

The Selective Serotonin Reuptake Inhibitor-Sertraline Diminishes Conspecific Aggression in Male Fighting *Betta splendens* Fish

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Abstract

In conspecific type of aggression the modulation of 5-hydroxytryptamine (5-HT) plays a main role. A decrease of 5-HT in the brain intensifies this type of aggression and in contrast, the increase of 5-HT reduces it. The aim of this study was to examine the effects of different concentrations of sertraline HCl on aggressive behavior of *Betta splendens* male fish. It was concluded that sertraline added to aquarium water in the dose of 0.4, 4.0 and/or 100.0 $\mu\text{g}\cdot\text{L}^{-1}$ BW during 14 days of exposition increased synaptic levels of 5-HT which in turn resulted in reduction of specific aggressive behavior in the environmental concentrations (0.4 μg) and then times higher. Sertraline caused a periodic, and sometimes even total weakening of the male-male type fight, which was a standard trial applied in ethological research on the Siamese fighting fish. In the current study, the most effective one is proved to be the dose of 4.0 $\mu\text{g}\cdot\text{L}^{-1}$ BW (parallel to earlier investigated fluoxetine in the same dose).

Keywords

Aggression, Fish, Serotonin Reuptake Inhibitor

1. Introduction

Siamese fighting fish (hereafter referred to by the common name “betta”) are found in fresh water ponds of

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Southeast Asia. They are also bred as ornamental fish and are readily available in pet stores around the world [1] [2]. Males of this species exhibit strong and stereotyped aggression in defending their territories against intruding conspecific males [3]. Patterns of aggressive behavior include frontal display (erecting the operculum, fins, and tail while facing an opponent accompanied by the erection of fins and tail), and physical attack by biting the intruder. Males may also intensify the coloration scale during an agonistic interaction [4] [5].

The expression of agonistic conspecific behaviors (strong and stereotyped aggression) in defending their territories against intruding male conspecifics, for example, allows the members of family on an exclusive access to food resources, nesting sites and mate if it is in correct place, which is very important for both reproduction and survival [6]. Territorial aggression may be expressed in various forms, including the vigilance and patrolling behavior, exhibition of stereotype displays, vocalization, chasing intruders, and engaging in physical combat [2].

Siamese fighting fish—*Betta splendens* has been a useful model species for 30 years, to study the mechanism of territorial aggression in the vertebrate classes. Their low cost and ease of performing this kind of studies on aggressive behavior in the laboratory are important. Aggressive behavior patterns in this species are easy to observe and quantify, and males exhibit a high level of conspecific aggression when presented with a mirror or an appropriate releaser of this behavior [4] [6].

For many years antidepressive drugs were used mainly by human. They are selective serotonin reuptake inhibitors (SSRI's), for example, of sertraline type. In this way sertraline and its metabolite desmethylsertraline and treatment plant effluents, are discharged into municipal wastewater, and are widespread in surface waters of the United States [1] [7]-[9]. Thus, aquatic animals may be exposed to SSRI's pharmaceuticals often detected in municipal wastewater at the levels high enough to possibly alter both, their behavior and physiology, because they can have the ability to bioaccumulate SSRI in organism in the concentrations that can have behavior symptoms [10] [11]. The epithelial absorption represents a plausible mechanism for the absorption of environmental contaminants in the body tissues of aquatic animals [1] [11].

The aim of this study was to determine the effects of waterborne sertraline hydrochloride indifferent concentrations on the aggressive behavior of betas and their correlations during 14 days of exposure. Bettas are an excellent model for studying the effects of environmental pollutants, because many ecologically relevant fish behaviors may be easily observed and quantified in controlled conditions [12]. Sertraline doses were tested at the aquatic ecological levels ($0.4 \mu\text{g}\cdot\text{L}^{-1}$), ten and twenty five times greater than the dose found in the environment, in order to more clearly quantify the relationship between the sertraline-induced changes in the expression of conspecific aggressive behavior and drug concentration.

