# Impact of pesticides on the respiration of *Planorbarius* (superspecies) *corneus* s. l. allospecies (Mollusca, Gastropoda, Pulmonata, Planorbidae) from the Ukrainian river network

To cite this article: Yu V Ikonnikova *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1254** 012116





# Impact of pesticides on the respiration of *Planorbarius* (superspecies) *corneus* s. l. allospecies (Mollusca, Gastropoda, Pulmonata, Planorbidae) from the Ukrainian river network

**Yu V Ikonnikova[1], O I Uvaieva[2] and T A Vakaliuk[2,3,4,5]**

[1] Zhytomyr Ivan Franko State University, 40 Velyka Berdychivska Str., Zhytomyr, 10008, Ukraine
[2] Zhytomyr Polytechnic State University, 103 Chudnivska Str., Zhytomyr, 10005, Ukraine
[3] Institute for Digitalisation of Education of the NAES of Ukraine, 9 M. Berlynskoho Str., Kyiv, 04060, Ukraine
[4] Kryvyi Rih State Pedagogical University, 54 Gagarin Ave., Kryvyi Rih, 50086, Ukraine
[5] Academy of Cognitive and Natural Sciences, 54 Gagarin Ave., Kryvyi Rih, 50086, Ukraine

E-mail: ikon_y@i.ua, bio-2016@ukr.net, tetianavakaliuk@gmail.com

**Abstract.** We studied the impact of different concentrations in water environment of the pesticides widely used in Ukraine (insecticide "Actor", fungicide "Scooter", and herbicide "Titus-C") on the features of pulmonary and surface respiration of *Planorbarius* (superspecies) *corneus* s. l. vicaristic genetic allospecies ("western" and "eastern"). Pesticides get into the mollusc organisms percutaneously through the covers of their body during their respiration and, in much less amounts, with the food. Both the allospecies are characterized by bimodal respiration mode. It was established that the used pesticides in the concentration range 10-50 mg/L caused the rapid development of pathological process (intoxication). The lethality of experimental animals occurred due to the asphyxia and heart paralysis, caused by the intensive mucus production and destruction of the respiratory epithelium: first in body covers, later – in lungs. "Eastern" allospecies appeared to be more sensitive and less durable for all used pesticides, so it tends more to regress under the high contamination of water environment by these toxicants.

## 1. Introduction

Pesticides now belong to the most widespread among artificially synthesized stable organic toxicants in Ukrainian river network. Many years of sometimes uncontrolled their use in agriculture in the last decade caused the contamination of surface waters and deterioration of their nature ecological balance [1]. They get into the water ecosystems due to the flow of melted, rain and ground waters from the treated soils and due to blowing by wind during cultivation of lands near water bodies. Because of the cumulative features, these toxicants are accumulated and circulate in the tissues and organs of almost all the hydrobionts, and involve into the trophic chains of water bodies, increasing their concentration in times [2].

The toxical impact of pesticides on the water animals largely depends on their ability to be consumed (bio-availability), to accumulate in organisms (bio-concentration) and in trophic

chains (bio-magnification), their dose power and stability in the environment [3]. The harmful action of these toxicants on the hydrosphere consists of the change of physico-chemical properties of water environment – changing its oxygen mode, decrease of phyto-/zooplankton amount, direct harmful action on ichthyofauna, gradual loss of turgor and death of higher aquatic plants [4–7].

Pesticides used for intensification of agriculture production are divided in three main groups: herbicides to fight the higher plants, insecticides for insect defense, fungicide against the fungal pathogens. Insecticide are the most acute toxic among all and lead to the decrease of water animal growth causing various metabolic and reproductive deteriorations [8, 9]. Fungicides are accumulated in water environment and in hydrobionts' food chain objects causing their intoxication and death. Herbicides worsen the water quality (bad taste and smell) decreasing the oxygen level, food supply and bioproductivity of hydrobionts [10].

