POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 32(3): 481–493, Y. 2017

EFFECTS OF NEONICOTINOID INSECTICIDE ACETAMIPRID ON SWIMMING VELOCITY, HEART RATE AND THORACIC LIMB MOVEMENT OF *DAPHNIA MAGNA*

Adam Bownik, Marlena Pawłocik, Natalia Sokołowska

Institute of Biological Basis of Animal Production
University of Life Sciences in Lublin, Poland

Key words: acetaprimid, neonicotinoid, Daphnia, behaviour, crustacean physiology.

Abstract

Little is known on the effects of nicotinoid pesticides on behavioural and physiological parameters of microcrustaceans. The aim of the study was to determine the effects of three concentrations (25, 50 and 100 mg l^{-1}) of neonicotinoid insecticide Mospilan 20 SP (containing 20% of the active ingredient acetamiprid) on swimming velocity and physiological parameters such as heart rate and thoracic limb activity in *Daphnia magna*. The results showed that acetamiprid induced concentration-dependent inhibition of swimming velocity and thoracic limb activity after 2 hours of the exposure. The insecticide depressed the heart rate at 100 mg l^{-1} after 24 hours of the exposure, however stimulation was noted at 25 and 50 mg l^{-1}. The study suggests that neonicotinoids may alter on behavioural and physiological parameters in *Daphnia magna* thereby increasing susceptibility of these animals to higher predator pressure.

WPŁYW INSEKTYCYDU NEONIKOTYNOWEGO ACETAMIPRIDU NA ZMIANY PRĘDKOŚCI PŁYWANIA, BICIA SERCA I RUCHU ODNÓŻY TUŁOWIOWYCH U *DAPHNIA MAGNA*

Adam Bownik, Marlena Pawłocik, Natalia Sokołowska

Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie, Polska

Słowa kluczowe: acetamiprid, neonikotynoid, Daphnia, behawior, fizjologia skorupiaków.

Address: Adam Bownik, University of Life Sciences in Lublin, ul. Akademicka 13, 20-950 Lublin, Poland, phone: +48 (81) 445 67 39, e-mail: adambownik@wp.pl

Abstrakt

Niewiele wiadomo na temat oddziaływania neonikotynoidów na behawioralne i fizjologiczne parametry mikroskorupiaków. Celem badań było określenie wpływu trzech koncentracji (25, 50 i 100 mg l^{-1}) neonikotynoidowego insektycydu Mospilan 20 SP (20% acetamipridu) na szybkość pływania i parametry fizjologiczne, takie jak: częstotliwość skurczów serca i aktywność odnóży brzusznych u *Daphnia magna*. Wykazano, że acetamiprid wywołuje zależne od koncentracji obniżenie szybkości pływania i aktywności odnóży brzusznych po 2 godzinach ekspozycji. Insektycyd w stężeniu 100 mg l^{-1} po 24-godzinnej ekspozycji obniża częstotliwość skurczów serca, jednak w stężeniach 25 and 50 mg l^{-1} zanotowano efekt stymulujący. Badania sugeują, iż neonikotynoidy mogą powodować zmiany parametrów behawioralnych i fizjologicznych u *Daphnia magna,* a tym samym zwiększać podatność tych zwierząt na presję ze strony drapieżników.

Introduction

Neonicotinoids are widely used water-soluble synthetic insecticides targetting pest insects on a wide range of crops (ELBERT et al. 2008) including compunds such as acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid, thiamethoxam. These molecules are very efficient insecticides able to bind and interact with acetylcholine receptors located in neurons of central nervous system in target insect causing paralysis leading to death (MATSUDA et al. 2001). Although their toxicity is known be restricted only to pest insects many controversies have emerged recently since they were shown to affect also non-target animals. It was shown that beneficial insects such as honey bees (SCHNEIDER et 2012, WILLIAMSON et al. 2014) and wild bees (WHITEHORN et al. 2012) exposed to these insecticides developed various dysfunctions, in foraging activity and reproduction (CHRISTEN et al. 2016) which, in a consequence resulted in a decreased pollination, lower reproduction and finally increased colony mortality due to a lack of food (MOMMAERTS et al. 2010). Neonicotinoids were also found to induce toxic changes in earthworms (ZHANG et al. 2017) and were linked to increased bird mortality associated with a reduction of insect populations (HALLMAN et al. 2014). Although acetamiprid is considered as safe ad not cancerogenic for humans, some cases of accidental human poisoning with multiorgan dysfunctions and erectile dysfunctions in males exposed to these insecticides were also described (IMAMURA et al. 2010, KUMAR et al. 2013, KAUR et al. 2015, XING et al. 2016).