2. Material and Methods

The procedures described below were performed with the approval of Local Ethical Commission for Care and Use of Laboratory Animals Welfare (N° 19/211). We used ninety two sexually mature male Siamese fighting fish—*Betta splendens* (male betta) of the domesticated strain purchased from a local pet supply store Zoomix (Warsaw, Poland). Each fish was housed in its own glass tank. All fishes were housed in separate tanks. The tanks were partitioned so the fish cannot see one-another. Each tank was filled with 2.0 liters of fresh water that was dechlorinated (pH 7.5). Fish in tanks were kept on a 12/12 light: dark cycle and fed once a day with dried chironomid larvae. They were held at a controlled room temperature of $22^{\circ}\text{C} - 24^{\circ}\text{C}$. The mean body mass of fishes used in this experiment was $1.70 \pm 0.13 \text{ g}$. Fishes were housed in the laboratory for 3 - 4 weeks prior (acclimatization period) to and then during 2 weeks of their participation in the experiment, and no fish was used for more than one treatment. To monitor the reactions of the fish a 10 minutes mirror test was used [6]. In one corner of the tank, a mirror ($25.4 \times 15 \text{ cm}$) was placed and the reaction of fish to its own image seen in the mirror was observed. After adaptation period, the fish were randomly divided into IV groups.

Following the mirror test, the fish served as control group ($n = 24$) were treated with 100 μl of teleost saline only, while first experimental group were treated with sertraline in $0.4 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 24$), second sertraline in $4.0 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 24$) and third sertraline in $100.0 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 24$) of water, daily during 14 days. An approximate body mass of fishes was 1.6 - 1.85 g after experimentation; approximately the same as in another investigation [13] [14].

Sertraline hydrochloride *in substantia* was purchased from Sigma-Aldrich (St. Louis, USA). In the experiment, fishes typically varied in mass by less than 10%. Hence, we used the same dosage of sertraline for individual fish assigned for specific treatment during 14 days. Fishes were randomly assigned to treatment groups,

and on the 7th day they were presented with 25.4×15 cm mirror for 10 min. Seven days later, on the 14th day of the experiment, we re-evaluated an aggressive behavior in each fish with the next mirror test (accordingly to earlier described methods-6, 13).

Mirror image stimulus tests are a standard protocol for eliciting aggression in betta. Results from mirror tests are significant predictors of reactions of other stimuli, such as videotaped or live males, as well as dominance in dyadic interactions [15] [16]. The duration (s) of opercula displays was recorded, because they are known to be associated with the fight outcome [17]. In a subset tests, we also measured the latency (in second) to respond to the mirror. All mirror tests and all applications of drug were performed between 12 and 16 EST.

Statistical analyses were performed using the SPSS 12.0 PL for Windows statistics package (IBM, Chicago, IL, USA). To compare the average results in the experimental and control groups one-way and two-way ANOVA were used and Bonferroni post-hoc test was applied when appropriate. Additionally, on account of major merits of the standard deviations from the mean values, Spearman's correlation was used. The results were considered as statistically significant at the $p \leq 0.05$ and highly significant at the $p \leq 0.001$.

3. Results

Before the experiment all fishes were subjected to the control mirror test. Then, every day for successive 14 days sertraline in the dose of $0.4 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 24$, group I), $4.0 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 24$, group II) and $100.0 \mu\text{g}\cdot\text{L}^{-1}$ was added to aquarium water in the volume of $100 \mu\text{L}$ of water ($n = 24$, group III) and $100 \mu\text{L}$ 0.9% NaCl in the control group ($n = 24$, group IV). After 7 and 14 days of the experiment a mirror test was performed (a mirror was placed in the aquarium in front of the fish for 10 minutes, **Figures 1-4**). During the test male fish showed various forms of aggression (ruffling fins, frontal attacks, and biting the intruder).

After 7 days of exposing fishes to the constant effect of sertraline concentration a characteristic prolongation of the latent time from the use of the mirror to the appearance of the first signs of aggression proportionally to the used dose of the drug from 17.8 (group I: $0.4 \mu\text{g}$; $p \geq 0.05$) to 20.0 (group II: $4.0 \mu\text{g}$) and 26.2 s (group III: $100.0 \mu\text{g}$) as compared to 10.5 s observed in the control group (group IV). Time to the appearance of the first attack caused by sertraline in water lengthened successively in the experimental II and III groups by 100 and 150%, respectively (**Figure 1**, $p \leq 0.05$).

