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Deltamethrin and Retene Toxicity to Excitability of Ventricular Myocytes in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Pyrethroids such as deltamethrin are widely used to control insect pests. Due to their selective toxicity to insects, they are considered harmless to birds and mammals. On the other hand, polycyclic aromatic hydrocarbons (PAHs) such as retene are widespread contaminants in aquatic ecosystems and more problematic to endothermic vertebrates. Both pyrethroids and PAHs might affect the developmental and functional processes in the cardiovascular system of fish. Therefore, deltamethrin and retene toxicity to the electrical excitability of ventricular myocytes were examined in the *Oncorhynchus mykiss* (rainbow trout) heart. Micromolar concentrations of deltamethrin and retene modified the action potential (AP) morphology in a time-dependent manner. After 5 min of application, both compounds strongly prolonged AP duration (APD) compared to control AP. However, deltamethrin prolonged APD by 42% and 25% more than retene at APD_{50} and APD_{90}, respectively. In contrast, retene reduced APD_{10} and the AP depolarization rate ($+dV\ dt^{-1}$) by 61% and 12% more than deltamethrin, respectively. Although deltamethrin and retene changed the ventricular AP shape in rainbow trout in the same way, their mechanism of action seems to be different. The findings revealed that deltamethrin mainly affects the outward potassium currents, while retene mainly affects the inward sodium and calcium currents. Further studies are needed to reveal the underlying ion currents/channels that might be involved in the AP attenuation of rainbow trout under the effect of deltamethrin and retene.
Keywords: Deltamethrin; retene; electrical excitability; cardiotoxicity; fish heart; aquatic toxicology.

1. Introduction

Organic chemicals are one of the most important causes of water pollution worldwide [1, 2]. Pollution is caused by human activity due to the extensive developments in industrial and agricultural applications [1, 3-5]. Among the organic chemicals, pyrethroids, that classified as highly effective and low-toxic biomimetic insecticides, are widely used to control pests in agriculture and residential areas. Deltamethrin (DM; $C_{22}H_{19}Br_2NO_3$) is one of the synthetic pesticides, which is widely utilized in agriculture, aquaculture, and animal rearing, because of its low toxicity and great effectiveness [6-8]. Consequently, it can enter different water sources like lakes and rivers, and therefore can be considered as one of the environmental threats to aquatic animals, including fish [9]. Indeed, previous studies revealed oxidative stress, excitation, cardiovascular system damage, cardiomyocyte injury, decrease in cardiac activity, cardiac arrhythmias, genotoxicity, behavioral changes, hematological and biochemical disorders, and histopathological damage in different aquatic organisms [10-14].

On the other hand, PAHs (polycyclic aromatic hydrocarbons) are ubiquitous pollutants with pyrogenic or petrogenic origins [15]. PAHs are commonly found in aquatic ecosystems due to human-related effluents, atmospheric deposition, surface runoffs, and naturally or accidentally accidental oil spills [16]. Therefore, the aquatic animals are directly or indirectly exposed to different PAHs when they are in touch with the surrounding water or sediment or when they prey on polluted organisms [17]. In different fish species, PAHs affect the function and development of the heart [18-23]. Among PAHs, retene (RET), alkylated phenanthrene, is one of the most common three-ring PAH compounds, which arise from the breakdown of resin compounds during wood burning in forest fires and from paper mill wastewater [24]. RET contamination has been found in downstream sediments and landfills [25-28]. It considers as AhR (aryl hydrocarbon receptor) agonist that stimulates AhR and induces toxicity in the early-life stages of fish. Moreover, it causes numerous changes in gene transcription that induce different developmental abnormalities in the cardiovascular system [18, 29-30].

