the other compounds, the interrenal fragments viability was
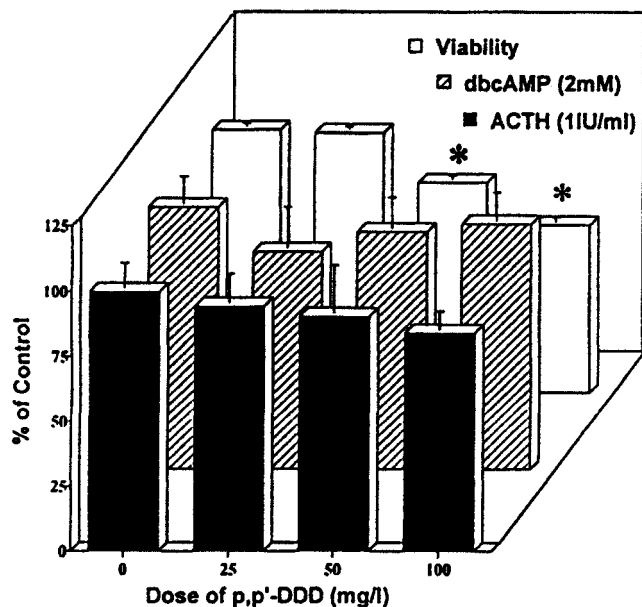

Fig. 4. Adrenocorticotropic hormone (ACTH)- and dbcAMP-stimulated cortisol secretion and viability (% control, mean ± SEM) following 5 h of exposure to *p,p*′-DDD. Statistical significance was evaluated by Dunnet's test (*p* < 0.05). Each control and viability bar represents the mean of 22 to 24 replicates and each *p,p*′-DDD bar represents 10 to 12 replicates.

altered at the doses of 50 and 100 mg/L *p,p*′-DDD (80.4 and 64.1% of controls, respectively).

Histological analysis of interrenal fragments following o,p′*-DDD treatment*

Interrenal fragments exposed for 5 h to 100 mg/L *o,p*′-DDD and the controls were observed. Preliminary results suggest that *o,p*′-DDD may decrease the size of the circumference of the nucleus (18.91 ± 0.43 vs 17.82 ± 0.43 μm for control and 100 mg/L *o,p*′-DDD, respectively; *n* = 3; mean ± SEM) of the interrenal cells; however, the difference was not significant. Further investigations will evaluate the effects of organochlorines on the cellular morphology of the interrenal cells.

DISCUSSION

A previous in vivo study demonstrated that the normal increase in cortisol secretion following stimulation of the hypothalamo-hypophyso-interrenal axis by confinement stress was inhibited in rainbow trout (*O. mykiss*) 14 d following a single ip injection of *o,p*′-DDD (Benguira and Hontela, unpublished data). Cortisol secretion is a complex process involving the hypothalamus, the pituitary, and the interrenal tissue. Since very few studies reported the effects of environmental xenobiotics on cortisol secretion in vitro in fish [31], it was important to investigate the direct effects of DDT compounds on the potential target tissue, i.e., the interrenals, which secrete corticosteroids. Hence, the aim of the present study was to determine the effects and the mechanisms of action of two DDD isomers, *o,p*′-DDD and *p,p*′-DDD, as well as the main constituent of technical grade DDT, *p,p*′-DDT, on the functional integrity and viability of the interrenal tissue of rainbow trout by using ACTH- or dbcAMP-stimulation.

In mammals, birds, and microorganisms, *p,p*′-DDT is metabolized in vivo to DDD isomers by dechlorination of the side chain of DDT [12]. There are no data available, however, on the metabolism of DDT in fish. Two metabolites of DDT, methysulfonyl-DDE and *o,p*′-DDD, have adrenolytic effects in some species of birds and mammals [6,7]. The teleost interrenal tissue, enriched with lipids originating from the steroid precursor cholesterol, may be particularly vulnerable to lipophilic xenobiotics such as DDT, as has been documented for mammalian adrenals [6].

The mechanisms of action of DDT compounds in the adrenals have been extensively studied in mammals and birds but less so in fish. In the only two studies to date reporting the effects of DDT in fish, Ilan and Yaron [8,30] suggested that *o,p*′-DDD impaired the ACTH response of the superfused interrenal tissue of tilapia at the level of the cAMP generating step. However, tissue viability, a measure of possible cytotoxic effects, was not assessed in these studies, even though it could account for the decrease in cortisol output. Furthermore, it was demonstrated in mammals and birds that the inhibition of conversion of 11-deoxycortisol to cortisol, an intramitochondrial step, was the cause of the reduced ACTH-stimulated corticosteroid output following *o,p*′-DDD treatment [6,7,25,26] rather than disruption of the cAMP production [23]. Our results demonstrated the inhibitory effects of *o,p*′-DDD on ACTH-stimulated cortisol secretion in rainbow trout (Fig. 2). The adrenotoxic effects were dose-related and provide evidence for a direct action, not involving the hypothalamus or pituitary gland, of *o,p*′-DDD. Furthermore, at the dose of 25 mg/L *o,p*′-DDD, the production of LDH, a measure of cell viability, was