After 14 days of fish staying in the environment of three sertraline concentrations in the groups of 24 animals, the time to the appearance of the first attacks increased proportionally to the applied drug dose from 13.8 s in the

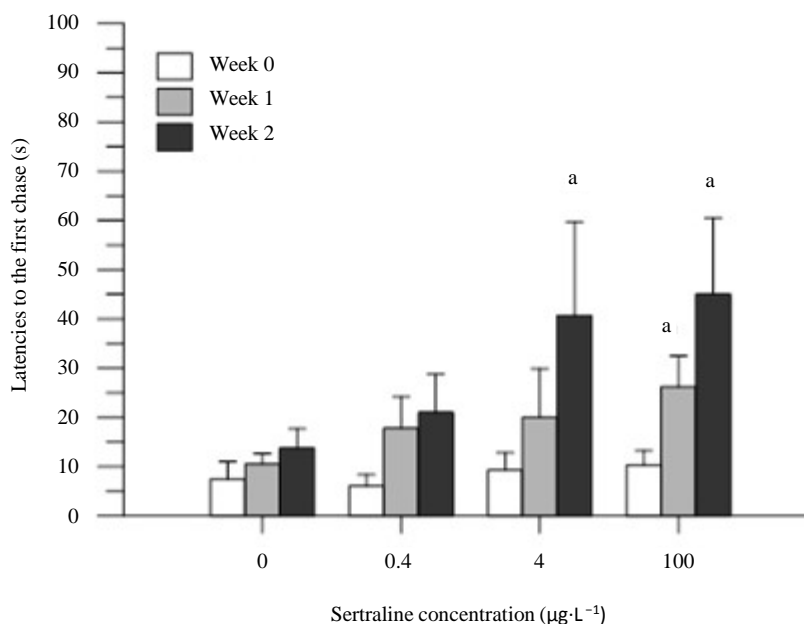


Figure 1. Influence of different sertraline concentration in water on latencies to the first chase of Siamese fighting fish during 2 weeks experiment. Week 0, $n = 24$, weeks 1 - 2, $n = 24$.

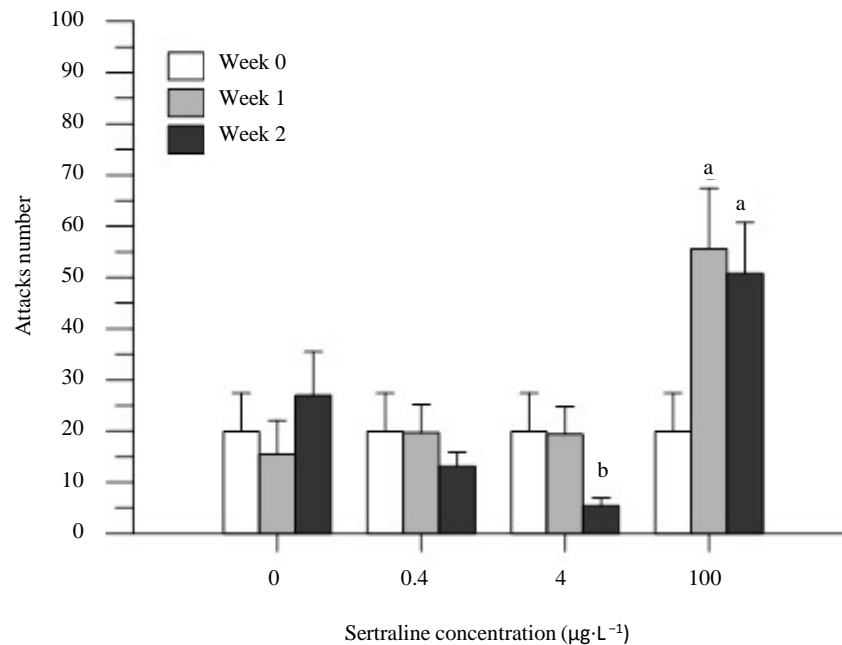


Figure 2. Influence of different sertraline concentration in water on attacks number of Siamese fighting fish during 2 weeks experiment. Observation time = 10 min. Week 0, n = 24, weeks 1 - 2, n = 24.

