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Behavioral response and gene expression changes in fipronil-administered male Japanese quail *(Coturnix japonica)*☆

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ABSTRACT

Fipronil is an important member of the phenylpyrazole group of insecticides and is widely used for various crops and vegetables to control insects, thereby exposing birds, animals, and humans to fipronil. Currently, there is limited information on the effects of fipronil exposure in Japanese quail. Therefore, our aim was to assess the reproductive toxicological effects of fipronil in the Japanese quail in a 15-day gavage study and then its recovery over a period of 60 days. Fipronil-administration led to significant losses in both feed intake and body weight. Whereas, the gonadosomatic index was not affected, and histological changes observed in the testes were reversible, particularly by day 45 and day 60 of recovery. Cloacal gland atrophy, reduced foam quantity and a reduction in fertility, sexual and aggressive behaviors, and serum testosterone with elevated estradiol (E2) hormone levels were also observed. All these changes gradually reversed during various recovery periods. Further, alterations in hepatic vitellogenin (Vtg) and estrogen receptor α (ERα) gene expression, assessed by quantitative polymerase chain reaction, were also observed. Specifically, ERα1 was induced after fipronil administration, while the Vtg transcript was elevated during both exposure and recovery periods. Our results showed that fipronil exposure has a profound negative influence on reproductive traits in the male Japanese quail and exhibits an estrogenic activity that can raise the incidence of infertility in males. Nevertheless, most of the changes could be reversed after a recovery period of 30–45 days.

1. Introduction

The hypothesis that environmental pollutants can upset endocrine function in wildlife and humans has attracted much consideration during the recent years. Various experiments and investigations have demonstrated alterations in the reproductive functions of species exposed to these chemicals released into the environment. These effects are often deceptive and hard to identify, and sometimes remain undetected until the population density of the affected species is critically reduced (Gray and Ostby, 1998). Endocrine disruptor chemicals (EDCs) can induce a variety of adverse effects by altering normal endocrine and physiological processes, such as growth, development, reproduction, and behavior, in humans, livestock, and wild animals, when they enter the organism either through ingestion or absorption (Zhang et al., 2013).

Fipronil, a new γ-aminobutyric acid (GABA)-disrupting insecticide, is a broad spectrum pesticide and a member of the phenylpyrazole class of insecticides that interfere with GABA-gated chloride channels in the nerve cell membranes causing neuronal hyper excitation and convulsions that eventually lead to death in insects (Das et al., 2006). The binding affinity of fipronil is much weaker in mammals than in insects, which makes it safer for mammals. As GABA and its receptors have an imperative role in neuroendocrine regulation, fipronil and other environmental pollutants that interfere with GABA signaling can upset normal regulation of the hypothalamic–pituitary–gonadal (HPG) axis and thus impair reproduction (Bencic et al., 2013). Disturbances in signaling, biosynthesis, and/or metabolism at any point along the HPG axis can conceivably influence reproduction, which in turn, determines population development and survival (Segner et al., 2006).

Fipronil is administered to control pests of various food crops

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and useful for the control of fleas, lice, and ticks on domestic animals (Bobe et al., 1997). Fipronil has also been widely utilized in commercial agriculture, veterinary practices, and household applications since 1993 (Tingle et al., 2003). In 1997, 480-metric tons of fipronil were manufactured by Rhône-Poulenc (Anon, 1997), and a report of Environmental Protection Agency (EPA) demonstrated that, between 1998 and 2008, its usage reached 68,039 kg of active ingredient. Consequently, this caused a high rate of potential contamination of the surrounding environment, especially in food and water sources (Brassard et al., 2011). Animals are usually exposed to fipronil indirectly through consumption of contaminated feed or fodder or directly when applied as an ectoparasiticide. Both of these applications pose considerable risk to human health as well (Lee et al., 2010).

Lately, despite its low mammalian toxicity, concerns about the potential adverse public health effects of fipronil have raised and led it to be classified as a class C chemical, i.e., a possible carcinogen. Besides, fipronil is highly toxic to many non-target organisms such as honeybees, aquatic invertebrates, fishes, and birds (US EPA, 1996), and fipronil-sulfone metabolites (degradation products formed through oxidation) exert more toxic effects on insects and non-target species than the parent compound (Das et al., 2006). Additionally, fipronil desulfinyl, which is one of its main degradation products in vegetation, soil, and water, is very persistent and as equally or more toxic than fipronil itself, and may, therefore, bioaccumulate in organisms (Tingle et al., 2003).

