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Mechanistic study of chlordecone-induced endocrine disruption: Based on an adverse outcome pathway network

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HIGHLIGHTS

- An adverse outcome pathway network was used to help reveal toxicity mechanism.
- Molecular docking was adopted to find possible molecular initiating event (MIE).
- Chlordecone acts as agonist of ERs and CYP19A in rare minnow.
- The results also pointed to other potential but important MIEs.

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ABSTRACT

The adverse outcome pathway (AOP) framework could be helpful for chemical risk assessment and mechanistic research. The aim of the present study was to unravel the mechanism of chlordecone-induced endocrine disruption by illustrating the main molecular initiating event (MIE)/perturbations responsible for the observed effects. *In silico* simulations were performed to predict the MIE(s), and the results pointed to agonistic interaction with estrogen receptors (ERα, ERβ), androgen receptor (AR), cytochrome P450 (CYP19A) by chlordecone. *In vivo* endocrine disruptions were evaluated in rare minnow (*Gobiocypris rarus*) exposed to 0.01, 0.1, 1 and 10 μg L^{-1} chlordecone from 2 h post-fertilization until sexually mature. In the females, increases of vitellogenin (*vtg*) mRNA levels in liver and gonad, plasma estradiol (E2), testosterone (T) and E2/T, and renalsomatic index confirmed the role of agonism of ER and CYP19A as MIEs, but the decreased gonadosomatic index, degenerated ovaries as well as the feed-forward response pointed to other potential but important MIEs and corresponding AOPs. In the males, increased E2/T ratio, increased testis *vtg* mRNA levels and occurrence of intersex confirmed the roles of agonism of ERα and CYP19A as main MIEs in chlordecone-induced endocrine disruptions. Our results also fetches out the limit of AOPs in predicting the adverse outcomes and explaining the mechanism of chemicals at present, thus reflected a critical need for expanding AOPs and AOP network before using it in chemical risk assessment.

1. Introduction

In the past decade, defining and/or understanding toxicity pathways of chemicals has been the focus of many studies, and the comprehensive understanding of information from such studies has become a key step toward the development of a new generation of predictive toxicology tools. Recently, the adverse outcome pathway (AOP) has been advocated for chemical risk assessment and mechanistic study (Ankley et al., 2010; Groh et al., 2015; Zhou, 2015). This approach presents a detailed progression of a series of

Abbreviations: AOP, Adverse Outcome Pathway; MIE, Molecular Initiating Event; KE, Key Event; AO, Adverse Outcome; T, Testosterone; E2, Estradiol; ER, Estrogen Receptor; AR, Androgen Receptor; VTG, Vitellogenin; CYP, Cytochrome P450; GSI, Gonadosomatic Index; RSI, Renalsomatic Index; GnRH, Gonadotropin-releasing hormones; DMRT1, Doublesex and mab-3 related transcription factor 1.

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key events (KE) following a molecular initiating event (MIE) leading to an adverse outcome (AO) within a logical framework (Knapen et al., 2015), thus facilitate greater understanding at theoretic level. Since a single AOP may not capture all events contributing to any relevant toxic effect, AOP networks are therefore required to present more realistically potential effects and to reveal previously unknown links between biological pathways (Knapen et al., 2015). However, there is still considerable work needed to demonstrate and maximize the utility of this framework. In the near future, comprehensive understanding of the toxicological mechanism was needed to help put the empirical support in context and/or help support and expand AOP network.

In this regard, chlordecone may provide good example for probing the utility and limits of the current AOPs. Chlordecone, also named kepone, was intensively used as an organochlorine insecticide to fight against banana root borer, apple scab and powdery mildew, tobacco wireworms as well as slugs, snails, and fire ants (NLM, 2004). Although banned since 1978 in the United States of America (IARC, 1979), chlordecone still could be detected in rivers (up to 4 μg L^{-1}), wild fish (up to 0.42 μg g^{-1}) and human (0.41 μg L^{-1} in cord blood) worldwide due to its high lipophilicity and resistance to degradation (Luellen et al., 2006; Guldner et al., 2010; Boucher et al., 2013). The UNEP (2007) have listed chlordecone into the Stockholm Convention on Persistent Organic Pollutants to call for specific concerns on its potential pollution and risk to wild animals as well as humans.