Blood circulation enables the rapid transfer of oxygen, nutrients, hormones, and temperature throughout the animal's body, and the heart acts as a muscular pump that powers the circulation. The fish heart is an electrically excitable muscle, i.e., a change of plasma membrane voltage (cardiac action potential, AP) sets the rhythmicity and contractility of the heart and regulates force production of cardiac myocytes [31]. Cardiac action potential is produced by a delicate series of interactions between different ion channels in the sarcolemma. The morphology of cardiac AP is determined by the opening and closing of specific ion channels (Na^+, Ca^{2+}, and K^+), which are time- and voltage-dependent. In fish ventricular myocytes, the main inward (depolarizing) currents are I_{CaL} (L-type calcium Ca^{2+} current) and the fast I_{Na} (Na^+ current), while the main

outward (repolarizing) currents are I_{Kr} (rapid component of the delayed rectifier potassium K^+ current) and I_{K1} (inward rectifier K^+ current) [32]. Any slight disturbance in ion channel function may cause conduction failures, cardiac arrhythmias, and compromise the heart's contractile force, which can be fatal for most fish. Because of the expanding environmental problems of pyrethroids and PAHs in various aquatic ecosystems that might potentially affect fish health, the current study examines the micromolar effect of DM and RET on the excitability (AP) of rainbow trout (*Oncorhynchus mykiss*) heart.

2. Materials and method

2.1. Animal

Rainbow trout (*Oncorhynchus mykiss*) were obtained from the Kontiolahti farm, Joensuu, Finland, reared in a 500-liter aquarium filled with aerated water (O_2 9.7 mg mL^{-1}) in the aquaria of the University of Eastern Finland, and fed 5 times $week^{-1}$ (Ewos, Finland). The aquarium was thermally regulated, and fish were acclimated at 12°C (Computec, Joensuu, Finland) under a light: dark photoperiod of 12:12-h. Experiments were carried out on myocytes from 8 rainbow trout (21.14 ± 1.50 g) with permissions no. STH252A and ESAVI/8877/2019 from the Finnish national animal board.

2.2. Ventricular myocytes isolation

The trout head was shocked by a bang, killed by pithing, and the heart was quickly excised and washed from blood in Ca^{2+}-free low Na^+ solution. The enzymatic digestion method was utilized to isolate ventricular myocytes as previously described in detail [33]. Briefly, for 7 min, the excised heart was perfused with Ca^{2+}-free low-Na^+ saline, then perfused by enzymatic solution for about 15 min. The enzymatic solution contained trypsin (Type IX), collagenase (Type IA), and fatty acid-free bovine serum albumin (0.5, 0.75, and 0.75 mg mL^{-1}, respectively; Sigma, St Louis, MO, USA). Thereafter, the ventricle was separated, cut into small portions with scissors, and dissociated into single ventricular myocytes by stirring them in fresh low-Na^+ solution through the Pasteur pipette's opening. Isolated ventricular myocytes were kept and used within 8 h from isolation.

2.3. Action potentials (APs) recording

APs were recorded by using the whole-cell patch-clamp method (current-clamp mode) in the ventricular myocytes, as previously described in detail [34]. Shortly, a small drop of myocyte suspension was added into the recording chamber, myocytes were allowed to settle down to the chamber, and then they were continuously perfused with external saline solutions. The external solution was thermally regulated at 12°C through the Peltier device (HCC-100A, Dagan, MN, USA) and recorded in the same computer file with electrophysiological data. Data were recorded and analyzed offline using the Clampex 9.2 and 10.4 software packages, respectively (Axon Instruments, Saratoga, CA, USA). Borosilicate glass was used to prepare the patch pipettes (King Precision, Claremont, CA) with a mean resistance of 2.87 ± 0.10 (± SEM) MΩ (n = 26) when filled with pipette solutions. The capacitive size of ventricular myocytes was 46.24 ± 4.78 pF (n = 26). When studying the effects of 10 µM from DM and RET (Sigma), each compound was added to the external solution and perfused through the recording chamber. The Ca^{2+}-free low Na^+ solution, the external (bath) saline solution, and the internal (pipette) saline solution were prepared as previously described [34].