Although neonicotinoids are susceptible to photoderadation (TISLER et al. 2009) and microbial biotransformation, they are considered as environmentally persistent in soil and water environment (LA et al. 2013) and may pose a risk to aquatic invertebrates after their transfer from soil to the aquatic environment (ARMBRUST and PEELER 2002). Toxic effect of neonicotinoids were documented in Ephemeroptera, Trichoptera and snails such as *Lymnea stagnalis*, however different sensitivity exists in these animals (ROESSINK et al.

2013, MORRISSEY et al. 2015, VEHOVSZKY et al. 2015). Some authors showed that ostracods, amphipods, mayfly nymphs and migde larvae are affected to these pesticides (SANCHEZ-BAYO et al. 2016). Although zooplanktonic organisms are susceptible to neonicotinoids they present different level of sensitivity (HAYASAKA et al. 2012). Lethal and sublethal exposure to these pesticides may impair predator-prey relationship and ecological balance in the aquatic ecosystems. For example, daphnids, important filter feeders subjected to neonicotinoids may develop disturbances in feeding which in a consequence may alter the food chain relationships.

Acetamprid (Figure 1) is one of the most widely used neonicotinoid (MORISSEY et al. 2015, YANG et al. 2016). The insecticide is persistent in water and may reach concentrations up to 41 μg l^{-1} (PPDB 2017). A number of studies indicate that Cladocera seem to be one of the most resistant invertebrates to neonicotinoids, however they present a wide range of sensitivity with LC50 (Lethal Concentration) from 4100 to >1000 000 μg l^{-1} (MORRISEY et al. 2015). LC50 for thiacloprid was estimated to be 4400 μg l^{-1} in *Daphnia magna* after 24-h exposure (BEKETOV and LIESS 2008) and LC50 (48 h) for acetamiprid was estimated to be 49.8 mg l^{-1}. Current knowledge on the effects of neonicotinoids od *Daphnia magna* is limited to mortality or immobilisation but very little is known on other parameters such as swimming behaviour and physiology. Therefore, the aim of the present study was to determine the influence of persistent neonicotionid formulation mospilan (20% acetamiprid) on swimming velocity and physiological parameters: heart rate and thoracic limb activity in *Daphnia magna*.

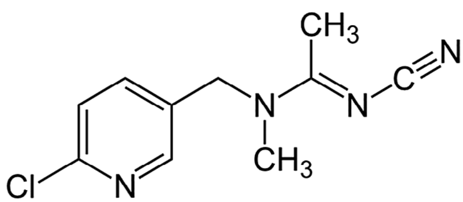

Fig. 1. Chemical structure of acetamiprid

Material and Methods

Culture method

D. magna were cultured in our laboratory in a continuous parthenogenetic reproduction for several generations in 6 L tanks with 5 L of aerated *Daphnia* culture medium under light: dark period of 16 h:8 h. Daphnia culture medium was prepared according to American Society of Testing and Materials standards (*Standard practice for*... 1986) with a temperature of 23±1°C. The number of cultured daphnids was about 30 animals per liter. Daphnia mothers were fed once daily with a few drops of powdered Spirulina (2 mg l^{-1}) per tank and supplemented with a few drops per tank of 10 mg l^{-1} stock suspension of baker's yeast. Neonates from fifth-sixth generation ≤ 24 h old were used in the study and were not fed 24 h before and during the exposure to the neonicotinoid.

Chemicals and experimental design

Mospilan 20 SP formulation (20% acetamiprid) was used in the form of the commercially available product in Poland. The insecticide was diluted in Daphnia culture medium to reach concentrations of 25, 50 and 100 mg l^{-1}, selected of the basis of toxicological data for acetamiprid (Material Safety Data Sheet). Swimming velocity, heart rate and thoracic limb movement frequency was determined after 2, 24, 48 and 72 hours of the exposure. The control daphnids were kept in clean medium only.

Determination of survival rate

Survival rate of the animals in each experimemtal group and the control was determined after 2, 24, 48, 72 h of the exposure. Daphnids were treated as dead when no heart beat was noted during examination under a light microscope.

