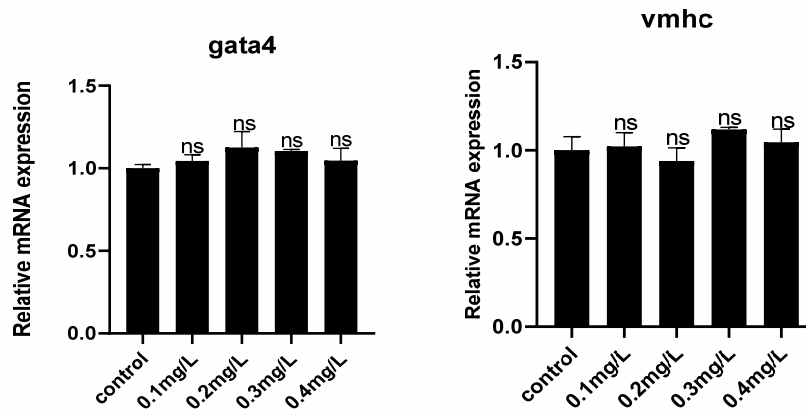


**Figure S1**

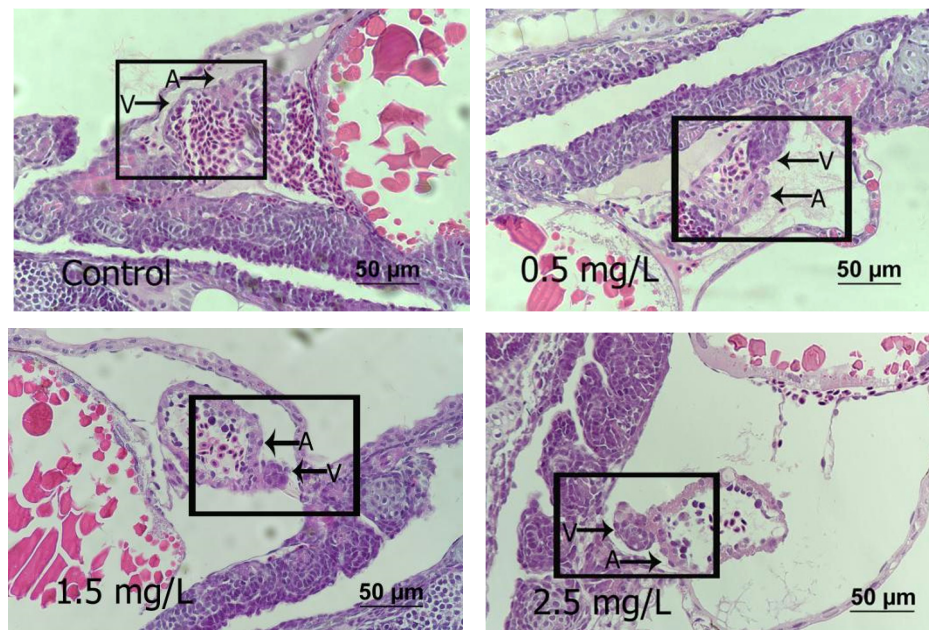


**Figure S1 Effect of diflubenzuron exposure on mRNA expression of the genes related to heart development**

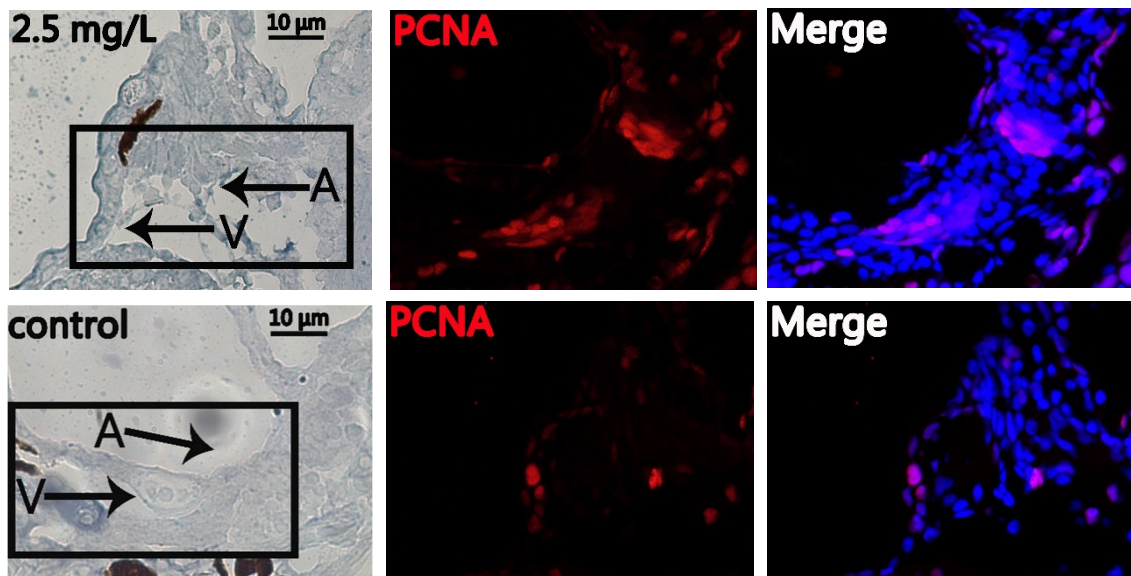
The transcription of *gata4* and *vmhc* in zebrafish embryos after exposure to various concentrations of diflubenzuron (0, 0.1, 0.2, 0.3 and 0.4 mg/L) at 96 hpf. Each bar represents mean  $\pm$  SD (n=24) of three independent experiments. *ns*: no significant difference.

Figure S2

(A)



(B)