Under improper use of pesticides, the danger arises for existence of the main water ecosystems, ecological links between water organisms are disturbed, their biodiversity is lost. To reveal the consequences of these toxins' impact on the water bodies and their inhabitants, more and more often the experimental method of bio-testing is used in water toxicology. This procedure consists of identification of toxicity of chemical compounds for hydrobionts by the quantitative changes of their vital functions. As test-objects one could use the animals highly-sensitive to the action of these compounds and able to accumulate them in organisms. The vicaristic genetic allospecies of *Planorbarius* (superspecies) *corneus* sensu lato, widespread in Ukrainian river network, belong to such animals. Until now, there were no data on functioning of their respiration system under the impact of the most widespread pesticides.

The aim of present study was to identify the features of impact of three different widespread in Ukraine pesticide groups (insecticides, fungicide, herbicide) in different concentrations on the indexes of pulmonary and surface (diffusive) respirations in "western" and "eastern" *P. corneus* s. l. allospecies, and to estimate the availability of these molluscs to be used as bio-indicators for monitoring the state of surface waters under their pollution of used toxicants.

## 2. Material and methods

### *2.1. Collection of molluscs*

We collected a total of 1358 individuals of P. *corneus* s. l. in July-August of 2021. Among them, 679 individuals were from the "western" allospecies, collected at the Hnyla river (Horodnytsia village, Ternopil region, $49°24'38.5''N$ $26°01'05.2''E$), and 679 individuals were from the "eastern" allospecies, collected at Psel river (Sumy, Sumy region, $50°54'26.5''N$ $34°48'18.1''E$). The allospecies were identified based on their conchiological traits.

### *2.2. Acclimatization and care of molluscs*

Upon collection, the molluscs were transported to the laboratory and subjected to a 15-day acclimatization period. During this time, they were housed in 10 L tanks at a density of 4 individuals per liter of water. The water temperature was maintained at $20 - 22°C$, with a pH range of 7.5-8.6 and oxygen levels of 7.5-8.6 mg/L. The molluscs were provided with daily environmental changes and were fed a diet consisting of *Cladophora sp., Miriophyllum spicatum* L., and *Alisma plantago-aquatica* L. mixture collected from the collection sites.

### *2.3. Experimental procedures and toxicants*

We conducted both pilot and main experiments following standard methods. As toxicants, we used the insecticide "Actor" and fungicide "Scooted" (from "Simeinyi Sad" Ltd, Ukraine), as well as the herbicide "Titus-C" (from "Dunlop" Ltd, Ukraine) at concentrations of 10, 20, 30, 40, and 50 mg/L. These solutions were prepared using two-days aged tap water from the Zhytomyr water supply. The exposure period was 7 days.

*2.4. Monitoring behavioral and physiological reactions*

To assess the effects of the pollutants, we monitored the behavioral and physiological reactions of the molluscs [11]. The number of "inspirations" was determined by counting the emergences of each individual from the water surface tension film to which they were attached using their pneumostome, a process accompanied by a clear clapping sound. This behavior signified the initiation of "inspiration", where atmospheric air was drawn through the pneumostome into the respiration syphon and then into the mollusc's pulmonary cavity.

*2.5. Surface respiration assessment*

Surface respiration intensity was estimated by measuring the survival duration of experimental molluscs that were deprived of pulmonary respiration. These molluscs were placed at the bottom of an aquarium in small, water-permeable boxes made from densely and finely perforated plastic sheathing, with weights attached to the bottom. We analyzed the obtained results using basic variation statistical methods.

**Table 1.** The impact of pesticides in different concentrations (mg/L) on indexes of lung respiration of P. *corneus* s. l. allospecies. "Western" allospecies.