Determination of swimming behaviour

Swimming velocity of *D. magna* neonates were determined after 2, 24, 48 and 72 hours according to the experimental setup previously described by SHIMIZU et al. (2002) and BOWNIK et al., (2015) with some modifications. 10 daphnids were placed in the observation culture dish of 55 mm diameter containing 6 ml of Mospilan 20 SP solution. Swimming behaviour of the animals was video recorded for a minimum of 1 min (with a speed of 30 frames per second) with a digital camera Nikon D3100 mounted on a stand over the dish and the recorded video was processed with motion analysis software, Tracker®, version 4.82. Vertical movement of Daphnia was negligible because of very small depth of the solution present in the observation dish. The video file with the recorded trajectories of swimming *D. magna* was analyzed by a frame-by-frame method with Tracker®. Briefly, by clicking with the cursor on Daphnia image in separate frames, the trail left by a single *Daphnia* (interpreted by the program as a mass point) and mean velocity (v) expressed in millimeters per second (millimeters per second) was measured. As the crustaceans moved in the observation dish virtually in two dimensions swimming behavior analysis was based on the trajectory represented by x and y coordinates. The velocities of ten daphnids calculated by the software were plotted in the separate graphs which were then superimposed. Mean velocity of the experimental group was obtained by statistical calculation of all measurements taken for each individual animal.

Heart rate and thoracic limb activity

Microscopic measurements of physiological parameters: heart rate and thoracic limb movement were performed at 2, 24, 48 and 72 hour of the experiment. A single daphnid was transferred in a 50 µl droplet of appropriate experimental solution to a microscope slide. After the individual daphnid was transported for the analysis, the excess of the medium was aspirated. The microscopic view of the examined daphnid was recorded for more than 1 min (with a speed of 30 frames per second) with a digital camera Nikon D3100 mounted on a light microscope. The magnification (30–100×) and camera resolution allowed to perform the analysis with a good visibility of the heart and thoracic limbs. Heart rate and thoracic limb movement were analyzed with multimedia player with a frame-by-frame method and separate heart contractions were counted for 1 min. Thoracic limb movement was also determined by a frame-by-frame video analysis with the multimedia player and the separate movements (beats) of the limbs were counted for 1 min.

Statistical analysis

The results are shown as means ± standard deviation (SD). Statistical analyses were performed using Develve software. All data were assessed for homogeneity of variance for ANOVA assumptions. Data were analysed using ANOVA followed by Tukey's test to find differences between means. The level of significance was set to be at least $p < 0.05$.

Results

Survival rate

The results showed (Figure 2) that survival rate of daphnids exposed to the insecticide at 100 mg l^{-1} was reduced to 80% after 48 hours, however no alive animals were noted after 72 hours. Less reduced survival rates (to 50% and 40%) at 72 hour were observed at 25 and 50 mg l^{-1} of the neonicotinoid, respectively.

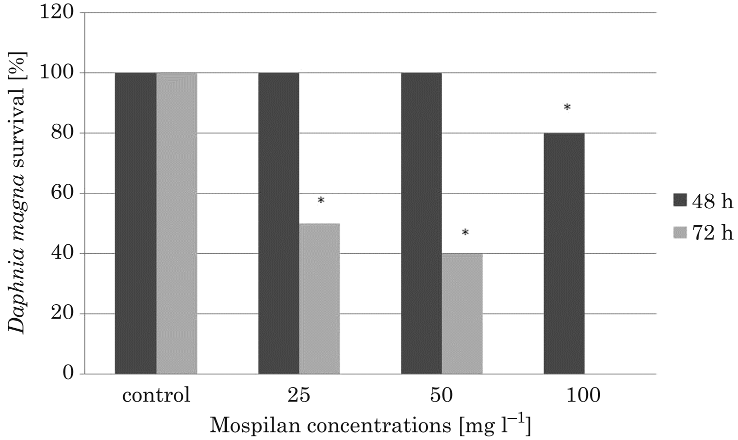

Fig. 2. Survival of *Daphnia magna* subjected to mospilan 20 SP (20% acetamiprid): $n = 10$; * – statistical significance between the experimental and control groups $p < 0.05$

Swimming velocity

The most significant reduction of swimming velocity was noted at 100 mg l^{-1}. The Figure 3 and graph images of velocity (Figure 4) obtained from Tracker® software demonstrate that this parameter was significantly inibited after 2-hour exposure (1.06 ± 0.6 cm s^{-1}) when compared to the control (4.98 ± 0.5 cm s^{-1}). More depressed swimming speed was observed after 24 hours (0.68 ± 0.7 cm s^{-1}). A significant reduction of the parameter at 2 hour was also observed at 50 mg l^{-1} (1.73 ± 0.4 cm s^{-1}), however motile daphnids were noted after 48 hours (0.93 ± 0.6 cm s^{-1}). The neonicotinoid at 25 mg l^{-1} induced only slight depression of swimming velocity (3.82 ± 0.5 cm s^{-1} and 2.33 ± 0.6 cm s^{-1} after 2 and 72 hours, respectively).

