Chemosphere 199 (2018) 16–25

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Developmental toxicity and neurotoxicity of synthetic organic insecticides in zebrafish (*Danio rerio*): A comparative study of deltamethrin, acephate, and thiamethoxam

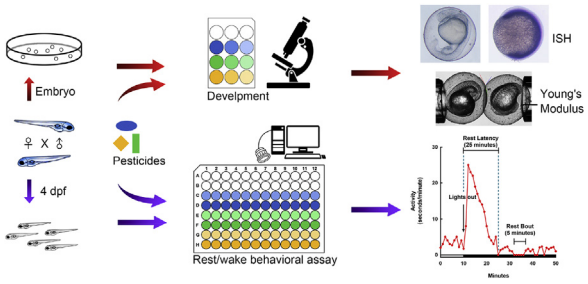
XingYu Liu [a, 1], QiuPing Zhang [b, 1], ShiBao Li [c, 1], Ping Mi [a], DongYan Chen [b, **], Xin Zhao [c, ***], XiZeng Feng [a, *]

[a] State Key Laboratory of Medicinal Chemical Biology, The Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Science, Nankai University, Tianjin 300071, China
[b] Tianjin Key Laboratory of Tumor Microenvironment and Neurovascular Regulation, Department of Histology and Embryology, School of Medicine, Nankai University, Tianjin 300071, China
[c] The Institute of Robotics and Automatic Information Systems, Nankai University, Tianjin 300071, China

## HIGHLIGHTS

- Deltamethrin and acephate can induce zebrafish developmental delay and malformation.
- Deltamethrin and acephate can decrease embryonic surface tension.
- Deltamethrin, acephate, thiamethoxam induce larval locomotor activity increase.
- Embryonic exposure to deltamethrin or acephate damages CaP axons.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 8 December 2017
Received in revised form
25 January 2018
Accepted 31 January 2018

Handling Editor: A. Gies

*Keywords:*
Synthetic organic insecticides
Zebrafish
Embryonic development
Locomotor behavior
Neurotoxicity

## ABSTRACT

Synthetic organic insecticides, including pyrethroids, organophosphates, neonicotinoids and other types, have the potential to alter the ecosystems and many are harmful to humans. This study examines the developmental toxicity and neurotoxicity of three synthetic organic insecticides, including deltamethrin (DM), acephate (AP), and thiamethoxam (TM), using embryo-larval stages of zebrafish (*Danio rerio*). Results showed that DM exposure led to embryo development delay and a significant increase in embryo mortality at 24 and 48 h post-fertilization (hpf). DM and AP decreased embryo chorion surface tension at 24 hpf, along with the increase in hatching rate at 72 hpf. Moreover, DM caused *ntl*, *shh*, and *krox20* misexpression in a dose-dependent manner with morphological deformities of shorter body length, smaller eyes, and larger head-body angles at 10 μg/L. TM did not show significant developmental toxicity. Furthermore, results of larval rest/wake assay indicated that DM (>0.1 μg/L) and AP (0.1 mg/L) increased activity behavior with different patterns. Interestingly, as an insect-specific pesticide, TM still could alter locomotor activity in zebrafish larvae at concentrations as low as 0.1 mg/L. Our results indicate that

* Corresponding author.
** Corresponding author.
*** Corresponding author.
*E-mail addresses:* chendy@nankai.edu.cn (D. Chen), zhaoxin@nankai.edu.cn (X. Zhao), xzfeng@nankai.edu.cn (X. Feng).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.chemosphere.2018.01.176

different types of synthetic organic insecticides could create different toxicity outcomes in zebrafish embryos and larvae.



## 1. Introduction

The application of insecticides is considered as one of the leading factors in the rise of agricultural productivity in the 20th century (Aktar et al., 2009). Since the 1940s, a great deal of synthetic organic insecticides has Cbeen brought into use. As of 2014, more than 1055 types of active ingredients have been registered as pesticides, and over 16,000 pesticide formulations have been put into the application (Chen et al., 2014). These insecticides possess advantages of high efficacy, crop safety, and wide-ranging applications. However, most insecticides have the potentiality to change ecosystems significantly (Stoate et al., 2009), and many are harmful to human beings and can be bioaccumulated along the food chain (Katagi, 2010).

There are various types of synthetic organic insecticides, including pyrethroids, organophosphates, neonicotinoids, etc. Deltamethrin (DM), a second-generation pyrethroid insecticide, was first synthesized in 1974 (Elliott et al., 1974) and has become a popular choice for agricultural and home pest control. The chemical structure of DM is similar to natural insecticide pyrethrins, the active constituent of pyrethrum *Chrysanthemum cinerariifolium*. Acephate (AP), an organophosphorus insecticide, was initially introduced in 1971 by Chevron Chemical (Magee, 1973). The compound performs its function as a weak acetylcholinesterase inhibitor (Spassova et al., 2000). Thiamethoxam (TM), a second-generation neonicotinoids insecticide, possesses a broad activity spectrum and high target specificity against types of insects, and its risk to non-target mammalian species is relatively low (Tomizawa and Casida, 2005).