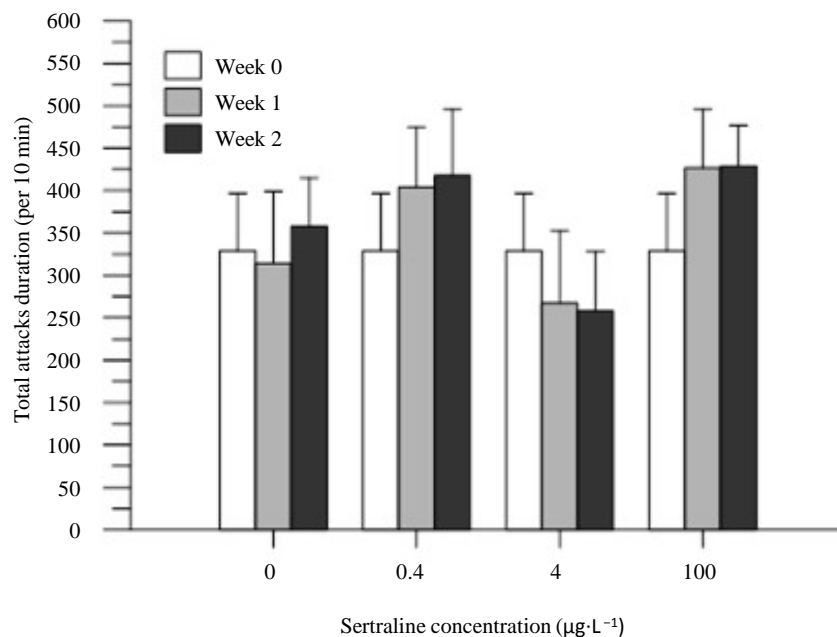


Figure 3. Influence of different sertraline concentration in water on total attacks duration (per 10 min) of Siamese fighting fish during 2 weeks experiment. Observation time = 10 min. Week 0, n = 24, weeks 1 - 2, n = 24.

control (group IV) successfully to 21 (group 0.4 µg; $p \geq 0.05$), 40.7 (group 4.0 µg) and 45 s (group 100.0 µg), *i.e.* by 100, 287.6% and 328.5% ($p \leq 0.01$), respectively.

After 14 day exposition of *Betta splendens* fish to the sertraline concentration of 0.4 µg·L⁻¹ (group I) the number of observed attacks decreased from 27/10 min in the control (group IV), to 13.2/10 min (-51.2%) in the remaining experimental groups (Figure 2, $p \leq 0.05$).

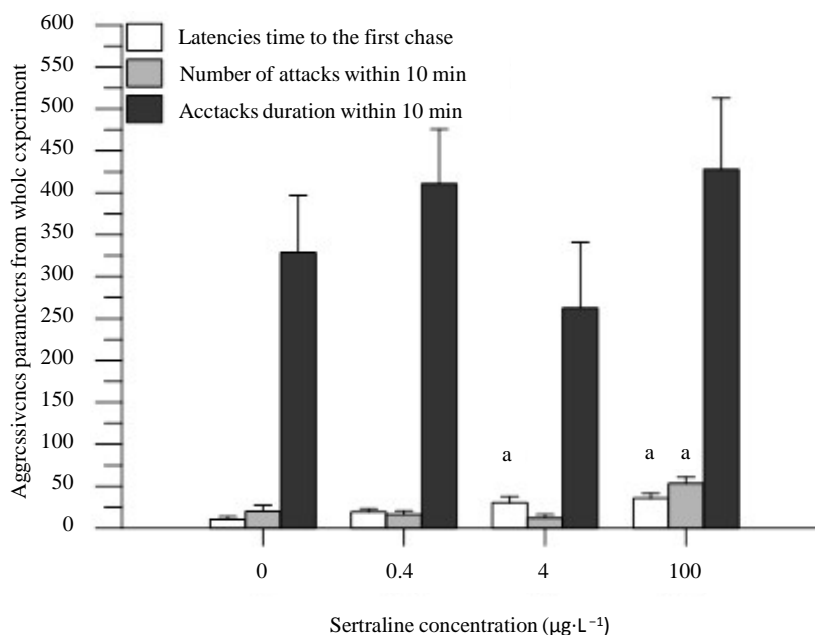


Figure 4. Influence of sertraline concentration given to the water on aggressiveness parameters of Siamese fighting fish. Summary observations from whole experiment. Means \pm SD. Means not shearing the same su[per]script letter differ at $p \leq 0.05$ level.