Observed chlordecone-induced reproductive effects include oligospermia, reduced sperm motility, and decreased libido in occupationally exposed men (Faroon et al., 1995). Similar results including testicular atrophy, altered sperm characteristics, persistent vaginal estrus, and anovulation were observed in chlordecone-treated laboratory animals (U.S. EPA, 1986). Degenerative changes including reduced growth of oocytes and appearance of atretic follicles were also reported in fish treated with chlordecone (Srivastava and Srivastava, 1994). These observations mimic similar effects produced by excessive estrogen (Scippo et al., 2004), and chlordecone has been proved to act as a competitive agonist *in vitroly* for both subtypes of estrogen receptors (ER α and β) (Kuiper et al., 1998). Besides, the database of Pubchem Bioassay (NIH, 2016), which mainly uses cell-based and biochemical *in vitro* tests, also provided evidence for agonistic and/or antagonistic activity of androgen receptor (AR) and Cytochrome P450 19A (CYP19A) by chlordecone. The agonism and/or antagonism of steroid receptors and CYP19A as MIE may lead to different series of KEs, some of which could be contrary as indicated by the already described AOPs (Knapen et al., 2015). Hence it is interesting to figure out the prospective MIE and unravel the process driving the observed effects upon chlordecone exposure. The definition of key MIE within the integrated strategy of AOP characterisation may be another challenge for the extension of this paradigm. *In silico* approaches such as molecular docking could provide clear view of the interactions between xenobiotic chemicals and biomolecules, therefore represent reasonable tools for predicting the potential MIE(s) of test chemicals.

Above all, the main purpose of this study is to predict the potential MIE of chlordecone by molecular docking, to evaluate the endocrine disrupting capabilities using rare minnow as an *in vivo* model fish, and to unravel the main MIE(s)/perturbations responsible for the observed effects.

2. Materials and methods

2.1. Chemicals

Chlordecone (purity > 99.5%) was purchased from Supelco Chemical Co. (St. Louis, MO, USA), and high-performance liquid chromatography-grade acetone was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanesulfonate (MS-222) were purchased from Sigma–Aldrich (Fluka, Shanghai, China).

2.2. Test fish and culture conditions

The rare minnow has been maintained in the laboratory of Aquatic Ecotoxicology in Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, for more than 10 years. The brood stock was kept in a flow-through system filled with dechlorinated tap water (pH 7.2–7.6; hardness 44.0–61.0 mg $CaCO_3$ L^{-1}) and subjected to a 16:8 h light:dark cycle at 25 ± 1 °C. The brood stock was fed newly hatched brine shrimp (Artemia nauplii) twice and granule food (TetraMin, Tetra Werke, Melle, Germany) once daily. Wastes and residues were removed daily, and the test equipment and chambers were cleaned once a week.

2.3. Experimental design

Embryos from one male-female pair with relatively stable spawning period, egg laying amount and fertility rate were used for experiments. Embryos that developed normally and reached the blastula stage (2 h post-fertilization, hpf) were equally divided into 6 groups and radomly distributed into 6 glass beakers (n = 80–100 each, depending on the total number of embryos) containing 200 mL exposure solution (water control, solvent control, or 0.01, 0.1, 1, 10 μg L^{-1} chlordecone, respectively). The exposure concentrations included environmental levels and the upper limit concentration showed no lethality in the 96 h acute test using larval rare minnow (7 days post fertilization, dpf). Such experiments were repeated for 10 times, in other words, there were ten beakers for each experimental and control group. At 14 dpf the larvae were transferred into a flow-through system and exposed to corresponding concentrations of chlordecone as previously described (Yang et al., 2010). Specifically, for each concentration group 100 larvae were radomly selected from ten beakers (10 from each), and assigned into 2 18-L glass tanks (50 each). There were 2 tanks for each group because of the limit of spaces and tanks. The remaining larvae were sampled for other analysis. Final concentrations of acetone in all treatments throughout the exposure experiment were lower than 1:100,000 (vol/vol). Although the water bone concentrations of chlordecone were not determined in the present study, we supposed that the actual concentrations were close to nominal concentrations based on the following reasons: First, chlordecone has been proved to be highly stable in water; second, the rates of stock solutions and dechlorinated tap water were strictly controlled in the flow-through system and adjusted every day which ensured the retention time shorter than 4 h or the renew times more than 6 in a day; third, according to earlier studies in our laboratory, the deviation of the concentrations could be controlled within ±15% (Yang et al., 2011).

As documented previously, rare minnow could be sexually matured at 4 month (Wang, 1992). During the flow-through exposure, the development of gonad (from 2 to 3 fish) were examined every two weeks since 3 month post hatch by observing the gonad after dissection. Therefore, after about 5 month post hatch, fish were considered sexually matured and sacrificed after anesthetized with 0.01% MS-222. For each fish, body length and weight were quickly determined, and blood was collected in heparinized microcapillary tubes. The samples were immediately centrifuged (8000 × g, 10 min, 4 °C), and plasma was collected and kept frozen at −80 °C until use. The abdomen of each fish was dissected, then liver, gonad and brain were carefully isolated and weighed. Three liver (or brain or gonad) of female or male fish from