| Pesticide | Concentraion, mg/L | $n$ | Daily number of inspirations $M \pm m$ | Interval between inspirations, min $M \pm m$ | Duration of inspiration, min $M \pm m$ | Volume of inspiration, number of bubbles $M \pm m$ |
|---|---|---|---|---|---|---|
| Insecticide "Actor" | Control | 20 | 15.78±1.32 | 64.42±1.16 | 20.59±1.44 | 18.46±1.18 |
| | 10 | 18 | 16.15±1.17 | 62.41±1.32 | 21.14±1.06 | 19.21±1.25 |
| | 20 | 19 | 17.37±1.11 | 55.12±1.28* | 23.41±1.15* | 21.39±1.23* |
| | 30 | 19 | 19.42±1.29* | 43.24±1.06** | 26.76±1.23** | 27.18±1.27** |
| | 40 | 20 | 12.23±1.07* | 93.62±1.18** | 15.79±1.16** | 11.25±1.33** |
| | 50 | 20 | 10.82±1.35* | 110.58±1.03** | 12.64±1.03** | 10.31±1.42** |
| Fungicide "Scooter" | Control | 20 | 16.06±1.12 | 63.54±1.14 | 21.26±1.18 | 19.33±1.24 |
| | 10 | 19 | 16.73±1.21 | 59.12±1.35 | 22.34±1.19 | 20.12±1.14 |
| | 20 | 19 | 17.86±1.19 | 52.37±1.11* | 24.12±1.13* | 22.22±1.31* |
| | 30 | 20 | 19.87±1.26* | 41.52±1.15** | 27.32±1.17** | 28.26±1.05** |
| | 40 | 20 | 12.57±1.14* | 91.71±1.27** | 16.12±1.20** | 11.76±1.23** |
| | 50 | 19 | 11.38±1.34* | 108.24±1.18** | 12.91±1.22** | 10.87±1.14** |
| Herbicide "Titus-c" | Control | 20 | 16.13±1.29 | 62.40±1.16 | 21.67±1.26 | 19.86±1.20 |
| | 10 | 19 | 16.89±1.42 | 57.67±1.39 | 22.63±1.24 | 20.27±1.16 |
| | 20 | 19 | 18.06±1.37 | 50.46±1.21* | 24.66±1.12* | 22.16±1.25* |
| | 30 | 20 | 20.22±1.02* | 40.25±1.36** | 28.05±1.28** | 29.10±1.09** |
| | 40 | 18 | 13.07±1.24* | 88.62±1.18** | 16.58±1.10** | 12.46±1.13** |
| | 50 | 19 | 12.16±1.03* | 100.36±1.14** | 13.46±1.19** | 11.63±1.34** |

Note: $n$ – number of individuals studied; MPC – maximum permissible concentration of ions in the water; $M \pm SE$ – mean value of index and its standard error; * – statistically significant differences ($p \leq 0.05$); ** – highly significant differences ($p \leq 0.001$).

*2.6. Ethical compliance*

Throughout the experiments, we strictly adhered to ethical norms and principles governing research involving living organisms, in full compliance with the current laws of Ukraine. As part of our ethical commitment, we emphasize the following:

1 *Invertebrate research*: the experimental molluscs in our study belong to the category of invertebrates, and we ensured that ethical considerations for their welfare were upheld.

2 *Compliance with Ukrainian laws*: we affirm that our research is fully compliant with the prevailing laws and regulations of Ukraine, including those governing the ethical treatment of research subjects.

3 *Ethical review*: this research received approval from the Human or Animal Ethics Committee at Zhytomyr Ivan Franko State University. The committee reviewed and approved the ethical aspects of our study to ensure the humane treatment of the experimental subjects.

**Table 2.** The impact of pesticides in different concentrations (mg/L) on indexes of lung respiration of P. *corneus* s. l. allospecies. "Eastern" allospecies.

