Antidepressants in the environment

Evaluation of embryotoxicity in zebrafish

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Antidepressiva läkemedel i miljön: utvärdering av embryotoxicitet hos zebrafisk

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Abstract

The increasing presence of antidepressants in aquatic environments has raised concerns about their ecotoxicological effects. This study investigates the developmental and behavioural effects of an environmentally relevant mixture of seven commonly detected antidepressants (Citalopram, Sertraline, Paroxetine, Mirtazapine, Amitriptyline, Venlafaxine, and Desvenlafaxine) on zebrafish embryos (*Danio rerio*). The exposure consisted of a dilution series based on concentrations reported in Swedish rivers. Zebrafish embryos were exposed from ~0 to 144 hours post fertilization (hpf) following OECD Test *No.* 236 (with additional endpoints). A ZFET test was conducted to test developmental endpoints, including early movement, heart rate, hatching time, and morphology. At 144 hpf, a light/dark test was performed to evaluate behaviour. Significant effects were observed at the highest exposure level (10 000 times higher than the environmental concentrations), including reduced early movement ($p \leq 0.05$), accelerated hatching ($p \leq 0.001$), and a decrease in swim distance during darkness ($p \leq 0.001$). No significant morphological deformities or mortality were detected across exposure groups.

The results may indicate a potential disruption of neurogenesis, increasing the risk for impaired development and a decline in fitness. Furthermore, the change in swim distance in darkness suggests a dampened stress response. However, at lower concentrations, the swim distance increased, albeit not significantly. The results may indicate an allocation of resources from non-essential to vital functions in the highest exposure group, keeping the larvae alive. This study highlights the need for further testing at lower concentrations, as well as the potential ecological risk in surface water with high pollution from pharmaceutical industries.

Keywords: Zebrafish, *Danio rerio*, ZFET test, Embryotoxicity, Pharmaceutical contamination, Antidepressants, Multi-compound exposure.

Abstract (Swedish/Svenska)

Den ökande förekomsten av antidepressiva läkemedel i akvatiska miljöer har väckt oro angående deras ekotoxikologiska effekter. Denna studie undersöker effekter på tidig utveckling och beteende hos zebrafiskembryon (*Danio rerio*) efter exponering av en miljörelevant blandning av sju vanligt förekommande antidepressiva ämnen: Citalopram, Sertralin, Paroxetin, Mirtazapin, Amitriptylin, Venlafaxin och Desvenlafaxin. Exponeringen bestod av en spädningsserie baserad på koncentrationer rapporterade i svenska ytvatten. Zebrafiskembryon exponerades från ~0 till 144 timmar efter befruktning (hpf) baserat på OECD Test *nr*. 236 (med ytterligare mätparametrar). Ett ZFET-test genomfördes för att undersöka utvecklingsparametrar, inklusive tidiga rörelser, hjärtfrekvens, kläckningstid och morfologi. Vid 144 hpf genomfördes ett ljus/mörker-test för att utvärdera beteende. Signifikanta effekter observerades vid den högsta exponeringsnivån (10 000 gånger högre än koncentrationerna rapporterade i svenska ytvatten), gällande minskad tidig rörelse ($p \leq 0,05$), tidig kläckning ($p \leq 0,001$), och minskad simsträcka i mörker ($p \leq 0,001$). Inga signifikanta resultat av morfologiska missbildningar eller dödlighet observerades i exponerings-grupperna.

Resultaten indikerar en möjlig störning av neurogenesen, vilket ökar risken för nedsatt utveckling och minskad fitness. Vidare tyder förändringen i simsträcka under mörker på en dämpad stressrespons. Vid lägre koncentrationer ökade dock simsträckan, om än inte signifikant. Resultaten kan tyda på en omfördelning av resurser från icke-vitala till livsviktiga funktioner i den högsta exponeringsgruppen, vilket kan bidra till en större chans för överlevnad. Denna studie belyser behovet av ytterligare tester vid lägre koncentrationer, samt den potentiella ekologiska risken i ytvatten med hög föroreningsgrad från läkemedelsindustrin.

Nyckelord: Zebrafisk, *Danio rerio,* ZFET-test, Embryotoxicitet, Läkemedelskontaminering, Antidepressiva läkemedel.

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Abbreviations

Abbreviation	Description
↑	*Increased/longer*
↓	*Decreased/shorter*
BL	*Body length*
DDD	*Daily defined dose*
DMSO	*Dimethyl sulfoxide*
EC_{max}	*Maximum environmental concentration*
EC_{mean}	*Mean environmental concentration*
EM	*Early movement*
hpf	*Hours post fertilization*
HR	*Heart rate*
HT	*Hatching time*
LOEC	*Lowest observed effect concentration*
M	*Mortality*
NOEC	*No observed effect concentration*
OCD	*Obsessive compulsive disorder*
OECD	*The Organization for Economic Cooperation and Development*
SD_D	*Swim distance in darkness*
SD_L	*Swim distance in light*
SNRIs	*Serotonin-norepinephrine reuptake inhibitors*
SSRIs	*Selective serotonin reuptake inhibitors*
TCAs	*Tricyclic antidepressants*
ZFET	*Zebrafish embryo toxicity*

1. Introduction

In the past couple of years, the annual consumption of antidepressants in Europe has dramatically increased (OECD 2023). After excretion from the body, both active compounds and their metabolites pass through wastewater treatment plants, which are not equipped to remove them completely (Lunghi et al. 2025). Antidepressants are among the most commonly detected pharmaceutical contaminants in surface water. Sampling data from Sweden's three biggest lakes and 24 of their associated rivers revealed several antidepressant residues, including Citalopram, Sertraline, Paroxetine, Mirtazapine, Amitriptyline, Venlafaxine, and Desvenlafaxine (Malnes et al. 2022). Through different methods, such as animal testing, researchers have discovered that even at low concentrations, these contaminants could disrupt the development, behaviour, and survival of aquatic organisms.

