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Neonicotinoid insecticides imidacloprid, guadipyr, and cycloxaprid induce acute oxidative stress in *Daphnia magna*

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ABSTRACT

Cycloxaprid (CYC) and guadipyr (GUA) are two new and promising neonicotinoid insecticides whose effects on *Daphnia magna* are as yet unknown. In this study, the acute toxicities of CYC and GUA to *D. magna,* including immobilization and embryo-hatching inhibition, and their effects on antioxidant enzymes and related gene expression were determined after a 48-h exposure. Imidacloprid (IMI) was evaluated at the same time as a reference agent. The 48-h EC_{50} values of IMI, GUA, and CYC for neonate immobilization were 13.0–16.5 mg/L and for embryo hatching were 11.3–16.2 mg/L. The specific activity of the enzymes superoxide dismutase (SOD) and catalase (CAT) were interfered by IMI, but not by GUA and CYC, while the activity of acetylcholinesterase (AChE) was significantly increased by IMI, but inhibited by GUA and CYC. The relative expressions of the *Sod-Cu/Zn*, *Sod-Mn*, *Cat*, and *Ache* genes were usually inhibited by IMI, GUA, and CYC, except for *Cat* by CYC, *Ache* by GUA, and *Sods* by IMI. For vitellogenin genes with a SOD-like domain (*Vtg1/2-sod*), relative expression was increased by IMI and inhibited by GUA and CYC, indicating that IMI, GUA, and CYC have potential toxicity toward reproduction. CYC and GUA are highly active against IMI-resistant pests, and considering the similar toxicity of IMI to *D. magna*, CYC and GUA are suitable for use in future integrated pest management systems.

1. Introduction

Neonicotinoid insecticides are one of the most important classes of synthetic pesticides and have outstanding potency in protecting crops against insects with piercing-sucking mouthparts (Casida, 2011). These insecticides are not intended for use in water, but they may pass into water bodies by spray drift, leaching or runoff potential after application (Morrissey et al., 2015). It is well known that in polluted ecosystems, such as soil, surface water, and groundwater, there are various risk factors for the health of humans and other non-target organisms that are hard to identify or assess simply. Pesticides, including neonicotinoid insecticides, are one of the most widely detected classes of pollutants in aquatic systems, and might present risks to non-target organisms or humans, following direct or indirect exposure (Tisler et al., 2009; Van der Sluijs et al., 2015).

Acute toxicity testing of chemicals to non-target organisms is mandatory during the pesticide registration process and studies have shown that neonicotinoids are potential hazards to aquatic systems. Thiamethoxam is a highly leachable compound and has frequently been detected in surface waters close to agricultural areas. Studies have shown that thiamethoxam is highly toxic to *Chironomus riparius*, with a 48-h median lethal concentration (LC_{50}) of 86.41 μg/L, and can reduce catalase (CAT) activity and lipid peroxidation levels (Saraiva et al., 2017). Imidacloprid (IMI) was the first neonicotinoid launched commercially, and generally has a very low acute toxicity to standard aquatic species such as *Daphnia magna*, rainbow trout, and zebrafish (Sánchez-Bayo et al., 2016). Nevertheless, IMI could inhibit the mobility of *D. magna*, interrupt the activity of the enzymes cholinesterase (ChE), glutathione *S*-transferase (GST), and CAT (Jemec et al., 2007) and impact the life traits of daphnids (Pavlaki et al., 2011).

Abbreviations: IMI, imidacloprid; GUA, guadipyr; CYC, cycloxaprid; CAT, catalase; *D. magna*, *Daphnia magna*; SOD, superoxide dismutase; AChE, acetylcholinesterase; *Sod-Cu/Zn*, gene of copper/zinc superoxide dismutase; *Cat*, gene of catalase; Ache, gene of acetylcholinesterase; *Vtg1/2-sod*, gene of *D. magna* dmagvtg1/2 vitellogenin fused with superoxide dismutase

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Neonicotinoids have attracted considerable attention in recent years, first due to their rapid growth in the marketplace, second due to the significant increase in the resistance of target insects, and third for their high toxicity to bees (Blacquière et al., 2012; Nauen and Denholm, 2005; Simon-Delso et al., 2015). Acetamiprid, thiacoprid, dinotefuran, and nitenpyram are newly developed and popular neonicotinoid insecticides that have entered the market in recent years to control many pests. Their toxicity to non-target organisms, like *D. magna*, fish, or bees varies. As shown in Supporting Table 3-1, they all have low toxicity to invertebrates and fish, but can remain in the water phase for many days, which might cause sublethal effects on these non-target organisms or even whole aquatic systems. Previous reviews and some databases have provided the most complete set of low mammalian toxicology information for the neonicotinoids (Supporting Table 3-2) (Sheets et al., 2016). Considering these advantages, the development of novel neonicotinoids and the comprehensive collection of their ecotoxicology data has generated much interest.

Guadipyr (GUA) and cycloxaprid (CYC) are two newly developed neonicotinoid insecticides that are as highly effective as IMI towards aphids (Liu et al., 2015a, 2015b; Qi et al., 2013; Su et al., 2012). As new active ingredients, data regarding their ecotoxicology and environmental fate are still lacking. Our earlier studies showed that GUA had low toxicity to *D. manga*, but could adjust the activity of acetylcholinesterase (AChE), GST, and CAT under acute conditions and delay maturation and inhibit reproduction at sub-lethal concentrations following exposures of less than 14 days (Qi et al., 2013). Liu et al. (2015a, 2015b) showed that the biota-sediment accumulation factor of CYC is between 0.59 and 0.82, which suggests its immobilization and easy degradation in the soil. Additionally, the elimination of CYC individual enantiomers from the earthworm *Metaphire guillelmi* was fitted to an availability-adjusted decay model, with a half-life of 9 days (Liu et al., 2015a, 2015b). This is the only available information regarding the impact of CYC on aquatic organisms.