Also in group II of the tested *Betta splendens* in the environment of tenfold higher sertraline concentration ($4.0 \mu\text{g}\cdot\text{L}^{-1}$) a significant decrease of the number of attacks was observed after 14 day exposition only, by 79.1% as compared to the control group (group IV), *i.e.* proportionally to the exposition time (Figure 2).

It was observed in the group of *Betta splendens* fish exposed to the highest sertraline concentrations amounting to $100.0 \mu\text{g}\cdot\text{L}^{-1}$ (group III) that both after 7 and 14 days of exposition the number of attacks significantly increased from 15.5 and 27/10 min in the control to 55.6 (+105.9%) and 50.8/10 min (+88.1%) respectively on 7 and 14 day of exposition (≤ 0.01).

The mean time of the attack in the group of control animals (group IV) and experimentally group I ($0.4 \mu\text{g}\cdot\text{L}^{-1}$) was the same both on the 7th and 14th day of observation and amounted to 358.4 ± 417.8 s/10 min of observation in the mirror test. In groups II ($4.0 \mu\text{g}\cdot\text{L}^{-1}$) the mean time of the attack length decreased significantly ($p \leq 0.05$) after one week amounting to 267.3 s/10 min and after two weeks to 258.1 s/10 min as compared to the betta attack time in the control group (group IV; Figure 3).

In the group of animals in the sertraline environment amounting to $100.0 \mu\text{g}\cdot\text{L}^{-1}$ (group III) the observed time of attack increased from 314 ± 171.8 s/10 min in the control (group IV) to 426.9 ± 139.2 s after 7 days and to 429.1 ± 93.8 s/10 min after 14 days of the experiment. These results were statistically significant ($p \leq 0.05$, Figure 3 and Figure 4).

Another, unwanted effects after sertraline application in three different doses during 14 days were not observed.

4. Discussion

Sertraline is a chemical compound belonging to antidepressive drugs from the SSRI group which inhibits the presynaptic 5-HT reuptake pump [18]. Thus they effectively increase the concentration of that neurohormone on the synapses of serotonergic neuron endings thus increasing the action of that indolamine and suppressing the aggressive actions [19] [20]. So far there were few investigations concerning the dependence between aggression and 5-HT concentration in aquatic animals. Perreault *et al.* [14] determined the effect of 5-HT on the aggression of bluehead wrasse (*Thalassoma bifasciatum*) a fish of the coral-reef. After a long application of SSRIs males of that species showed a decrease of aggression against the intruder. Also the period of latency until the first attack became longer as compared with the control males. In males receiving SSRIs for a long time one

could observe a significantly decreased frequency as well as the time of the contact with the mirror (intruder). The authors noted that the decrease of aggression level in the coral-reef fishes can be obtained both after a single or long-term administration of fluoxetine [14]. The obtained results on the sertraline daily added to the aquarium containing 2 L of water for 14 days in the dose of $0.4 \mu\text{g}\cdot\text{L}^{-1}$ in which the betta fishes of the investigated group were kept showed a insignificant decrease of both the mean number of attacks in a 10 min mirror test and a highly significant statistically prolongation of time until the first attack as the reaction to the intruder appearance (Figure 4). It is interesting that such a small dose of sertraline in the concentration observed in the spring or river pools as well as storm waters was contaminated with chemical compound, *i.e.* polluting the environment of aquatic animals may awake the aggressive behavior of animals. It is very important because sertraline, similarly as the above described fluoxetine [6], as the SSRIs, apart from the inhibition of aggression in aquatic animals may at the same time disturb their reproductive ability [19].

5-HT—as the indoleamine—is a transmitter of the central and peripheral nervous system and an important regulator of the aggressive behavior in vertebrates. Among many various species of vertebrates the activity of the serotonergic system is higher in less aggressive males and lower in more aggressive males [19]. An experimental increase of the 5-HT concentration or the serotonergic system activity also caused the inhibition of aggression in reptiles, birds and mammals [14] [17] [21] [22].