1.1 Zebrafish as a model organism

Zebrafish (*Danio rerio*) is a useful model organism in toxicology. Other than being an affordable vertebrate model, its small size, large egg production, and rapid development make it an efficient model. Furthermore, zebrafish eggs are fertilized externally and are optically transparent during early life stages, which allows for direct observation of embryo development in vivo (Teame et al. 2019). Moreover, different endpoints, such as spontaneous coiling, heart rate, and behaviour, are easily recognised and quantifiable.

1.1.1 Normal development

After cell division and the formation of distinct tissues, the embryo's rudimentary organs begin to take shape. By 24 hours post fertilization (hpf), the body straightens from its curvature around the yolk sac, fins begin to form, and early pigmentation may be visible. At this time, early movements (i.e., spontaneous tail coiling) can be observed, but note that these muscle contractions are involuntary. Heartbeat may also be detected this early, but the circulation is still weak. By 48 hpf, the circulation becomes stronger, and the heart rate is easier to quantify. Zebrafish transition from the embryonic stage to the larval stage after hatching (at around 48-72 hpf) when incubated at a temperature of ~28°C. At this point, the basic structure of zebrafish morphology is almost complete. Other changes include swim bladder inflation and further development of the mouth and gut. In contrast to the embryonic stage, the early larvae will become more active and begin to swim, and move their pectoral fins, jaw, eyes, and operculum (the latter is related to respiration). These behavioural and physiological changes mark the onset of essential functions such as food-seeking and active avoidance behaviours (Kimmel et al. 1995).

1.1.2 Disrupted development

Aquatic organisms have receptors for SSRIs that have been conserved throughout evolution, meaning that these organisms might react similarly when exposed to SSRIs as humans (Riberio et al. 2015). Furthermore, serotonin is a significant signal molecule during early neurogenesis, such as the development of spinal motor neurons in zebrafish (Zindler et al. 2019). Assessing early movements after exposure to antidepressants can therefore be an indicative endpoint for neurotoxicity.

Environmental stressors, such as antidepressants, can have a negative influence on developmental milestones, e.g. the hatching of embryos. By modifying their hatching time, embryos can enhance their survival by weighing the protective benefits of staying within the chorion against the potential advantages of becoming free-swimming larvae. However, early hatching can result in underdeveloped larvae, thereby reducing their fitness (Wisenden et al. 2022).

1.1.3 Behaviour

A common behavioural test is the light/dark test, where zebrafish larvae are exposed to alternating periods of light and dark. A sudden change from light to dark elicits a stress-like response with an instant increase in swimming velocity and distance. The opposite reaction is usually seen when shifting from dark to light environments. Then, an immediate halt in activity is typically observed. The dark period evaluates how larvae respond to stress, while the light period gives insight into how well the larvae stabilise after being exposed to the stressor. The light/dark test is useful in drug screening, especially when testing substances affecting behaviour (Rock et al. 2022). Furthermore, measuring swim distance (regardless of lighting) can show a potential effect on the overall locomotion of the larvae.

1.2 Antidepressants: classification and effect

Antidepressants are divided into different classes based on their mechanism of action. They work by inhibiting the reabsorption of neurotransmitters, such as serotonin, norepinephrine, and dopamine, making these mood-regulating signal molecules more abundant in the brain. The three classes used in this study are tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs).

1.2.1 Tricyclic antidepressants

TCAs (including Amitriptyline and Mirtazapine), represent an older class of antidepressants with a more diverse pharmacological use. These drugs are mainly used to treat depression in humans who do not respond well to SSRIs or SNRIs. TCAs can also be prescribed for treating chronic pain and insomnia. The primary mechanism of TCAs is the inhibition of serotonin- and norepinephrine transporters in the synapse, which will block their reabsorption and thereby increase the levels available in the brain. Unlike SNRIs and SSRIs, TCAs can have undesirable interactions with other receptor systems. It is believed that this unwanted effect is why they generally produce more side effects compared to the other two classes. TCAs have therefore fallen out of popularity as there are often better alternatives available (Fasipe 2018). However, they have increased in popularity in Sweden, with Mirtazapine being the most popular of the seven antidepressants used in this study (Swedish National Board of Health and Welfare 2025).

Sampling data from Swedish surface water showed that Amitriptyline and Mirtazapine had a mean environmental concentration (EC_{mean}) of 7.7 ng/L and 5.2 ng/L, respectively (Malnes et al. 2022). Both substances have been reported to elicit toxic effects in the zebrafish embryo toxicity (ZFET) tests at concentrations below the EC_{mean}. Amitriptyline has been found to cause a higher mortality rate and a decrease in body length after exposure to 1 ng/L (*Appendix 2*). At 10 ng/L, Amitriptyline also prolonged hatching time (Yang et al. 2014). Mirtazapine has shown similar effects, including a decrease in spontaneous coiling and total swim distance. These effects were observed below the environmental level, at 3.9 ng/L (Zhou et al. 2023).

1.2.2 Selective serotonin reuptake inhibitors

SSRIs are a popular class of antidepressants and include Citalopram, Paroxetine, and Sertraline. Besides depression, SSRIs are used to treat obsessive-compulsive disorder (OCD), anxiety, and bulimia. The main mechanism of action for SSRIs is the inhibition of serotonin reabsorption. Unlike TCAs and SNRIs, this class does not block norepinephrine reuptake. Side effects from SSRIs include anxiety, sleep disturbances, sexual dysfunction, gastrointestinal disturbances, and intense restlessness (Fasipe 2018).