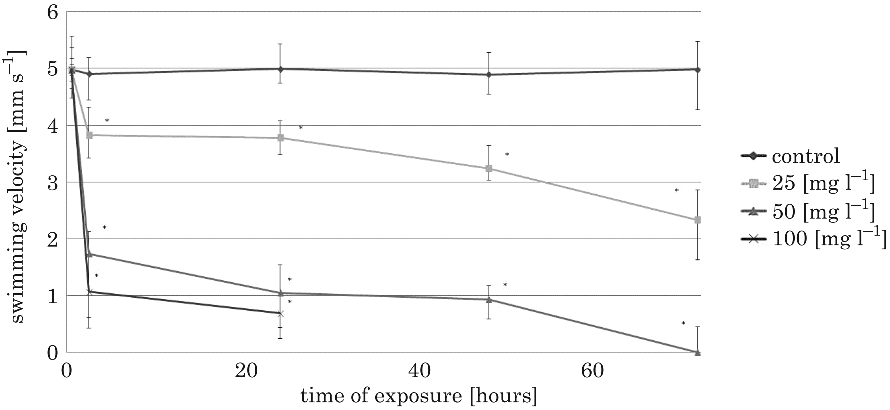

Fig. 3. Swimming velocity of *Daphnia magna* exposed to Mospilan 20 SP. The results are presented as means ± SD; $n = 10$; * – statistical significance between the experimental and control groups $p < 0.05$

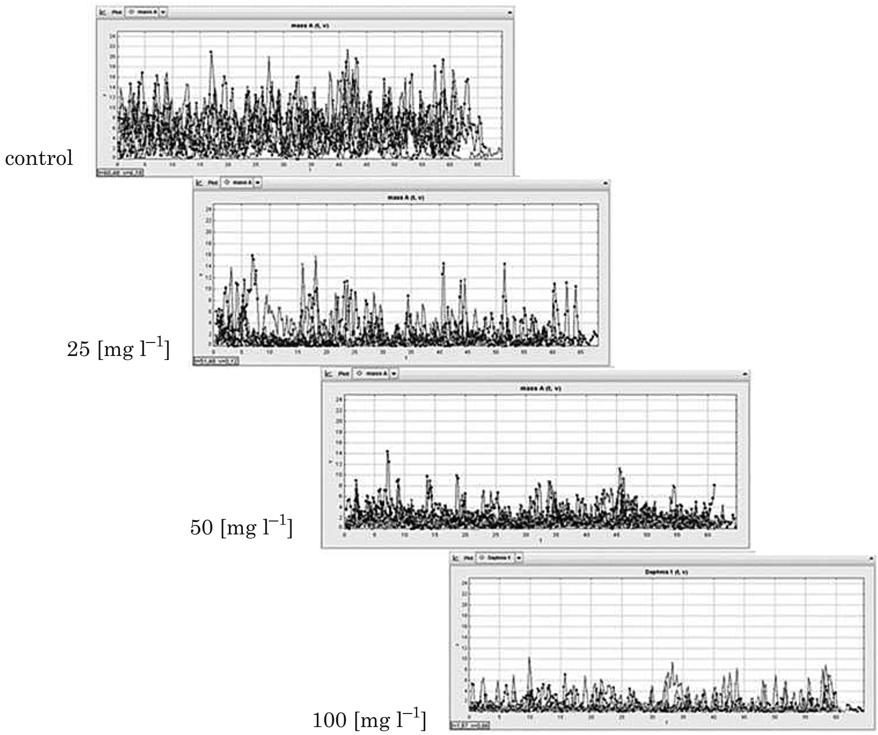

Fig. 4. Exemplary amplitudes of swimming velocity of 10 *Daphnia magna* exposed for 2 hours to different concentrations of neonicotinoid insecticide Mospilan 20 SP. Data from 10 individuals were obtained from the frame-by-frame- analysis in Tracker® software. The graphs present the superimposed amplitudes swimming velocities of 10 animals