| Pesticide | Concentraion, mg/L | $n$ | Daily number of inspirations $M \pm m$ | Interval between inspirations, min $M \pm m$ | Duration of inspiration, min $M \pm m$ | Volume of inspiration, number of bubbles $M \pm m$ |
|---|---|---|---|---|---|---|
| Insecticide "Actor" | Control | 20 | 14.23±1.28 | 78.64±1.12 | 18.56±1.13 | 17.34±1.25 |
| | 10 | 19 | 14.61±1.19 | 75.36±1.21 | 19.37±1.36 | 18.04±1.33 |
| | 20 | 20 | 15.34±1.08 | 68.13±1.25* | 21.46±1.18* | 20.08±1.15* |
| | 30 | 19 | 17.49±1.35* | 54.61±1.11** | 24.38±1.11** | 25.12±1.29** |
| | 40 | 19 | 11.15±1.25* | 112.46±1.06** | 13.18±1.21** | 10.52±1.17** |
| | 50 | 20 | 9.85±1.23** | 130.02±1.13** | 11.12±1.36** | 9.03±1.06** |
| Fungicide "Scooter" | Control | 20 | 14.41±1.32 | 76.82±1.27 | 19.06±1.38 | 17.53±1.12 |
| | 10 | 20 | 15.16±1.25 | 71.12±1.35 | 20.48±1.39 | 18.78±1.31 |
| | 20 | 19 | 16.32±1.23 | 63.16±1.02* | 22.14±1.20* | 20.41±1.32* |
| | 30 | 19 | 18.11±1.42* | 51.21±1.11** | 25.16±1.19** | 26.82±1.23** |
| | 40 | 20 | 11.57±1.10* | 106.27±1.19** | 13.52±1.27** | 11.06±1.33** |
| | 50 | 19 | 10.12±1.19* | 126.08±1.22** | 11.63±1.45** | 9.89±1.25** |
| Herbicide "Titus-c" | Control | 20 | 14.78±1.21 | 74.41±1.13 | 19.72±1.24 | 18.12±1.22 |
| | 10 | 19 | 15.48±1.32 | 69.25±1.17 | 20.64±1.12 | 19.21±1.16 |
| | 20 | 19 | 16.62±1.20 | 60.12±1.01* | 22.82±1.06* | 21.42±1.13* |
| | 30 | 19 | 18.55±1.13* | 47.82±1.15** | 26.79±1.36** | 27.14±1.31** |
| | 40 | 20 | 11,62±1,27* | 104,29±1,33** | 13,74±1,12** | 11,16±1,41** |
| | 50 | 20 | 10,59±1,38* | 120,10±1,08** | 12,08±1,17** | 10,11±1,28** |

Note: $n$ – number of individuals studied; MPC – maximum permissible concentration of ions in the water; $M \pm SE$ – mean value of index and its standard error; * – statistically significant differences ($p \leq 0.05$); ** – highly significant differences ($p \leq 0.001$).

## 3. Results and discussion

Pesticides can get into the molluscs' organisms percutaneously through their body covers of during respiration and in much less amounts from their food. Allospecies of P. *corneus* s. l. possess the bimodal respiration mode. The lungs are the adaptive acquisition in these molluscs for the new environment, where they found themselves in ancient times due to their forced transition from water to land environment, acquiring the ability to breathe by atmospheric air. The pulmonary respiration is performed by them via the periodical emergences under the water surface tension film to take the air through pneumostome. The surface respiration is established due to the diffusive income of water-solved oxygen in the molluscs' blood through their epithelial body covers and adaptive gill of quite large area. It is known, that efficiency of oxygen supply is almost equal for both modes: 0.025 and 0.03 $O_2$ (mg)/L (per 1 g of fresh mass, respectively).

We established that pesticides in the concentration range from 10 to 50 mg/L caused the rapid development of pathological process in ramshorn allospecies. Under the 10 mg/L concentration, obtained indexes of both pulmonary and surface respirations were close to those found in control group (tables 1-4), which corresponds with a latent phase of intoxication.

Under the concentrations of mentioned pesticides of 20-30 mg/L, the indexes of both respiration modes demonstrated the development of adaptive process in form of stimulation of all studied indexes in studied allospecies, which increased with increasing of used toxicant's concentration. This corresponds with the next phase of pathological process – stimulation. Studied molluscs showed the increase of daily number of "inspirations" and of their duration

**Table 3.** The impact of pesticides in different concentrations (mg/L) on indexes of direct diffusive respiration of *P. corneus* s. l. allospecies. "Western" allospecies.

| Pesticide | Concentration, mg/L | $n$ | $M \pm m$ |
|---|---|---|---|
| Insecticide "Actor" | Control | 18 | 47.36±2.65 |
| | 10 | 17 | 48.45±3.41 |
| | 20 | 19 | 50.62±2.48 |
| | 30 | 20 | 55.02±2.67* |
| | 40 | 18 | 22.53±3.12** |
| | 50 | 17 | 20.14±2.79** |
| Fungicide "Scooter" | Control | 20 | 47.89±3.36 |
| | 10 | 19 | 49.06±3.28 |
| | 20 | 17 | 51.24±3.13 |
| | 30 | 18 | 56.12±2.78* |
| | 40 | 19 | 23.20±3.46** |
| | 50 | 17 | 20.72±3.61** |
| Herbicide "Titus-c" | Control | 18 | 48.06±2.03 |
| | 10 | 19 | 49.57±2.39 |
| | 20 | 18 | 52.15±3.08 |
| | 30 | 20 | 57.34±2.82* |
| | 40 | 18 | 23.65±2.81** |
| | 50 | 19 | 21.23±3.08** |

Note: $n$ – number of individuals studied; MPC – maximum permissible concentration of ions in the water; $M \pm SE$ – mean value of index and its standard error; * – statistically significant differences ($p \leq 0.05$); ** – highly significant differences ($p \leq 0.001$).