The EC_{mean} for Sertraline, Citalopram, and Paroxetine were 4.8 ng/L, 9 ng/L, and 27 ng/L, respectively (Malnes et al. 2022), which are much lower than the reported lowest observed effect concentrations (LOEC) in ZFET tests. The no observed effect concentration (NOEC) for Citalopram has been observed at <20 000 ng/L. At ≥20 000 ng/L, Citalopram decreased the swim distance in darkness (*Appendix 2*) (Steele et al. 2018).

1.2.3 Serotonin-norepinephrine reuptake inhibitors

The antidepressant Venlafaxine and its active metabolite Desvenlafaxine are examples of SNRIs. Although Desvenlafaxine is not currently allowed for marketing in Sweden, significant traces of it were found in Swedish surface water (Malnes et al. 2022). When Venlafaxine is metabolised and excreted, about 29 % of it comes in the form of Desvenlafaxine (Singh D. & Saadabadi A. 2024), which could explain its presence in Swedish lakes and rivers. Other than treating depression, SNRIs can also be prescribed to treat generalized anxiety disorder, stress urinary incontinence, and vasomotor symptoms of menopause. SNRIs work by inhibiting the reabsorption of serotonin and norepinephrine. At low doses, they function similarly to SSRIs by selectively inhibiting serotonin reabsorption. As the dose increases, they also block the reuptake of norepinephrine. Small quantities of dopamine can be carried by norepinephrine transporters; therefore, inhibition by SNRIs can indirectly increase dopamine transmission, particularly in the prefrontal cortex, where dopamine transporters are scarce. However, this influence is weak and is only seen in higher doses of SNRIs. Even though TCAs also block norepinephrine transporters, their effect on dopamine transmission is not as apparent, presumably due to their broader spectrum of receptor interactions (Fasipe 2018).

Venlafaxine and Desvenlafaxine have been measured in surface water at concentrations of 58 and 25 ng/L, respectively, as reported by Malnes et al. (2022). Low concentrations (16 ng/L) of Venlafaxine have caused spinal deformities in the ZFET tests (*Appendix 2*) (Rodrigues et al. 2020). Changes in behaviour have also been reported after exposure to Venlafaxine. At 1 000 ng/L, Venlafaxine increased the total swim distance of zebrafish larvae but decreased after exposure to 10 000 and 100 000 ng/L in a dose-dependent manner (Tang et al. 2021). Desvenlafaxine has produced similar effects as its parent compound, Venlafaxine, but at a lower potency (Atzei et al. 2021).

1.3 The aim of the study

Most studies on antidepressants focus on single-compound exposures. Although informative, this approach does not reflect the complex mixture of compounds that aquatic organisms are typically exposed to in the environment. These chemicals may exert additive, synergistic, or antagonistic effects, making the process of risk assessment more complex. To address this, the current study investigated early developmental toxicity in zebrafish from exposure to a mixture of seven commonly detected antidepressants: Citalopram, Sertraline, Paroxetine, Mirtazapine, Amitriptyline, Venlafaxine, and Desvenlafaxine. The concentrations of each antidepressant were based on surface water concentrations previously reported by Malnes et al. (2022).

2. Method

2.1 Experimental design

Dimethyl sulfoxide (DMSO) was chosen as the solvent for the antidepressants. Each antidepressant was dissolved and mixed into a stock solution 100 000 000 times stronger than their EC_{mean}. A 1:10 dilution series was then made from the stock solution to create the exposure mixtures. For the embryo exposures, each mixture was diluted 1:10 000 in carbon-filtered tap water to minimize solvent exposure (i.e., 0.0001% DMSO). The strongest exposure mixture was 10 000 times stronger than the EC_{mean}, and the lowest was 10 times weaker. In total, six exposure mixtures were prepared (*Table 1*) and named based on their dilution factor from the EC_{mean}. Carbon-filtered water was used as the control for the solvent.

Table 1. Exposure mixtures with the respective antidepressant concentrations (ng/l)

Antidepressant	10 000x	1 000x	100x	10x	1x	0.1x
Amitriptyline	77 000	7 700	770	77	7.7	0.77
Citalopram	85 000	8 500	850	85	8.5	0.85
Desvenlafaxine	250 000	25 000	2 500	250	25	2.5
Mirtazapine	52 000	5 200	520	52	5.2	0.52
Paroxetine	270 000	27 000	2 700	270	27	2.7
Sertraline	48 000	4 800	480	48	4.8	0.48
Venlafaxine	580 000	58 000	5 800	580	58	5.8

2.1.1 Zebrafish Embryo Toxicity Test

The ZFET test was based on the protocol of OECDs test *No.* 236, but extended with additional endpoints from Carlsson et al. 2013 and Pohl et al. 2019. The eggs needed for the test were obtained by moving groups of adult zebrafish into a stainless-steel net cage inside a 10 L glass aquarium the day before starting the exposure. The next morning, the cages were transferred to new 10 L glass aquariums, where the fish were allowed to reproduce for 1 hour. Eggs were then collected, and the newly fertilized eggs were selected for the exposure studies. Thereafter, Petri dishes were prepared with 40 mL of carbon-filtered water and 4 µL of each exposure mixture, as well as for the DMSO solvent control. The same volume was used for the water control. In total, eight Petri dishes were prepared. There was no significant difference between the water and DMSO control. Thus, only the DMSO control was shown in the results.

Thereafter, 24 newly fertilized zebrafish embryos (4-16 cell stage) were added to each Petri dish. Individual embryos were then transferred into wells of two 96-well plates in a pre-planned randomized block design. Each well contained 250 µL of exposure solution. The plates were then covered with Parafilm and kept in a 12-hour light/dark cycle at a temperature of 26 ± 1 °C. The exposure started at ~0-4 hpf and continued until 144 hpf without renewing the exposure solutions.