Heart rate

The most depressed heart rate was noted after 48 hours in daphnids treated with 100 mg l^{-1} (132 ± 40 bpm) – Figure 5. The animals exposed to the neonicotinoid at 25 mg l^{-1} showed stimulated heart rate in comparison to the control (445 ± 25 bpm). The highest values were noted after 24 (538 ± 20 bpm) and 48 hours (502 ± 32 bpm), however it decreased to 380 ± 55 bpm after 72 hours. Very similar stimulatory effects were also noted at 50 mg l^{-1}, however at 72 hour the reduction of heart rate was more significant (300 ± 45 bpm). All the experimental animals showed no heart arrhythmia (data not shown).

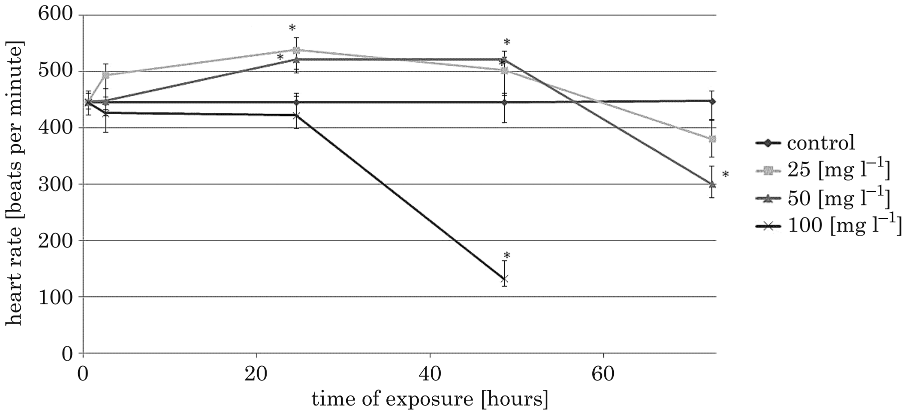

Fig. 5. Heart rate of *Daphnia magna* exposed to Mospilan 20 SP. The results are presented as means ± SD; n = 10; * – statistical significance between the experimental and control groups $p < 0.05$

Thoracic limb activity

Daphnia exposed to all tested concentrations of the neonicotinoid showed a time- and concentration-dependent decrease of thoracic limb beat frequency (Figure 6.). The highest inhibition of this activity was observed at 100 mg l^{-1} after 48 hours (12 ± 6 bpm) of treatment, however a significant reduction also appeared after 2 hours (204 ± 46 bpm) when compared to the control (357 ± 25 bpm). Lower concentrations of the insecticide also induced supressory effect and after 48 hours of the exposure the limb activity was 224 ± 55 and 280 ± 30 bpm at 50 and 25 mg l^{-1}, respectively. The parameter was more inhibited after 72 hours and was 180 ± 33 and 220 ± 45 bpm at 50 and 25 mg l^{-1}, respectively.

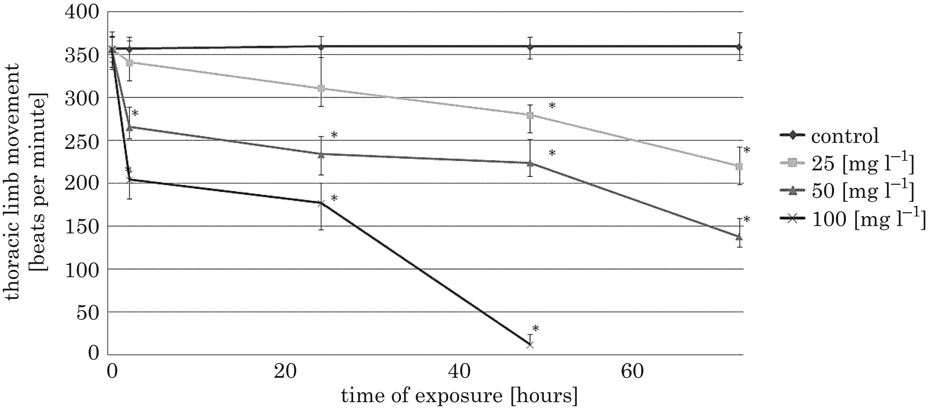

Fig. 6. Thoracic limb activity of *Daphnia magna* exposed to Mospilan 20 SP. The results are presented as means ± SD; $n = 10$; * – statistical significance between the experimental and control groups $p < 0.05$