Under control conditions, AP was recorded for at least 5 min to obtain steady-state values of AP parameters when using the EGTA-free pipette solution. Therefore, 10 µM of DM or RET was allowed to act for 5 min. AP parameters were analyzed off-line after 1, 3 and 5 min of exposure to both toxins as follows: resting membrane potential (V_{rest}; mV), threshold potential for AP initiation (TP; mV), critical depolarization (CD = TP - V_{rest}; mV), AP overshoot (OS; mV), AP amplitude (Amp; mV), AP duration at 10, 50, and 90% repolarization level (APD_{10}, APD_{50}, and APD_{90}; ms), the maximum rate of AP depolarization (+dV dt^{-1}; mV ms^{-1}), and the maximum rate of AP repolarization (-dV dt^{-1}; mV ms^{-1}; Figure 1).

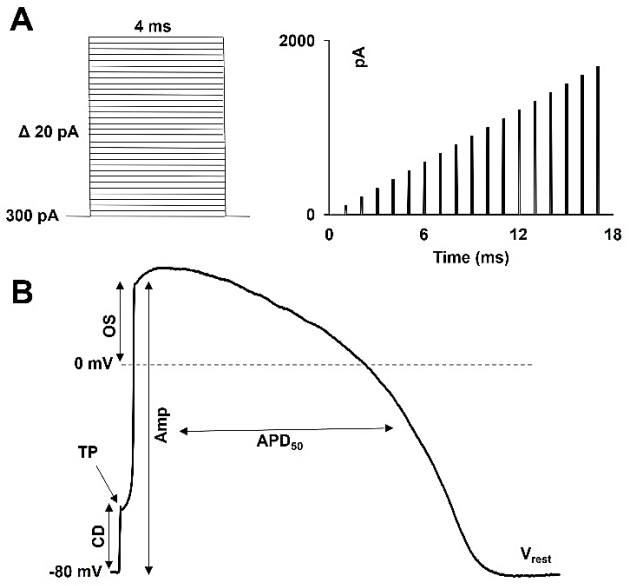

Figure 1. Action potential (AP) waveform of ventricular myocytes in rainbow trout heart at 12°C. (A) The protocol of stimuli that used to trigger APs. (B) model registration of normal ventricular AP and showing the different AP parameters: V_{rest}, resting membrane potential (mV); TP, threshold potential (mV); CD, critical depolarization (mV); OS, AP overshoot (mV); Amp, AP amplitude (mV); APD_{50}, AP duration at 50% repolarization level (ms).

2.4. Statistics

IBM software (version 27.0) was applied to perform the statistical analyses. After checking the normality and homogeneity of data, one-way ANOVA was used to check the statistical differences of mean values of AP parameters between control and under the effect of DM or RET at different times; and between control, DM, and RET after 5 minutes of application with Tukey's or Dunnett's T3 post hoc tests when equal or unequal variances assumed, respectively. The results are expressed as means ± SEM, and the variations between mean

values were deemed statistically significant when $p < 0.05$.