2.1.2 Developmental quantifications

Observations on development were made using a stereo microscope (Leica EZ4D). At 24 hpf, early movements were assessed by counting the number of spontaneous coiling events per 30 seconds and then converting it to movements per minute. Similarly, heart rate was observed at 48 hpf by recording the time for 30 heartbeats and then converting the value to beats per minute (bpm). Observations of dead and deformed individuals were documented throughout the experiment. Starting at 24 hpf, the plates were observed via a microscope to count the newly hatched embryos. The observations were made at specific time points (24, 48, 52, 56, and 72 hpf) until all healthy embryos were hatched. The time points were chosen to correspond to the normal hatching period (48-72 hpf).

2.1.3 Light/Dark behaviour test

At 144 hpf the larval swim distance was measured using automated video tracking in the DanioVision system (Noldus, NL) and EthoVision 16 software (Noldus, NL). Each 96-well plate was placed in the Zebrabox recording chamber. The program was set to start with a 10-minute light period for acclimatization (swim distance was not recorded). Thereafter, the swim distance was recorded during four 5-minute alternating dark and light cycles. The overall sum of swim distance during dark periods, swim distance during light periods, and the total swim distance during both light and dark periods were calculated for each larva. Dead or malformed individuals were excluded from behavioural analysis.

2.1.4 Morphological measurements

After measurement of swim distance, the larvae were euthanized with 1 g/L of ethyl 3-aminobenzoate methane sulfonate salt (MS-222, Sigma-Aldrich). Thereafter, they were laid flat on filter paper to measure body length (mm), eye area, and swim bladder (μm^2) using the DanioScope 1.2 program (Noldus; NL).

2.2 Statistical analysis

Each embryo was treated as an individual experimental unit. Categorical data were analysed using Fisher's exact test to evaluate the prevalence of affected individuals. For data exhibiting a normal distribution, a one-way ANOVA was applied, followed by a two-sided Dunnett's post hoc test to determine statistically significant differences between the exposure groups and the control, with the significance set at $p \leq 0.05$. In cases of non-normal distribution, the Kruskal–Wallis test was used, followed by the Mann–Whitney U-test with Bonferroni correction applied to the p-values. The Kruskal–Wallis test was specifically employed for analysing hatching time and swim distance in the light environment, while the Mann–Whitney U-test was used to assess early movement. Data analysis was performed with Minitab 21. Statistical significance is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

3. Results

3.1 Early Movement

The highest exposure group (10 000x) showed a significant decrease ($p \leq 0.05$) compared to the control (*Fig. 1 and Appendix 1*). Early movements increased in groups 1x and 10x (non-significantly), before decreasing as the dose increased.

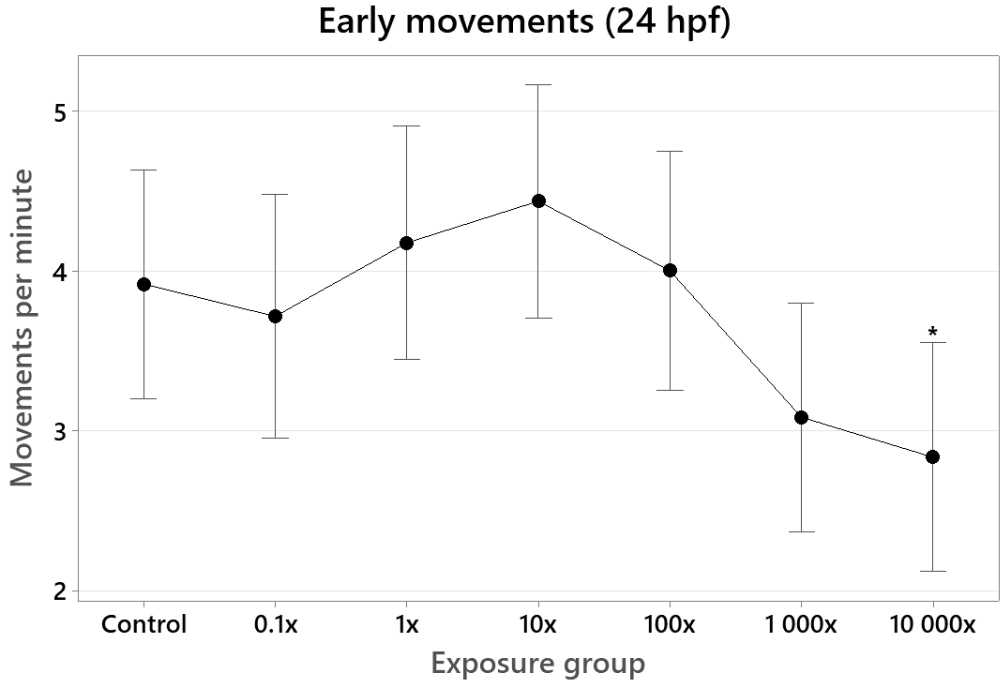

*Fig. 1. Early movements per minute in zebrafish embryos (24 hpf) per exposure group. Statistical significance is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.*

3.2 Heart Rate

There were no significant results in heart rate. However, there was an increase in the three highest exposure groups (100x, 1 000x, and 10 000x) compared to the control (*Fig. 2 and Appendix 1*). The results for exposure groups 0.1x to 10x were relatively similar to that of the control. The heart rate increased first after exposure >10x. The biggest increase compared to the control was ~5 % in the highest exposure group.