Discussion

Survival rate or immobilisation of *Daphnia magna* are common endpoints used for determination of toxicity induced by various compounds. They are easy for to detection without special equipment or advanced training with a naked eye. However these parameters may not be suitable as biomarkers in assessment of sublethal effects in sensitive systems of exposed animals such as nervous system. Currently, more sensitive parameters as swimming activity and physiological activity of *Daphnia* such as heart rate or thoracic limb activity are becoming more frequently used early biomarkers required for more detailed information on toxic effects in aquatic invertebrates (BOWNIK et al. 2015, BOWNIK and STĘPNIEWSKA 2015). This is the first study showing that neonicotinoids may affect nervous system of *Daphnia magna* resulting in changes in swimming behaviour and physiological activity.

Survival rate

Our study confirmed that acetamiprid is moderately toxic compound to *Daphnia magna*. The insecticide induced time- and concentration dependent reduction of survival rate which is in agreement with the LC50 value estimated for acetamiprid (49.8 mg L^{-1} after 48 h). These cladocerans are more resistant to this insecticide when compared to LC50 values for other aquatic invertebrates such as *Ephemeroptera* (3.9 μg l^{-1}), *Trichoptera* (6.9 μg l^{-1}), *Chironomus dilutus* (9.3 μg l^{-1}) (LEBLANC et al. 20120, ROESSINK et al. 2013). Although environmental concentrations of acetamiprid should not be expected to reach LC50 values, its high persistence, and time-cumulative toxicity to invertebrates (RONDEAU et al. 2014) may pose a risk of intoxication to sensitive cladoceran species.

Swimming behaviour

Swimming behaviour is a complex endpoint used for assessment of toxicity in aquatic invertebrates and vertebrates. It can be easily monitored with the use of frame-by-frame video analysis and its impairments may be clearly detected (SHIMIZU et al. 2002, ZEIN et al. 2014, BOWNIK et al. 2015). Our study indicated that although *Daphnia* could survive at high levels of acetamiprid these animals responded after 2 hours with inhibition of swimming velocity. A quick response indicates that *Daphnia* neuromuscular system is sensitive to neonicotinoids. The inhibition of motility is a consequence of interaction of acetamiprid with nicotine receptors leading to inhibition of neuronal transmission and depression of swimming velocity. On the contrary, acetamprid-induced stimulation of locomotor activity in honey bees (EL HASSANI et al. 2008). This may suggest that nicotinic receptors in *Daphnia* and honey-bees have different structure or different mechanisms of neuronal signal transduction. Our study also indicated that, signalling through nicotinic receptors seems to be an important pathway in neuromotor activity in *Daphnia magna*.

Heart rate

Microscopic observation of *Daphnia* heart activity is a simple method used for determination of effects induced by various chemicals (VILLEGAS-NAVARRO et al. 2003, BOWNIK et al. 2015). Our study showed that acetamprid induced concentration- and time- dependent chronotropic effect. Interestingly, stimulation of heart rate was noted at 25 mg l^{-1} and 50 mg l^{-1} of the neonicotinoids, on the other hand, negative chronotropic effect was noted at its highest concentration (100 mg l^{-1}). Although no results on the effects of neonicotinoids on cladoceran heart activity can be available, our findings are similar to those obtained by BAYLOR (1942), who found stimulatory of higher concentrations or inhibitory effects at lower levels of nicotine on heart rate of *Daphnia magna*. Various responses in different species of aquatic invertebrates may also be induced towards another ligand of the nicotinic receptor, acetylcholine. It was found that although the molecule inhibited heart rate in *Daphnia* (BAYLOR

1942), its positive chronotropic effect was found in decapods (DAVENPORT 1941). These opposite reactions of various invertebrate species indicate that different physiological mechanisms are involved in heart responses to neuroleptic molecules.

Thoracic limb activity

We found that acetamiprid inhibited activity of thoracic legs in a concentration- and time-dependent manner which probably suggests inhibitory action of the neonicotinoid on daphnid cholinergic system. Thoracic limbs play an important role in cladoceran feeding and ventilation (SEIDL et al. 2002) and since their activity is modulated by various chemicals they may serve as a sensitive physiological endpoint in ecotoxicology. However, little is known on the influence of neurotoxicants. The nicotinoid-induced inhibition of thoracic limbs may in a consequence cause feeding problems and ventilation disturbances leading to anoxia.