The dose of sertraline tenfold higher from the so called, “ecologic” observed in the river pools caused the strongest antiaggressive action in *Betta splendens*, significantly increasing the time until the appearance of the first attack as well as decreasing the number of attacks in 10 min of the mirror test and reducing the mean time of the attack from 358.4 to 258.1 s for 10 minute test ($p \leq 0.05$).

Interesting and difficult to explain they are the results obtained by auer after 14 days of sertraline application in the concentration 50 fold higher (called by auer as ecotoxic) than environmental concentration. Both after 7 and 14 days of exposing fishes to sertraline the mean number of attacks increased from 27/10 min in animals from the control group to 55.6 and 50.8/10 min respectively after 7 and 14 days of exposition to the drug, *i.e.* sertraline applied in that dose nearly doubled the number of attacks. At the same time the dose of $100.0 \mu\text{g}\cdot\text{L}^{-1}$ increased in a highly significant way until the first attack, but it doesn't have significant effects on the total time of aggression duration measured for 10 minutes with the mirror test (Figure 3).

Drugs which are selective serotonin reuptake inhibitors (fluoxetine, sertraline) in a lower dose inhibit mainly the 5-HT reuptake, thus prolonging their receptor effects and as a final effect they show the antidepressive action. In the higher doses they probably act in an inhibitory way also on the NE reuptake, similarly to the inhibitors of the 5-HT and NE type of venlafaxines or milnacipran [23] [24]. In a high dose the SSRIs would act similarly to tricyclic antidepressants with the exception that they do not bind together with many various receptors of the muscarinic, histaminergic or dopaminergic type as is the role played by tricyclic *antidepressiva*. Thus it is possible that sertraline applied in a high dose by blocking the α_2 -adrenergic receptors causes the NE neuronal release which is followed by the stimulation of the motivational brain structures, including the intensification of aggression.

The sertraline antiaggressive effect presented on the model of the mirror test of conspecific aggression in *Betta splendens* males shows that in environmental concentration $0.4 \mu\text{g}\cdot\text{L}^{-1}$ dose, is ten-fold higher than that of drug extends the 5-HT action on the receptors in the nervous structures connected with the aggressive behavior in fishes. The role of 5-HT in the conspecific aggression of *Betta splendens* was presented by Clotfelter *et al.* [17] proving that fluoxetine—another SSRI—acted in an antiaggressive way by intensifying the serotonergic transmission. A single application of 5-HT as well as 8-OH-DPAT antagonist of the 5-HT_{1A} receptor decreased the aggression in *Betta splendens*. A long application of fluoxetine caused not significant changes in the aggressive behavior and significantly decreased the 5-HT and 5-HIAA concentration in the betta brain [17]. However, on the other hand, supplying with feed the L-tryptophan, a 5-HT precursor, causes various changes both in aggressive behavior and in the concentration of monoamines in the brain of *Betta splendens*. Thus, the role of 5-HT in the expression of aggressive behavior of *Betta splendens* is ambiguous and needs further research on the molecular level.

The analysis of individual reactions of *Betta splendens* males to the mirror test showed that in tested 10 fishes out of 96 no reaction was observed to the presence of the “intruder”, in 7 fishes the number of attacks on the average exceeded 10/10 min and in 2 it amounted to 44 and 54/10 min respectively. There is a suggestion to use for the further tests only the last mentioned reagents. Similarly clear individual differences were observed in the control animals during the total time of attacks (2.03 and 9.20/10 min, respectively). It is also difficult to defi-

nately prove the suppression of the aggressive behaviors in this species of fish, *i.e.* the antiaggressive action of sertraline. That action is evident as compared to the control in our own investigated group. Comparing it with the course of aggression in the control group during 2 weeks of the experiment and 4 weeks after its ending those actions are not so evident. Similarly as other authors [25], it was noted in the research that in the control group the aggression of animals increased until the 14th day of the experiment and then spontaneously but significantly subsided as to the number of attacks. However, the total time of aggression did not become shorter and even showed the growing tendency. In order to broaden the assessment of sertraline added to water in which the fish live, in the environmental doses $0.4\mu\text{g}\cdot\text{L}^{-1}$ was confirmed in rivers or lake pools both the number of tested animals and the scope of concentrations of the applied sertraline should be increased.

Conflict of Interest Statement

Authors have no conflicts of interest relevant to this article.

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