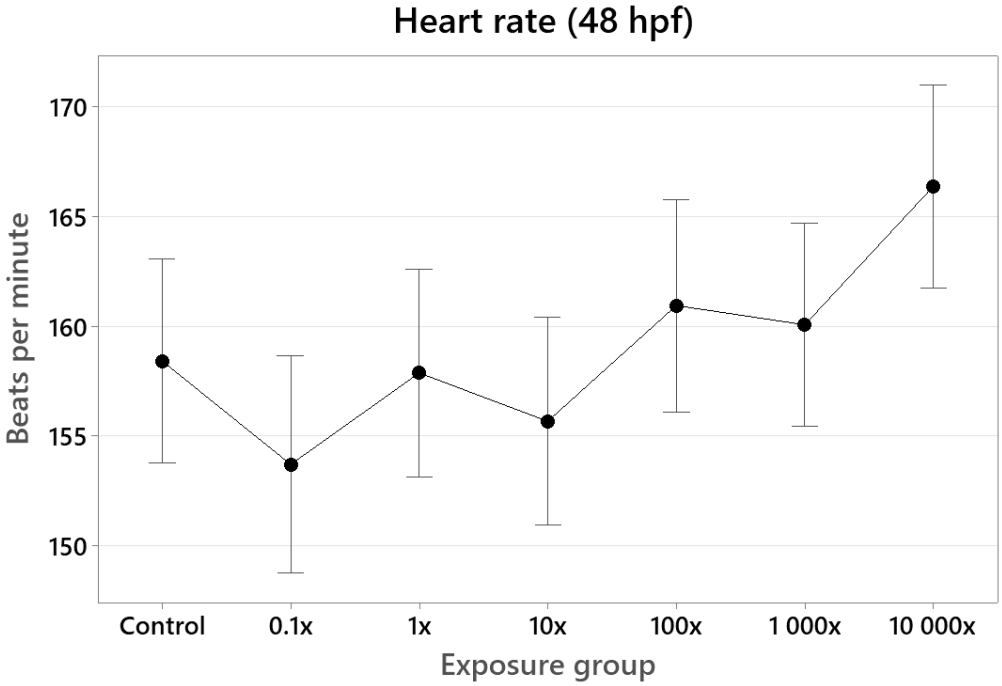

*Fig. 2. Mean heart rate (beats per minute) of zebrafish (48 hpf) per exposure group. Statistical significance is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.*

3.3 Morphology and Effect

There were no significant differences concerning body length (µm), eye area (µm^2), or swim bladder area (µm^2) in any exposure group. Similarly, there were no differences in malformations or mortality (*Appendix 1*).

3.4 Hatching time

None of the groups showed signs of delayed hatching. The time it took for embryos in the highest exposure group to hatch was significantly faster than that of the control ($p \leq 0.001$ *at 48 and 52 hpf,* $p \leq 0.01$ *at 56 hpf*) (*Fig. 3 and Appendix 1*). The results indicate a possible dose-dependent acceleration in hatching after the concentration reached levels 100 times stronger than the EC_{mean}.

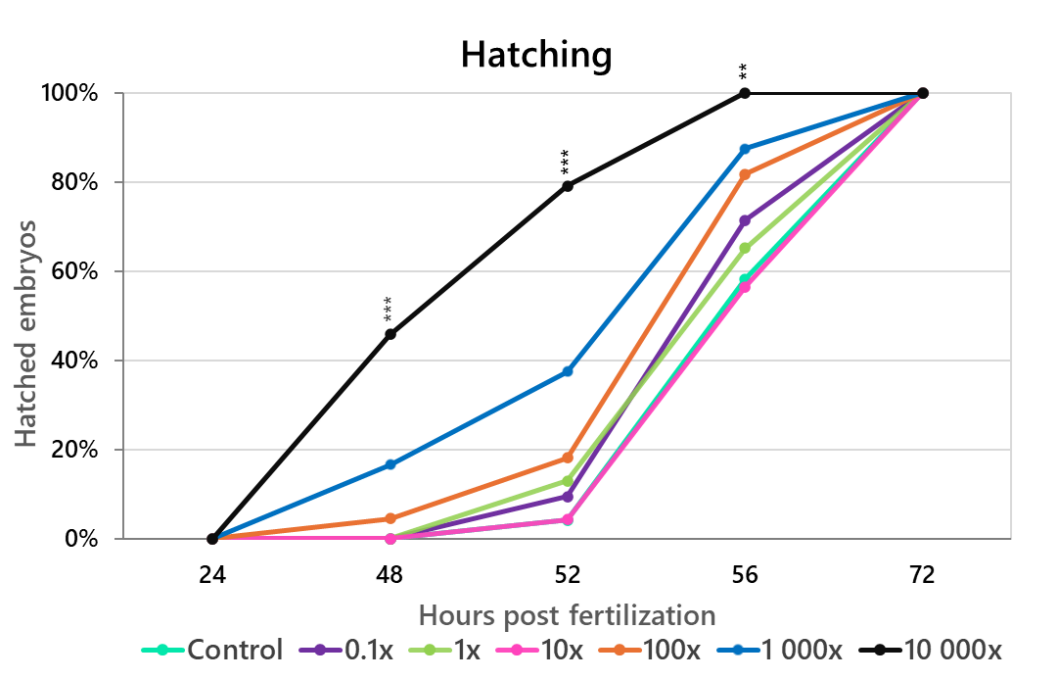

*Fig. 3. Cumulative percentage of hatched zebrafish embryos at different time points (24, 48, 52, 56, 72 hpf) per exposure group. Statistical significance is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.*

3.5 Behaviour (light/dark test)

The light/dark test showed a highly significant decrease in swim distance ($p \leq 0.001$) for larvae in the highest exposure group (10 000x) during the dark periods of the test (*Fig. 4 and Appendix 1*). There were no significant results in the other exposure groups in the dark environment. However, the overall results showed a trend of dose-dependency, with an increase in lower concentrations and then a decrease in higher concentrations.