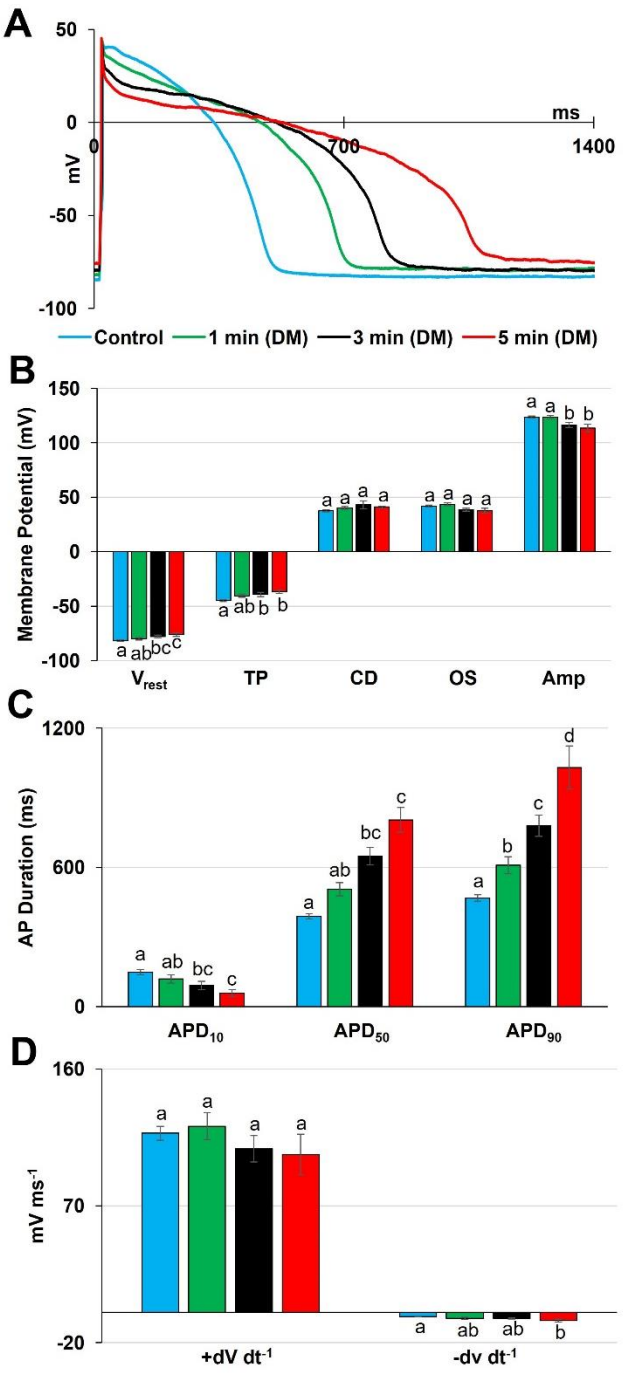

Figure 2. Effects of DM (10 µM) on APs of ventricular myocytes in rainbow trout at 12°C after 1, 3 and 5 min. (A) Representative AP tracings under the control conditions and in the existence of DM (10 µM) after 1, 3, and 5 min. (B) Mean values of V_{rest}, TP, CD, OS, and Amp. (C) Mean values of APD_{10}, APD_{50}, and APD_{90}. (D) Mean values of +dV dt^{-1} and -dV dt^{-1}. Data were presented as means ± SEM of 14 ventricular myocytes (4 fish). The statistically significant differences between mean values were expressed with dissimilar letters (one-way ANOVA; $p < 0.05$).

3. Results

3.1. Action potentials (APs)

APs recordings were stable throughout 5 min without significant changes in different AP parameters when using free-EGTA pipette solution. Effects of DM (10 µM) and RET (10 µM) on AP parameters were time-dependent ($p < 0.05$), i.e., different AP parameters gradually increased or decreased and reached their maximum effect at 5 min (Figures 2A and 3A).

After 5 min, DM significantly depolarized resting membrane potential (V_{rest}), decreased the AP amplitude (Amp) by about 8%, and depolarized the threshold potential (TP) by 18% ($p < 0.05$; Figure 2B). Moreover, DM increased and decreased (not statistically significant) the critical depolarization (CD) and AP over-shoot (OS), respectively ($p > 0.05$; Figure 2B). DM shortened APD_{10} (AP duration at 10% repolarization level) by 65% and strongly prolonged the APD_{50} and APD_{90} by 97% and 111%, respectively ($p < 0.05$; Figure 2C). DM slightly decreased the maximum depolarization rate of AP (+dV dt^{-1}) ($p > 0.05$) and increased the maximum repolarization rate of AP (–dV dt^{-1}) ($p < 0.05$; Figure 2D).