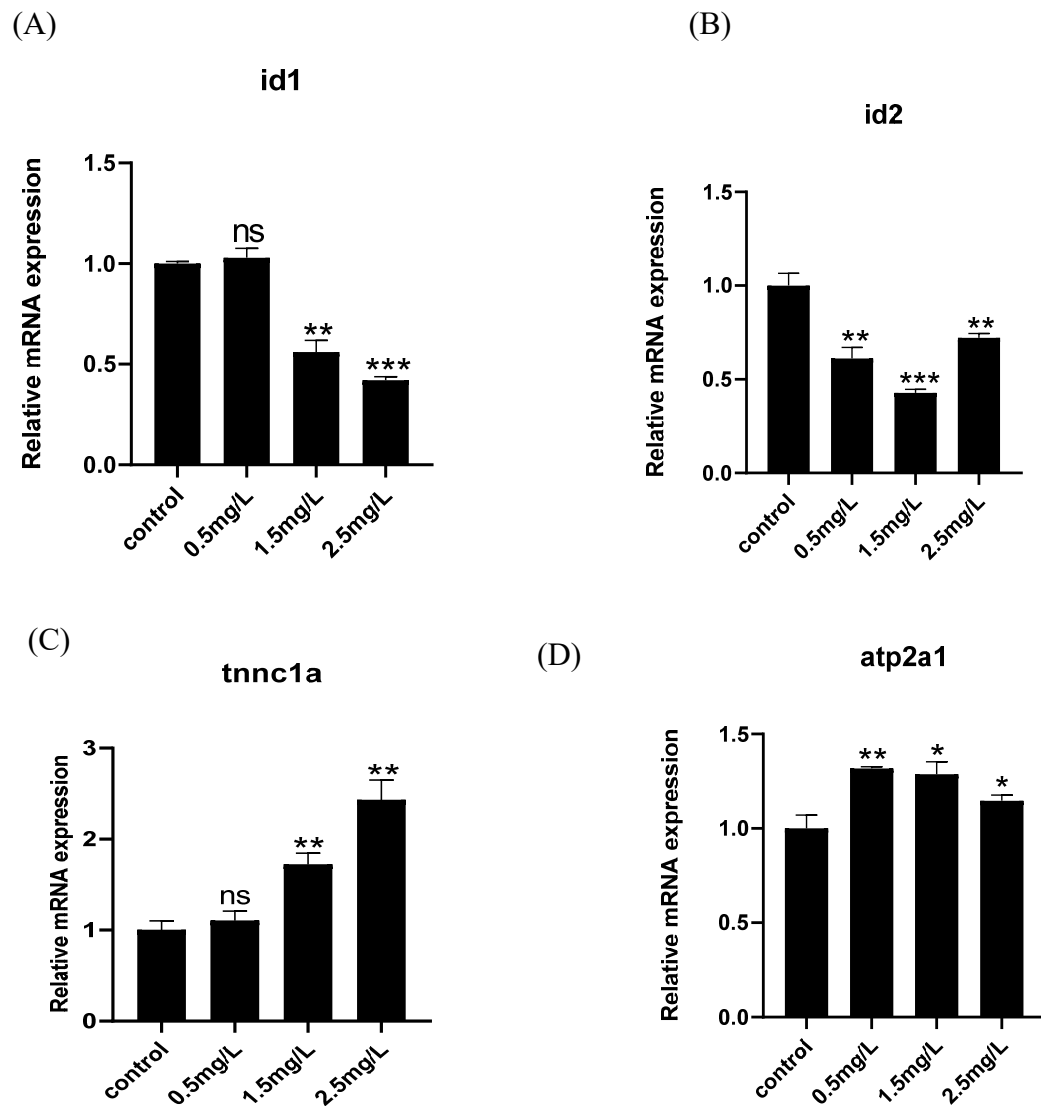


**Figure S2 Exposure to diflubenzuron induces cardiac developmental toxicity in zebrafish embryos.**

H&E staining of heart sections in control and 0.5, 1.5 and 2.5 mg/L diflubenzuron-exposed zebrafish at 96 hpf (A), The heart slices were prepared, incubated with PCNA

primary antibodies and stained with cy3-tagged second antibodies **(B)**. DAPI was used to stain the nucleus. Each of experiment was repeated at least three times.

Figure S3



**Figure S3 Exposure of diflubenzuron affects the gene expression of embryos in BMP signaling pathway and calcium dependent signaling pathway**

Q-PCR was used to detect the genes in BMP signaling pathway (*id1* and *id2*) (**A and B**) and calcium dependent signaling pathway (*tnnc1a* and *atp2a1*) (**C and D**). Each bar represents mean  $\pm$  SD (n=24) of three independent experiments. Asterisks indicate significant differences from control, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Table S1

**Sequences of the qRT-PCR primers used in this study.**

Target gene	Prime sequence (5'-3')