There were no significant results in swim distance for the light period for any group (*Fig. 5 and Appendix 1*). When the total swim distance was combined, the significance in the highest exposure group seen in the dark periods was cancelled out by the results from the light periods (*Fig. 6 and Appendix 1*). The overall results in the combined swim distance were not significant for any group.

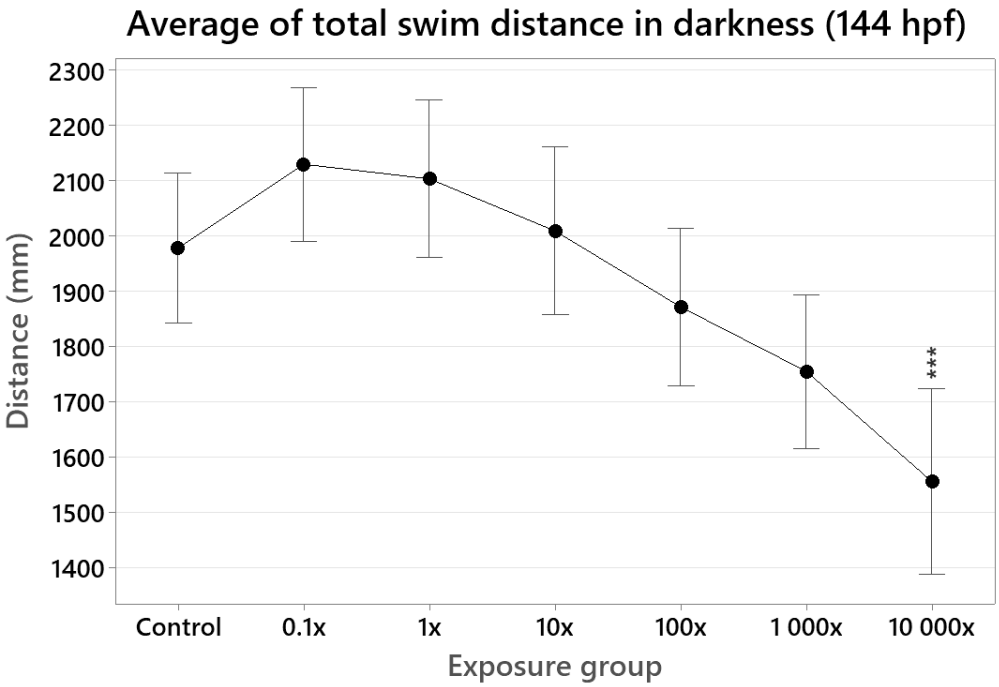

*Fig. 4. The average swim distance (mm) of zebrafish larvae (144 hpf) during the dark periods of the light/dark test per exposure group. Statistical significance is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.*

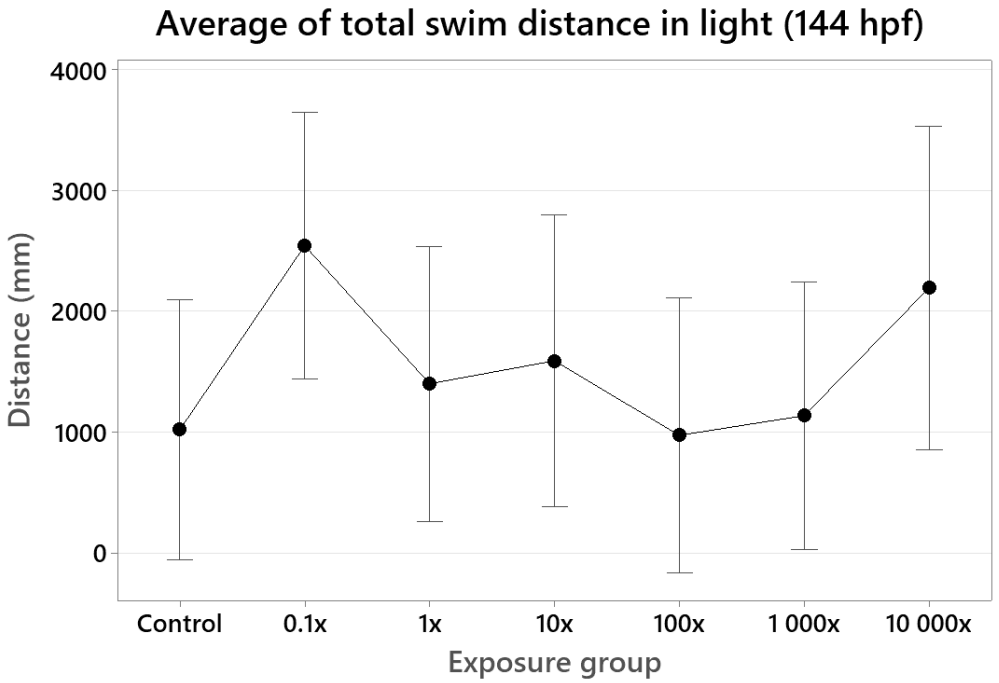

*Fig. 5. The average swim distance (mm) of zebrafish larvae (144 hpf) during the light periods of the light/dark test per exposure group. Statistical significance is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.*