RET had no significant effect on V_{rest}, TP, OS, and Amp ($p > 0.05$), but significantly increased the CD by 15% ($p < 0.05$; Figure 3B). RET had the same effect on APD parameters as DM, as APD_{10} was shortened by 82%, and APD_{50} and APD_{90} were prolonged by 54% and 86%, respectively ($p < 0.05$; Figure 3C). Moreover, RET strongly decreased the maximum upstroke velocity (+dV dt^{-1}) by 27% ($p < 0.05$) and increased the maximum rate of AP repolarization (–dV dt^{-1}) ($p < 0.05$; Figure 3D).

When the effects of DM and RET were compared, DM had a slight depolarizing effect on V_{rest}, while RET slightly increased CD after 5 min of application ($p > 0.05$; Figure 4A). Moreover, DM had a more pronounced effect on APD_{50} and APD_{90}, which were prolonged by 42% and 25%, respectively, compared to RET ($p < 0.05$; Figure 4B). In contrast, RET had a more pronounced effect on APD_{10} and +dV dt^{-1} by decreasing these parameters by 61% and 12%, respectively, compared to DM ($p < 0.05$; Figure 4B and C).

4. Discussion

The current findings reveal that both DM and RET strongly affect, and basically in the same way, the ventricular AP shape in the heart of rainbow trout. However, DM prolonged AP duration more than RET. DM had significant effects on APD which occurred as: (1) a shortening of APD_{10}, and (2) elongation of APD_{50} and APD_{90}. APD is regulated by a harmonious interaction between the inward of Ca^{2+} ions through L-type Ca^{2+} channels (I_{CaL}) and outward of K^+ ions through the fast-component of delayed rectifier K^+ current (I_{Kr}) and I_{K1} [35]. At the AP plateau, the resistance of the sarcolemma is high (small fluxes of Ca^{2+} and K^+); therefore, any small changes in activation or inactivation rates of Ca^{2+} and K^+ currents and the AP amplitude will affect the APD [36].