It has been reported that the levels of TM in surface water were below 0.0165 mg/L (Schaafsma et al., 2015), and DM in groundwater was below 0.104 μg/L (Shakerkhatibi et al., 2014). To our knowledge, few data of AP in the environment were found. Although the concentrations of insecticides can be very low in the water environment, insecticide users could be exposed to higher levels. Previous studies have shown that DM metabolites were found in the urine from pregnant women from an agricultural area (Castorina et al., 2010; Qi et al., 2012) and children under residential pesticide exposure (Lu et al., 2006). Also, Osaka et al. (2016) found TM metabolites in the urine from young children. Therefore, exploring the consequences of synthetic organic insecticides exposure during development is crucial. Richardson et al. (2015) evaluated the behavioral effects in 6-week-old male mice after developmental exposure to DM, and observed increased locomotor activity and defected working memory. In addition, hyperactivity and fish surfacing were observed in adult zebrafish after DM exposure (Huang et al., 2014). As for AP, administration of 28 mg/kg/day AP to female mice by gavage for 4 weeks resulted in maternal and developmental defects (Farag et al., 2000). Also, methamidophos, an organophosphate insecticide as AP, caused neurotoxicity in the early stages of zebrafish by the effects on neurodevelopmental genes and the cell apoptosis activation in the brain (He et al., 2016). Besides, anxiety behavior increase was observed in rats after TM treatment (50 or 100 mg/kg) for seven consecutive days (Rodrigues et al., 2010). Early developmental exposure to imidacloprid, a neonicotinoid insecticide as TM, decreased swimming activity in zebrafish larvae (Crosby et al., 2015). Together, these studies suggest that synthetic organic insecticides may possess developmental neurotoxicity and finally cause neurobehavioral deficits.

As a vertebrate organism, zebrafish has been one of the promising models for research in developmental processes (Olivares et al., 2016), drug discovery (MacRae and Peterson, 2015), neuroscience (Stewart et al., 2014), and chemical toxicity (Abe et al., 2017; Hanigan et al., 2017; Velki et al., 2017). This organism has strong fecundity and high genetic and physiological similarities to mammals (Sood et al., 2006). Compared with adult zebrafish, embryos and larvae are more proper for high-throughput and large-scale studies. Moreover, zebrafish have been used to evaluate the developmental toxicity (DeMicco et al., 2010; Pamanji et al., 2015) and neurotoxicity (Eddins et al., 2010; Truong et al., 2016) of various insecticides. However, to the best of our knowledge, there have been no studies on the effects of AP or TM exposure on zebrafish larval neurobehavior. Hence, we chose zebrafish of embryo-larval stages to conduct the comparative research of three synthetic organic insecticides in embryonic development and larval neurobehavior.

In this study, a wide range of concentrations (0.01 μg/L to 100 μg/L of DM, and 0.01 mg/L to 100 mg/L of AP and TM) was applied to investigate the developmental and neurobehavioral toxicity effects of the three synthetic organic insecticides. Developmental effects on zebrafish embryos and larvae were examined by mortality, morphological analysis, and gene expression. Furthermore, a high-throughput and rapid video tracking system was used to monitor to evaluate the neurobehavior impact on zebrafish larvae of the synthetic organic insecticides. Finally, the immunofluorescence assay of larval motoneuron at 28 hpf was conducted to assess the motoneuron damage caused by the synthetic organic insecticides. Together, the results of this research could provide a reference for the assessments of the three synthetic organic insecticides.

## 2. Materials and methods

### 2.1. Zebrafish

Adult zebrafish (strain AB) were maintained in a standard circulating water system (KCl 0.05 g/L, NaHCO3 0.025 g/L, NaCl 3.5 g/L, and CaCl2 0.1 g/L, pH 7.0–7.2) on a cycle of 14:10-h light: dark photoperiod at 28.5 °C. All experimental procedures involving zebrafish were approved by the Committee for Animal Experimentation of the College of Life Science at Nankai University (no.2008) and were conducted by the NIH Guide for the Care and Use of Laboratory Animals (no.8023, revised in 1996).

### 2.2. Chemicals and insecticide exposures

DM (purity 99.0%), AP (purity 99.1%), and TM (purity 98.2%) were purchased from Beijing Qinchengyixin Technology Co. Ltd (Beijing, China). DM was initially dissolved in DMSO at 1000 mg/L, and TM and AP were dissolved in standard system water at 1000 mg/L, respectively. Stock solutions were stored in darkness at 4 °C. Serial dilutions were prepared in system water to yield final levels of 0.01, 0.1, 1, 10, 100 mg/L for TM and AP respectively, and