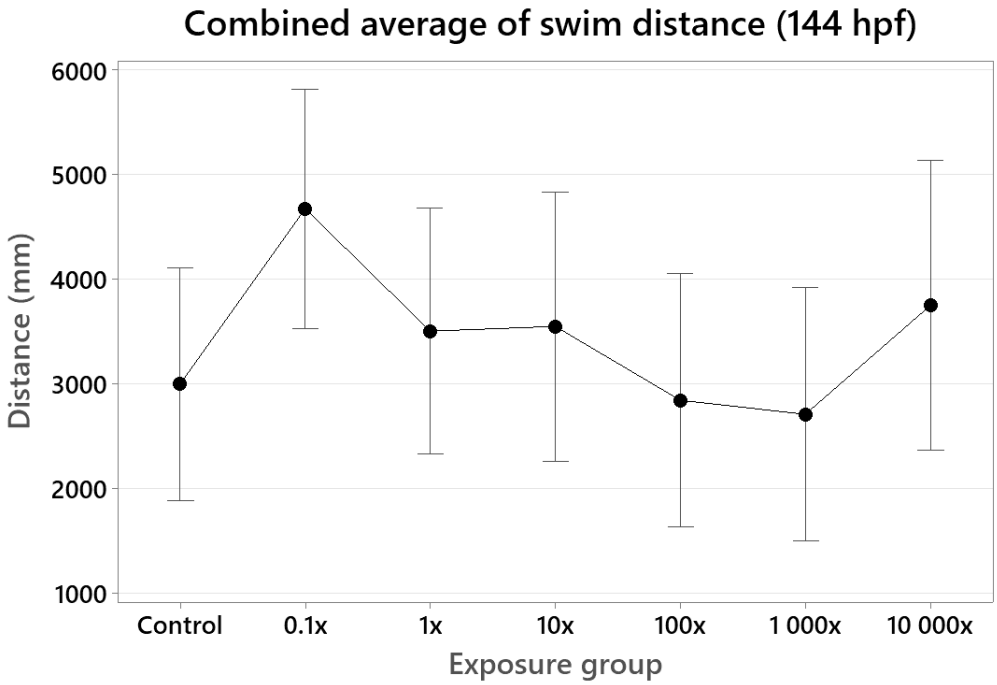

*Fig. 6. The combined average swim distance (mm) of zebrafish larvae (144 hpf) during the light and dark periods of the light/dark test per exposure group. Statistical significance is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.*

4. Discussion

In this study, zebrafish embryos were exposed to a mixture of Citalopram, Sertraline, Paroxetine, Mirtazapine, Amitriptyline, Venlafaxine, and Desvenlafaxine in their respective EC_{mean} (*Table 1*). The experiment lasted for seven days (excluding one day for preparation). At 24 hpf, early movements were quantified, and at 48 hpf, heart rate was measured. On the last day, a light/dark test was performed, and measurements of body length, eye area, and swim bladder area were taken. Observations of hatching and death were made throughout the experiment. Similarly, observed effects, such as spine deformation and the lack of a swim bladder, were also documented. The results showed that exposure to concentrations 10 000 times higher than what has been detected in surface water by Malnes et al. (2022) significantly affected hatching time, swim distance in dark environments, and early movements in zebrafish embryos. The NOEC was seen in exposure group 1 000x, with concentrations 1 000 times stronger than the EC_{mean}. Thus, LOEC was seen at concentrations 10 000 times stronger than the EC_{mean}.

4.1 Ecotoxicological implications

The light/dark test showed a significant decrease in swim distance for larvae in the highest exposure group in darkness (*Fig. 4 and Appendix 1*). However, the lower concentrations (0.1x to 10x) produced a slight increase in swim distance, albeit non-significant. Venlafaxine (presumably also Desvenlafaxine) has been observed to increase swim distance in light environments at 10 000 ng/L while decreasing swim distance at 100 000 ng/L in both light and darkness (Tang et al. 2021) (*Appendix 2*). This effect may be more related to a resource allocation from non-essential functions, like swimming, to functions essential for life, such as keeping the heart beating. This phenomenon may arise as a direct mechanistic effect of antidepressant exposure; however, it might not be a direct causation but rather the effect of the stress caused by the overall chemical exposure. Since the trend implies increased swimming in low concentrations, it would be valuable to perform the same test at an even lower concentration range. Regardless, the behavioural shift between the control and the highest exposure group indicates a subdued response to a stressor. The stress response works as a mechanism to increase fitness, as it can help the organism escape dangerous situations such as predation.

The results showed a significant decrease in early movements in the highest exposure group (*Fig. 1 and Appendix 1*). The decrease could indicate a disruption in neurogenesis and potentially have negative effects on locomotion. The same group also hatched significantly earlier which can cause the larvae to be underdeveloped. Furthermore, the initial results for early movements also showed significance in the second highest exposure group, but not after performing Bonferroni-Holm corrections. The survival rate in the wild, where aquatic vertebrates are susceptible to predation, may therefore be lower after exposure to antidepressants. It would be informative to follow the larvae into adulthood to observe how the indications of developmental impairment would translate. The results also suggest that concentrations up to 10 times higher than the measured EC_{mean}, increase the number of early movements in embryos; however, the results were not significant. Thus, similarly to that of swim distance in darkness, early movements should be tested in a lower concentration series to determine the full effect seen in the results.

4.2 International perspective of toxicity

Sweden has a relatively small population and many lakes and rivers; this combination would theoretically dilute the pharmaceutical residues that are released. Therefore, it would be interesting to compare the results of this study with international concentrations of antidepressants in surface water to assess the risk of toxicity outside of Sweden.

For example, samples from Monjas River in Ecuador have reached concentrations of 590 000 ng/L and 400 000 ng/L for Desvenlafaxine and Venlafaxine, respectively (Voloshenko-Rossin et al., 2015). In the highest exposure mixture used in this study, the concentrations for the same antidepressants were 250 000 ng/L and 580 000 ng/L, respectively. Citalopram has been measured at ~76 000 ng/L in the Isakavagu-Nakkavagu rivers in India (Fick et al. 2009), circa 10 000 times higher than what was measured by Malnes et al. (2022).