Concluding remarks

The present study revealed that acetamiprid is a potent neuromodulator altering behavioural and physiological parameters of *Daphnia magna*. High persistence in water and the ability to induce cumulative toxicity suggests that this neonicotinoid may interact with nicotinic receptors and thereby affect nervous system of sensitive species of cladocerans which may result in behavioural and physiological changes. Our results also indicate that parameters such as swimming velocity, heart rate and thoracic limb activity may be treated as sensitive, early biomarkers of neurotoxicity in cladocerans.

Translated by ADAM BOWNIK

Accepted for print 5.03.2017

References

ARMBRUST K.L., PEELER H.B. 2002. *Effects of formulation on the run-off of imidacloprid from turf*. Pest Manag. Sci., 58(7): 702–706.

BAYLOR E.R. 1942. *Cardiac pharmacology of the cladoceran, Daphnia*. Biol. Bull., 83(2): 165–172.

BEKETOV M.A., LIESS M. 2008. *Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods*. Environ Toxicol Chem., 27(2): 461–470, http://dx.doi: 10.1897/07-322R.1.

BOWNIK A., STĘPNIEWSKA Z., SKOWROŃSKI T. 2014. *Protective effects of ectoine on heat-stressed Daphnia magna.* J. Comp. Physiol. B., 184: 961–976.

Bownik A., Stępniewska Z., Skowroński T. 2015. *Effects of ectoine on behavioural, physiological and biochemical parameters of Daphnia magna*. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 168: 2–10.

Bownik A., Stępniewska Z. 2015. *Ectoine alleviates behavioural, physiological and biochemical changes in Daphnia magna subjected to formaldehyde*. Environ. Sci. Pollut. Res. Int., 22: 15549–15562.

Christen V., Mittner F., Fent K. 2016. *Molecular effects of Neonicotinoids in honey bees (Apis mellifera)*. Environ Sci Technol., 50(7): 4071–4081, http://dx.doi: 10.1021/acs.est.6b00678.

Davenport D. 1941. *Effects of acetylcholine, atropine and nicotine on the isolated heart of the commercial crab, Cancer magister Dana*. Physiol. Zool., 14(2): 178–185.

Elbert A., Haas M., Springer B., Thielert W., Nauen R. 2008. *Applied aspects of neonicotinoid uses in crop protection*. Pest. Manag. Sci., 64(11): 1099–1105.

El Hassani A.K., Dacher M., Gary V., Lambin M., Gauthier M., Armengaud C. 2008. *Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (Apis mellifera)*. Arch. Environ. Contam. Toxicol., 54: 653–661.

Hallmann C.A., Foppen R.P., van Turnhout C.A., De Kroon H., Jongejans E. 2014. *Declines in insectivorous birds are associated with high neonicotinoid concentrations*. Nature, 7509: 341–343, http://dx.doi:10.1038/nature13531.

Imamura T., Yanagawa Y., Nishikawa K., Matsumoto N., Sakamoto T. 2010. *Two cases of acute poisoning with acetamiprid in humans*. Clin. Toxicol. (Phila), 48(8): 851–853, http://dx.doi:10.3109/15563650.2010.517207.

Kaur R.P., Gupta V., Christopher A.F., Bansal P. 2015. *Potential pathways of pesticide action on erectile function. A contributory factor in male infertility*. As. Pac. J. Rep., 4(4): 322–330.

Hayasaka D., Korenaga T., Suzuki K., Sánchez-Bayo F, Goka K. 2012. *Differences in susceptibility of five cladoceran species to two systemic insecticides, imidacloprid and fipronil*. Ecotoxicology, 21: 421–427.

Kumar A., Verma A., Kumar A. 2013. *Accidental human poisoning with a neonicotinoid insecticide, imidacloprid: A rare case report from rural India with a brief review of literature*. Egypt. J. Forensic Sci., 3(4): 123–126.

La N., Lamers M., Bannwarth M., Nguyen V., Streck T. 2014. *Imidacloprid concentrations in paddy rice fields in northern Vietnam: measurement and probabilistic modeling*. Paddy Water Environ., 13(2): 1–13.

Leblanc H.M.K., Culp J.M., Baird D.J., Alexander A.C., Cessna A.J. 2012. *Single versus combined lethal effects of three agricultural insecticides on larvae of the freshwater insect Chironomus dilutus*. Arch. Environ. Contam. Toxicol., 63(3): 378–390.

Matsuda K., Buckingham S.D., Kleier D., Rauh J.J., Grauso M., Sattelle D.B. 2001. *Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors*. Trends Pharmacol. Sci., 22(11): 573–580.