$\beta$ -actin-F	AGCACGGTATTGTGACTAACTG
$\beta$ -actin-R	TCGAACATGATCTGTGTCATC
nppa-F	GCTCCTGGTTTGGCAGCAG
nppa-R	GAGCTGCTGCTTCCTCTCGG
gata4-F	CTACAGGCACCCCAGCAGA
gata4-R	AGAGCCCGAGACCCGAAAT
vmhc-F	GAGCTTGATGAGGCAGAAG
vmhc-R	CAGCATAACGGAGACACAG
tbx5-F	AGTGACAGCGAACC AAAAG
tbx5-R	GTCTGGATGGACATAAAGC
myh6-F	CACCAGCAGACACTGGATG
myh6-R	GCTCCAAGTCCATTCTGAC
tbx2b-F	GGGGAACAATGGATGGCTAA
tbx2b-R	CCTAAGTGGGCTGGAAACC
klf2a-F	CCGTCTATTTCCACATTTTCG
klf2a-R	TCCAGTTCATCCTTCCACCT
bax-F	TCGAACATGATCTGTGTCATC
bax-R	TATGGCTGGGGTCACTTTTCTC
bcl2-F	TGGCGTCCCAGGTAGATAAT
bcl2-R	ACCGTACATCTCCACGAAGG
p53-F	CCCGGATGGAGATAACTTG
p53-R	CACAGTTGTCCATTCAGCAC
casepase3-F	GAGACCGCTGCCCATCACTAG
casepase3-R	ATCCTTTCACGACCATCT
casepase9-F	GCCTTTCTTGATTCCCTGCG
casepase9-R	TCTTGGCCTGGTTGGTCTCA
id1-F	CAGCAAAGTTGGAGGAGAGG
id1-R	AGCCGTTCTCCACAGAGATGCT
id2-F	GAGTGTAACGACGACGGAGC
id2-R-F	TGATGCAGGCTGGTTATCGC
bmp4-F	GGTCATTTTATTATGCCAAGT
bmp4-R	AAACGAATCGCAGAGGAGT
atp2a1-F	CCGACAAA ACTGGCACCTTG
atp2a1-R	CTGCAGTCAACTTTGGCACC
tnnc1a-F	GGCAGAGCAACTCACCGAT
tnnc1a-R	GTAGGGTTCTGGCCCAACAT