Experimental toxicologic studies show that fipronil, apart from acting on the central nervous system (CNS), is also an EDC because it induces endocrine disruption and adversely affects the female reproductive function in rats by modifying their estrus cycle, reducing pregnancy incidence (Ohi et al., 2004), and disturbing their maternal aggressive behavior (Magalhães et al., 2015). In addition, fipronil also induces spermatoxicity in rats through DNA damage and apoptosis of the spermatozoa (Khan et al., 2015) and disrupts thyroid function that results in the formation of thyroid neoplasia secondary to hormone imbalance (Leghait et al., 2009). Other studies have shown that fipronil can alter normal regulation of the HPG axis in fathead minnow larvae (Beggel et al., 2012) and Japanese medaka larvae (Sun et al., 2014), thus impairing fish reproduction, apart from inducing developmental neurotoxicity in zebra fish (Stehr et al., 2006). *In vitro* studies have reported that an exposure to micromolar concentrations of fipronil and its sulfone and sulfide metabolites induced cytotoxicity in a human intestinal epithelial cell model (Caco-2 cells) and in the SHSY5Y neuronal cell line (Vidau et al., 2009, 2011). Furthermore, it affects the central behavioral response in rats (Terçariol and Godinho, 2011), triggers physiological and behavioral alterations in honey bees (Le Faouder et al., 2007), induces oxidative stress in the mouse kidney (Badgujar et al., 2015), rat liver (Mossa et al., 2015), and causes acute human poisoning (Fung et al., 2003; Mohamed et al., 2004). A recent dose-response study has evaluated the toxic impact of sub-lethal exposure to fipronil on Japanese quails at the doses of $^1/_2$, $^1/_5$ and $^1/_{10}$ of LD_{50}. It showed that fipronil increased the percentage of DNA damage and histopathological alterations in liver tissue particularly in $^1/_2$ LD50 group (Mohammed et al., 2016). Published data demonstrate that there is a high species-specific variability in fipronil sensitivity in the few avian species studied, which ranges from being highly toxic in Galliformes (LD_{50} 11.3, 31 and 34 mg/kg for bobwhite quail, ring-necked pheasant, red-legged partridge, respectively) to non-toxic in the mallard duck (LD_{50} > 2150 mg/kg) (US EPA, 1996). This variability makes the prediction of fipronil toxicity in unexplored species exceedingly difficult.

The Japanese quail *(Coturnix japonica)* is considered an ideal biological and experimental model due to its quick development. As a research animal, the Japanese quail has been widely used in reproductive toxicity testing. Quails are considered as the representative of terrestrial birds and are acknowledged models for evaluating the effects of pesticides and different chemicals in wild birds (EPA, 1996). Spermatogenesis in quail is well-characterized (Lin and Jones, 1992). Additionally, quails have well-developed neuroendocrine systems that share fundamental properties with other vertebrate species, including mammals (Ball and Balthazart, 2010).

Avian exposure to fipronil occurs mainly by consumption of contaminated seeds and insects (EPA, 2001). There are only a few reports on the toxicological effects of fipronil in avian species, and very limited information is available on the morphological, reproductive, and behavioral responses to fipronil ingestion. For these reasons, we used the adult male Japanese quail (*Coturnix japonica*) as a model to determine the *in vivo* toxic effects of oral dosing of fipronil on growth, energy utilization, behavioral response, reproductive function, interrelated hormonal changes, and testicular tissue architecture. We also examined the possible endocrine disrupting effects of fipronil exposure by evaluating molecular changes in genes that serve as appropriate molecular biomarkers for endocrine disruption such as the vitellogenin (Vtg) and estrogen receptor α (ERα) genes. Additionally, a subsequent 60-day recovery period was considered to evaluate the possible reversal of the altered responses to baseline levels.

2. Materials and methods

2.1. Chemicals and biochemical reagents

Fipronil insecticide (98.5%–98.8% purity, CAS No. 120068-37-3) was provided by Sigma-Aldrich Co. (St. Louis, MO, USA). Fipronil was suspended in corn oil and used for gavage feeding in the experiment. All other chemicals and reagents were of the highest available commercially grade and were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Housing and rearing of birds

Seventy-two adult males (10 weeks old) and 72 receptive egg-laying females (12 weeks old) Japanese quail (*Coturnix japonica*) were obtained from the Poultry farm, Faculty of Agriculture, Zagazig University, Egypt. Prior to the experiment, the quail was acclimatized for two weeks, wherein the birds were held under a controlled photoperiod (12 h light/dark cycle) at 25 ± 2 °C with a relative humidity of 50 ± 5%. The birds were provided with standard breeder ration (Al-Qahera Feeds, Egypt) and water *ad libitum*. The experiments were conducted strictly according to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH, USA) and the study protocol was approved by the local authorities of Cairo University, Egypt.

2.3. Experimental groups and treatments

The male birds were randomly divided into two groups. The birds in group 1 (n = 12) (control birds) were administered the corn oil (vehicle) alone using a gastric tube and those in group 2 (fipronil group) (n = 60) received fipronil orally at a dose equivalent to $^1/_5$ LD_{50} (2.26 mg/kg; LD_{50} = 11.3 mg/kg (Tingle et al., 2003), daily, for 15 days. After the exposure period of 15 days, 12 birds were sacrificed in the fipronil group and the remaining 48 birds were used in the recovery study. These birds divided into four equal groups (n = 12 each), such that, after the 15-day exposure, group R_{15} had 15 days of recovery, R_{30} had 30 days of recovery, R_{45} had 45 days of recovery, and R_{60} had 60 days of recovery. Throughout the study, feed consumption was recorded to estimate average feed intake,