Mommaerts V., Reynders S., Boulet J., Besard L., Sterk G., Smagghe G. 2010. *Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior*. Ecotoxicology, 19(1): 207–215, http://dx.doi:10.1007/s10646-009-0406-2.

Morrissey C.A, Mineau P., Devries J.H., Sanchez-Bayo F., Liess M., Cavallaro M.C., Liber K. 2015. *Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review*. Environ. Int., 74: 291–303, http://dx.doi:10.1016/j.envint.2014.10.024.

PPDB. 2017. *Pesticide properties database*. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm, access: 10.04.2017.

Roessink I., Merga L.B., Zweers H.J., van den Brink P.J. 2013. *The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs*. Environ. Toxicol. Chem., 32(5): 1096–1100.

Rondeau G., Sanchez-Bayo F., Tennekes H.A., A. Decourtye Ramirez-Romero R., Desneux N. 2014. *Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termites*. Sci. Rep., 4: 5566.

Sanchez-Bayo F., Goka A., Hayasaka D. 2016. *Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems*. Front. Environ. Sci., 4: 1–14.

Schneider C.W., Tautz J., Grünewald B., Fuchs S. 2012. *RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of Apis mellifera*. PLoS 7: e30023, http://dx.doi:10.1371/journal.pone.0030023.

SEIDL M.D., PIROW R., PAUL R.J. 2002. *Water fleas (Daphnia magna) provide a separate ventilatory mechanism for their brood.* Zoology (Jena), 105(1): 15–23.

SHIMIZU N., OGINO C., KAWANISHI T., HAYASHI Y. 2002. *Fractal analysis of daphnia motion for acute toxicity bioassay*. Inc. Environ. Toxicol., 17(5): 441– 448.

Standard practice for conducting static acute toxicity tests on wastewaters with Daphnia: annual book of ASTM standards. 1986. American Society of Testing and Materials. Philadelphia, 11.04: D4229–D4284.

TISLER T., JEMEC A., MOZETIC B., TREBSE P. 2009. *Hazard identification of imidacloprid to aquatic environment*. Chemosphere, 76(7): 907–914.

VEHOVSZKY Á., FARKAS A., ÁCS A., STOLIAR O., SZÉKÁCS A., MÖRTL M., GYÖRI J. 2015. *Neonicotinoid insecticides inhibit cholinergic neurotransmission in a molluscan (Lymnaea stagnalis) nervous system.* Aquat. Toxicol., 167: 172–179, http://dx.doi:10.1016/j.aquatox.2015.08.009.

VILLEGAS-NAVARRO A., ROSAS L.E., REYES J.L. 2003. *The heart of Daphnia magna: effects of four cardioactive drugs*. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 136(2): 127–134.

WHITEHORN P.R., O'CONNOR S., WÄCKERS F.L., GOULSON D. 2012. *Neonicotinoid pesticide reduces bumble bee colony growth and queen production*. Science, 336(6079): 351–352.

WILLIAMSON S.M., WILLIS S.J., WRIGHT G.A. 2014. *Exposure to neonicotinoids influences the motor function of adult worker honeybees*. Ecotoxicology, 23(8): 1409–1418, http://dx.doi: 10.1007/s10646-014-1283-x.

XING P., XU J., LI L., MA K. 2016. *Acute severe multiorgan dysfunction syndrome with oral acetamiprid poisoning*. J. Formos. Med. Assoc. (in press), http://dx.doi:10.1016/j.jfma.2016.06.008.

YANG X.Q., WU Z.W., ZHANG Y.L., BARROS-PARADA W. 2016. *Toxicity of six insecticides on codling moth (Lepidoptera: Tortricidae) and effect on expression of detoxification genes*. J. Econ. Entomol., 109(1): 320–326, http://dx.doi:10.1093/jee/tov297.

ZEIN M.A., MCELMURRY S.P., KASHIAN D.R., SAVOLAINEN P.T., PITTS D.K. 2014. *Optical bioassay for measuring sublethal toxicity of insecticides in Daphnia pulex*. Environ. Toxicol. Chem., 33(1): 144–151, http://dx.doi:10.1002/etc.2404.

ZHANG Y., ZHANG L., FENG L., MAO L., JIANG H. 2017. *Oxidative stress of imidaclothiz on earthworm Eisenia fetida*. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 191: 1–6, http://dx.doi:10.1016/ j.cbpc.2016.09.001.