These rivers are connected to pharmaceutical industries and are highly polluted. Based on the results of the current study, the high concentrations of antidepressant residues in these rivers could pose a serious ecological risk for the aquatic organisms in these waters and may threaten the local food chain.

4.3 For future perspectives

According to the OECD No. 236 test, for the fulfilment of a legitimate test regime there should be a maximum of 10 % affected individuals in the control group. In the present study, ~16.7 % were affected. Thus, to fulfil the requirements, the present study needs to be repeated to verify the present results. Furthermore, it would have been helpful to perform a chemical analysis throughout the days of the experiment to better understand the change in the chemical configuration of the mixtures over time. The exposure solution was not renewed at any point in the experiment; therefore, it is unknown how the different antidepressants deteriorated during the exposure period. Furthermore, even if it is presumed that they were most potent at the start of the experiment, that does not take into consideration that there may have been antagonistic relationships between the antidepressants. Another aspect is the chemical's ability to penetrate the chorion and potentially affect embryo development. The chorion protects the embryo from its environment, but some chemicals can pass through this protective layer. Paroxetine has been observed to penetrate the chorion and cause early hatching at 10 000 ng/L in ZFET tests (Nowakowska et al. 2020) (*Appendix 2*). The highest exposure group in this study had 270 000 ng/L of Paroxetine, and the second highest contained 27 000 ng/L. Significant results of accelerated hatching was only seen in the highest exposure group. However, the second highest exposure group did show a significant acceleration of hatching ($p \leq 0.05$) after the Fischer exact test at 52 and 56 hpf. After the Bonferroni-holm correction, the significance at 56 hpf disappeared, and the corrected p-value for 52 hpf was $p = 0.0509$, which is just above the significance level. Although it is impossible to make any legitimate assumptions on whether the effect of Paroxetine in single-compound exposure was conserved during the embryonic period in this study, the results could motivate further investigation of causation for early hatching.

Moreover, in the present study, only one type of aquatic organism was used for toxicological testing. Sensitivity to antidepressants may differ for other types of aquatic organisms. For example, a single-compound study where sea urchins (*Paracentrotus lividus)* were exposed to 10 000 ng/L of Sertraline resulted in a significantly lower larval length ($p \leq 0.05$) compared to the control group. Furthermore, the same study saw an increase in morphological deformation ($p \leq 0.05$) after exposure to 640 ng/L of Sertraline (Riberio et al. 2015). This may indicate that sea urchins are more sensitive to antidepressants, thereby underlining the importance of including organisms of different taxonomies.

In conclusion, at levels 10 000 times higher than the measured concentrations from Swedish waters, the antidepressants have the potential to disrupt the development and behaviour of aquatic vertebrates, thereby lowering the fitness in non-target organisms. Furthermore, the results highlight the need for further testing at lower concentrations and the potential ecological risk in surface water with high pollution from pharmaceutical industries.

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Appendix

Appendix 1. Mean values of all tests per exposure group.

	Control	0.1x	1x	10x	100x	1 000x	10 000x
Early movement	3.92	3.71	4.17	4.43	4.00	3.08	2.83
Heart rate	158	154	158	156	161	160	166
Swim distance in 1st dark period (mm)	1 010	1 090	1 070	1 020	961	882	797
Swim distance in 2nd dark period (mm)	968	1 040	1 040	993	911	873	759
Total swim distance in dark (mm)	1 980	2 130	2 100	2 010	1 870	1 760	1 560
Swim distance in 1st period (mm)	507	1 180	847	451	494	404	1 320
Swim distance in 2nd light period (mm)	512	1 360	553	1 140	479	731	874
Total swim distance in light (mm)	1 020	2 540	1 400	1 590	972	1 140	2 190
Total combined swim distance (mm)	3 000	4 670	3 500	3 600	2 840	2 890	3 750
Eye area (µm²)	88 100	88 400	86 200	86 000	89 300	89 900	87 600
Swim bladder area (µm²)	54 600	58 000	57 800	62 200	55 500	59 602	56 000
Body length (µm)	3 400	3 870	3 940	3 920	3 900	3 830	3 830
Individuals with spine deformation	4	2	4	7	4	2	10
Individuals without swim bladder	3	1	5	3	4	1	4
Mortality (all dead by 24 hpf)	0	2	1	1	2	0	0
Affected individuals (144 hpf)	4	5	6	8	6	2	10
Hatched by 48 hpf	0%	0%	0%	0%	5%	17%	46%
Hatched by 52 hpf	4%	10%	13%	4%	18%	38%	79%
Hatched by 56 hpf	58%	71%	65%	57%	82%	88%	100%
Hatched by 72 hpf	100%	100%	100%	100%	100%	100%	-

Appendix 2. Reported concentrations causing toxic effects. Abbreviations: Increased/longer (↑), Decreased/shorter (↓), Mortality (M), Early movement (EM), Swim distance in darkness (SDD), Swim distance in light (SDL), Heart rate (HR), Hatching time (HT), Body length (BL).

Antidepressant	Max exp. conc.	Reported toxic conc.	Source
Amitriptyline	77 000	1 (↑M, ↓BL)	Yang et al. 2014
		10 (↑HT)	
Citalopram	85 000	20 000 (↓SD_D)	Steele et al. 2018
Mirtazapine	52 000	3.9 (↓EM, ↓SD_D, ↓HR)	Zhou et al. 2023
Paroxetine	270 000	10 000 (↓HT)	Nowakowska et al. 2020
Venlafaxine	580 000	16 (↑SC_D)	Rodrigues et al. 2020
		10 000 (↑SD_D)	Tang et al. 2021
		100 000 (↓SD_D↓SD_L)	

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