Neonicotinoids present risks for aquatic organisms. It is therefore important to thoroughly assess the risks of these agrochemicals toward aquatic organisms and elucidate the response patterns of organisms at different levels. The aims of this study were: (1) to investigate the acute effects of IMI, GUA, and CYC on *D. magna*, including immobilization, embryo hatching, and development, (2) to assess and compare the effects of IMI, GUA, and CYC on changes in the activity of CAT, superoxide dismutase (SOD), and AChE, and (3) to evaluate the expression of the related genes *Cat*, *Sod-Cu/Zn*, *Sod-Mn*, *Ache*, and two vitellogenin genes containing the Sod-like domains (*Vtg1-sod* and *Vtg2-sod*). The data collected provide an insight into the detoxification mechanisms.

2. Materials and methods

2.1. Chemicals

IMI (97% purity) was obtained from the Zhejiang Haizheng Company (China). CYC (> 99% purity) and GUA (97% purity) were provided by the East China University of Science and Technology and China Agricultural University, respectively. The structures of these chemicals are shown in Fig. 1. Chromatographically pure acetone was used as a solvent to prepare aqueous solutions of the three pesticides.

In the water-based analyses, analytical grade formic acid was used, while both acetonitrile and water were chromatographically pure. All other chemicals used for the enzyme activity tests were of the highest commercially available purity and were purchased from the Beijing Chemical Regent Company (China).

2.2. Test organisms

The culturing and maintenance of *D. magna* was as described in our earlier studies (OECD, 2004; Qi et al., 2013). Generally, *D. magna* were fed daily with 3.0×10^6 cells/mL of *Scenedesmus obliquus* in dechlorinated tap water (pH 7.5 ± 0.5, dissolved oxygen > 5.8 mg/L, hardness of 250 ± 50 mg/L $CaCO_3$) and maintained at 21 ± 1 °C, with a photoperiod at 16 h light: 8 h dark. The culture medium was renewed three times a week, and juveniles less than 24 h old were used for toxicity tests.

2.3. Acute toxicity tests

The acute toxicity tests of IMI, CYC, and GUA to *D. magna* were performed in the laboratory according to the modified OECD guideline 202 (OECD, 2004; Qi et al., 2013). Based on range-finding tests, 0–100% deaths of juveniles after exposure to IMI, GUA, and CYC occurred between concentrations of 1–20 mg/L. Thus, five nominal concentrations of 1.25, 2.5, 5.0, 10.0, and 20.0 mg/L plus one blank control and a solvent control (1% acetone, v/v) of IMI, GUA, and CYC were used for the definitive test. Three replicates × 10 daphnids were placed in a 50-mL glass beaker containing 30 mL of test solution for 48 h under static conditions. The 48-h half maximal effective concentration (EC_{50}) values of the pesticides on immobilization were calculated using probit analysis.

2.4. Embryonic toxicity test

Collection of embryos from adult *D. magna* was performed according to the procedure used by Ton et al. (2012). To calculate the EC_{50} of IMI, GUA, and CYC on embryo hatching, a range finding test was also performed and the 0–100% hatching inhibition concentrations were also between 1–20 mg/L for each pesticide. Thus, four concentrations of 2.5, 5.0, 10.0, and 20.0 mg/L of IMI, GUA, and CYC (1.25 mg/L had no obvious effect) were used for the embryonic toxicity test. After collection and washing, embryos were randomly transferred into 24 wells of tissue culture plates containing 1.5 mL of control medium or each test solution. The plates were incubated under the same culture conditions as the *D. magna*. Embryo hatching and development were observed at 48 h under an Aigo digital viewer GE-5 computer system. The 48-h EC_{50} values of the pesticides for hatching rate were used as endpoints for the analysis of embryo toxicity.

2.5. Determination of enzyme activity and gene transcription

One aim of this research was to assess and compare the differences in enzyme activity due to IMI, CYC and GUA exposure. Based on the

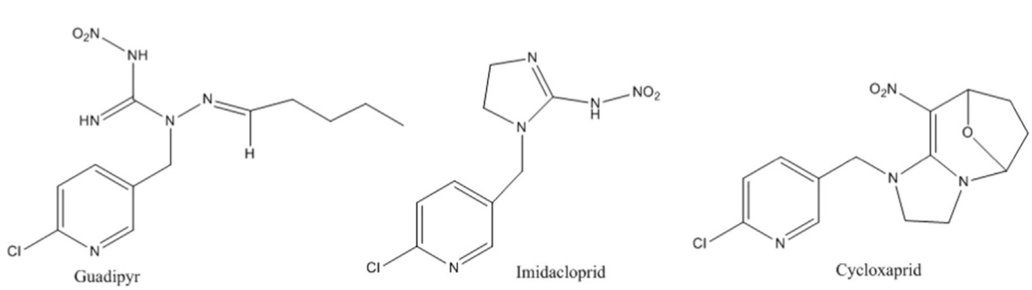

Fig. 1. Chemical structures of guadipyr (GUA), imidacloprid (IMI), and cycloxaprid (CYC).