not different from controls. While the treatment with o,p'-DDD at 25 mg/L impaired the response to ACTH, the response to dbcAMP was not significantly different from the controls not treated with the toxicant. A significant effect of o,p'-DDD on cortisol secretion in only ACTH-stimulated but not in dbc-AMP-stimulated interrenal fragments suggests that o,p'-DDD may act on the cAMP generating step by either damaging the membrane ACTH receptors in the interrenal cells or inhibiting the adenylate cyclase that produces cAMP via ATP or by both. However, at the higher doses tested (50, 100 mg/L o,p'-DDD), tissue viability was significantly decreased and stimulation with dbcAMP could not correct the adverse effects of o,p'-DDD. These results suggest that, at higher doses, the organochlorine may have an irreversible cytotoxic effect, as previously described in mammals and birds [6,7,26,29]. To further investigate the cytotoxic effects, o,p'-DDD treated fragments were analyzed by microscopic image analysis. To our knowledge, no study has ever reported the effects of o,p'-DDD on the interrenal cells of rainbow trout. Preliminary results suggested that o,p'-DDD did not alter the morphology of the interrenal cells, although the size of the nuclei seemed smaller in the o,p'-DDD treated fragments (100 mg/L). Further histological analyses are necessary to clarify these results.

There is evidence that the various DDT compounds formulated in technical grade DDT generate adrenotoxic effects that vary with the tested animal species [6]. The major constituent of technical grade DDT, about 77%, is p,p'-DDT [12]. Our results, shown in Figure 3, demonstrated a significant inhibition in ACTH-stimulated cortisol secretion following treatment with 100 mg/L p,p'-DDT but not with doses of 25 and 50 mg/L, in contrast with o,p'-DDD. Previous findings in bovine and rat adrenal homogenates demonstrated that, as for o,p'-DDD, p,p'-DDT interfered at the binding site of steroids to the cytP450 and reduced corticosteroid biosynthesis [34]. In a more recent study with the gray seal, p,p'-DDT as well as o,p'-DDD at the same concentration (40 mM) inhibited the 11β-hydroxylation of cortisol (25% of controls) [26]. However, p,p'-DDT (50 mg/L) did not suppress the ACTH-stimulated cortisol secretion of interrenal fragments in tilapia [30]. Our data indicate that p,p'-DDT induced adrenotoxicity with less potency than o,p'-DDD (25 mg/L and 100 mg/L for o,p'-DDD and p,p'-DDT, respectively) and that, similarly to o,p'-DDD, dbcAMP could not reverse the effects on ACTH-stimulated cortisol secretion when tissue viability was significantly decreased. Surprisingly, at the dose of 50 mg/L, a significant reduction in viability of tissue is observed without a significant effect on cortisol output following ACTH or dbcAMP stimulation. Steroidogenic cells of teleost fish are scattered as islets in the head kidney and constitute few percent of the total cells in interrenal fragments [14]. Since viability of the tissue is assessed by the release of LDH measured in the pronephros fragments constituted by chromaffin cells, lymphocytes, melanomacrophages, and red blood cells as well as steroidogenic cells, the measurement of viability probably mainly represents toxicity to cells other than steroidogenic cells. Steroidogenic cells could be also less sensitive to DDT compounds than other cells; however, cell-specific cytotoxicity has not been evaluated thus far in the head kidney. Some evidence for such a phenomenon of specific cytotoxicity has been previously reported in medullary catecholamine producing cells in the adrenal gland of rats fed technical grade DDT [29] but not in other mammalian species [6,25]. Moreover, in birds, which like fish have intermingled interrenal and medullary cells, the

specific target cells could not be identified [7]. Future investigations in fish should compare the cell-specific effects of various organochlorines on fish adrenals.

Since p,p'-DDD is another major metabolite of DDT, its effects on cortisol secretion were also determined in vitro. To our knowledge, no study has ever reported the effects of p,p'-DDD on steroidogenesis in the interrenal tissue of fish. In our study, treatment with p,p'-DDD did not affect the cortisol response to ACTH or dbcAMP at any of the doses tested (Fig. 4). However, viability of tissue was decreased significantly at 50 and 100 mg/L, suggesting that there may be head kidney cells more sensitive to p,p'-DDD than the steroidogenic cells. A previous study in the dog demonstrated that treatment with any of the three isomers, o,p'-DDD, p,p'-DDD, or m,p'-DDD, had an inhibitory effect on the response to ACTH (50% of controls) [27]. However, p,p'-DDD was the least potent, and the decrease in steroid output was observed only 4 to 18 h after treatment, compared with 27 and 87 min for m,p'-DDD and o,p'-DDD, respectively [27]. More recently, covalent binding of o,p'-DDD and p,p'-DDD and subsequent toxicity to the adrenal cortex of mink and otter [25], as well as dog, chicken, mink, and man but not in mouse and rat [6], were reported. These results suggest major species differences in the types of metabolites that generate adrenotoxic effects. Our data indicate that the adrenal steroidogenesis of rainbow trout is insensitive to p,p'-DDD.

In conclusion, our study demonstrated that o,p'-DDD, p,p'-DDT, and p,p'-DDD induce irreversible cytotoxic effects to the head kidney of rainbow trout (*O. mykiss*) at the doses of 50 and 100 mg/L. The metabolite o,p'-DDD was the most potent in inhibiting the response to ACTH but its isomer p,p'-DDD had no significant effect in decreasing cortisol output in this species. Our results suggest that DDT and its metabolites have two distinct effects on the interrenal tissue of fish endocrine disruption via interference with the synthesis of cortisol and cytotoxicity at the level of the interrenal cells. However, comparison of the concentrations required in the present study to disrupt cortisol synthesis in vitro and the contamination of fish at sites where interrenal dysfunction has been diagnosed [14,35] does not indicate that DDT compounds are solely responsible for the impairment of corticosteroid synthesis diagnosed at these sites.

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