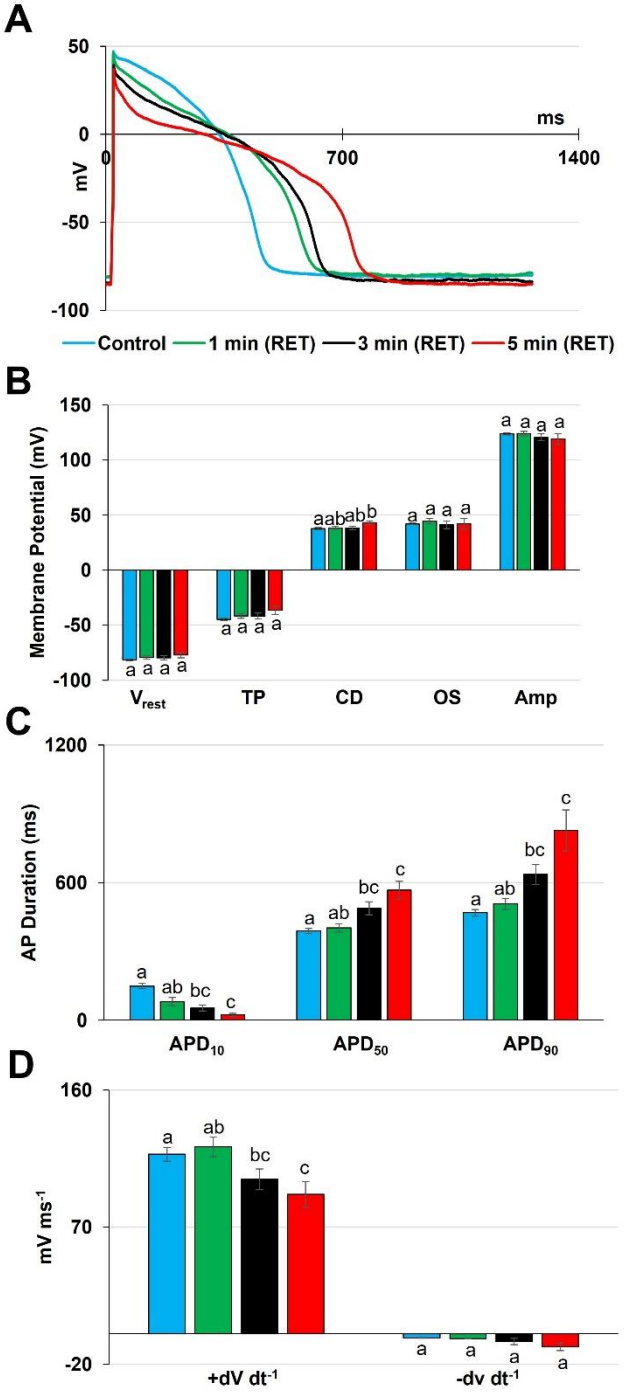

Figure 3. Effects of RET (10 µM) on APs of ventricular myocytes in rainbow trout at 12°C after 1, 3 and 5 min. (A) Representative AP tracings under the control conditions and in the existence of RET (10 µM) after 1, 3, and 5 min. (B) Mean values of V_{rest}, TP, CD, OS, and Amp. (C) Mean values of APD_{10}, APD_{50}, and APD_{90}. (D) Mean values of +dV dt^{-1}, and -dV dt^{-1}. Data were presented as means ± SEM of 12 ventricular myocytes (4 fish). The statistically significant differences between mean values were expressed with dissimilar letters (one-way ANOVA; $p < 0.05$).

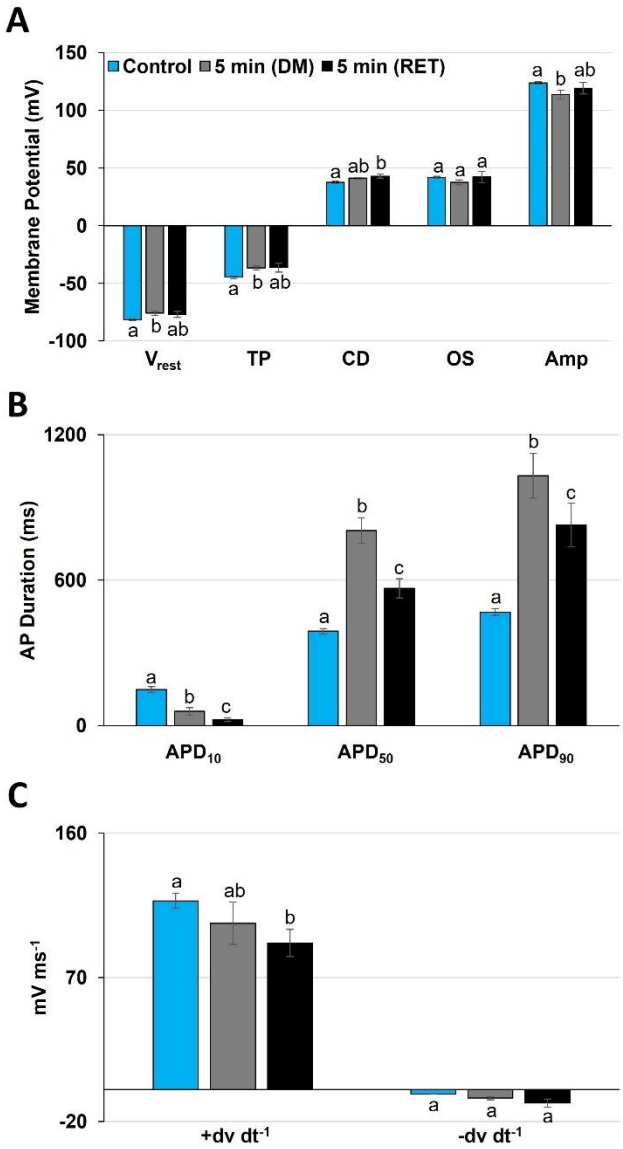

Figure 4. Comparison between different AP parameters in a control condition and under the effect 10 µM of DM and RET after 5 min of application. (A) Mean values of V_{rest}, TP, CD, OS and Amp. (B) Mean values of APD_{10}, APD_{50}, and APD_{90}. (C) Mean values of +dV dt^{-1}, and -dV dt^{-1}. The results are means ± SEM of 12-14 myocytes from 8 fish. The statistically significant differences between mean values were expressed with dissimilar letters (one-way ANOVA; $p < 0.05$).

At the zero-voltage level, the shortening of APD_{10} indicates that DM is depressed I_{CaL} more than I_{Kr}. Furthermore, the prolongation of APD (APD_{50} and APD_{90}) suggests a reduction in I_{Kr} and I_{K1} by DM, which cause a delay at the beginning of phase 3 of AP repolarization. Despite the delay, the repolarization rate of AP (-dv dt^{-1}) was remarkably increased by DM, perhaps due to depression of the antagonizing I_{CaL}. The prolongation of ventricular AP can explain the prolonged QT interval (ventricular AP duration in ECG) with about 35%, which was found in the electrocardiogram of zebrafish (*Danio rerio*) after the application of 2 µg L^{-1} of DM [11]. Also consistent with the present findings, the QT interval of the epicardial electrocardiogram was prolonged by about 25% after

the application of 10 µM of DM in the isolated heart of crucian carp (*Carassius carassius*) [37]. The application of DM significantly decreased the AP amplitude (Amp) and slightly but significantly reduced +dv dt^{-1} (maximum rate of AP depolarization), suggesting a significant inhibition of Na^+ current (I_{Na}). Consistent with this, about 30% and 50% depression in the density of the cardiac Na^+ current by DM was found in rainbow trout and crucian carp, respectively [10, 37]. In addition, resting membrane potential (V_{rest}) was dramatically depressed by DM, suggesting a direct effect of DM on the inward rectifier potassium current (I_{K1}) that maintains V_{rest}.

Resembling other 3-rings polyaromatic hydrocarbons (e.g., phenanthrene), RET (alkylated phenanthrene) had no effect on V_{rest} which is consistent with the effect of phenanthrene on bluefin tuna (*Thunnis orientalis*) and rainbow trout cardiomyocytes [22, 38]. This suggests that - unlike DM - RET does not inhibit/affect I_{K1}. On the other hand, RET had qualitatively similar effect as DM on APD: the shortening of APD_{10} and the prolongation of APD_{50} and APD_{90}, suggesting a significant reduction of I_{CaL} and I_{Kr}, respectively. Indeed, Brette et al. reported that micromolar of phenanthrene causes a dramatic decline in the density of I_{CaL} and I_{Kr} in the bluefin tuna cardiomyocytes [22]. Also, consistently with the present findings, phenanthrene and RET decreased I_{Ca} and I_{Kr} in rainbow trout cardiomyocytes [38]. Similar to DM, RET slightly decreased AP amplitude and significantly depressed +dv dt^{-1} (maximum rate of AP depolarization), indicating a significant reduction in I_{Na}. Notably, both of DM and RET increased the critical depolarization (CD), which in turn will attenuate the excitability of ventricular myocytes of rainbow trout. However, further studies are needed to reveal the mechanism by which both DM and RET compromise the electrical excitability in the heart of trout and understand the underlying ion currents/channels that might be involved. Both chemicals seem to compromise sarcolemmal ion currents with a mechanism that might be partly similar to the toxicity mechanism of cadmium (Cd^{2+}) in rainbow trout ventricular myocytes, where the AP prolongation was an indirect consequence for I_{Ca} inhibition [39].

In conclusion, the current findings revealed the toxicity of DM and RET on the excitability of the fish heart that might give indications about how cardiac arrhythmias are initiated in fish heart and might consider as an indicator for assessing the chemical risks of DM and RET of other aquatic vertebrates.

Acknowledgments

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Conflict of interest

We declare that no conflict of interest might affect the present study.

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