**Table 4.** The impact of pesticides in different concentrations (mg/L) on indexes of direct diffusive respiration of *P. corneus* s. l. allospecies. "Eastern" allospecies.

| Pesticide | Concentration, mg/L | $n$ | $M \pm m$ |
|---|---|---|---|
| Insecticide "Actor" | Control | 19 | 41.12±2.35 |
| | 10 | 18 | 42.36±3.78 |
| | 20 | 18 | 44.12±3.16 |
| | 30 | 19 | 48.56±3.67* |
| | 40 | 17 | 19.23±2.05** |
| | 50 | 18 | 18.21±2.28** |
| Fungicide "Scooter" | Control | 19 | 41.36±3.45 |
| | 10 | 18 | 42.82±3.13 |
| | 20 | 17 | 45.06±2.75 |
| | 30 | 18 | 48.86±3.12* |
| | 40 | 19 | 20.13±2.16** |
| | 50 | 18 | 18.72±2.25** |
| Herbicide "Titus-c" | Control | 18 | 41.58±2.19 |
| | 10 | 19 | 43.04±2.71 |
| | 20 | 18 | 45.69±3.28 |
| | 30 | 19 | 49.03±3.63* |
| | 40 | 17 | 20.48±2.49** |
| | 50 | 19 | 19.02±3.10** |

Note: $n$ – number of individuals studied; MPC – maximum permissible concentration of ions in the water; $M \pm SE$ – mean value of index and its standard error; * – statistically significant differences ($p \leq 0.05$); ** – highly significant differences ($p \leq 0.001$).

by 1.1 times, intervals between them by 1.1-1.3 times, and "inspiration" volume by 1.3 times ($p \leq 0.05$–$0.001$). We registered also the increase of the surface respiration indexes (estimated by the survival rate without pulmonary respiration) by 1.1 times ($p \leq 0.05$).

However, under the impact of higher toxicants' concentrations (40 and 50 mg/L), the level of mentioned way of defense appeared to be insufficient to successfully defeat the harmful action of toxic agent. Due to that, the experimental molluscs rapidly developed the depressive phase of intoxication process, which was caused by structural and functional damages of both their lung and cover epitheliums; it was quickly replaced by sub-lethal and lethal phases. Comparing to the control group, experimental P. *corneus* allospecies demonstrated the decrease of daily "inspiration" number by 1.2-1.3 times, interval between them by 1.6 times, "inspiration" duration by 1.3-1.4 times, and "inspiration" volume by 1.6 times ($p \leq 0.05$–$0.001$). Noteworthy, the survival of the studied allospecies without ability to perform the pulmonary respiration decreased by 2.1 times ($p \leq 0.001$).

Changes in both respiration modes in studied molluscs were followed with manifestations of fast ethological and physiological responses-reactions to the toxic environment impact. Avoiding of toxic environment is one of the first fast behavioral defensive reactions, which such molluscs possess. It is caused by the presence in P. *corneus* s. l. allospecies the neural connection between their organs receipting the chemical stimuli (osphradia) and the muscles responsible for movement in space as a result of stimulus obtained. This reaction, however, was observed during long time only under the low (10 and 20 mg/L) and moderate (30 mg/L) concentrations of used

pesticides, and quite quickly fully slowed down under the high (40 and 50 mg/L) concentrations.

The lethality of experimental molluscs occurred due to the asphyxia and heart paralysis, caused by the intensive mucus production and destruction of the respiratory epithelium: first in body covers, later – in lungs. In both studied allospecies the fast and powerful watering of epithelium cells caused first the rapid growth of cell elements volume due to edema, which, although, strongly crimpled and destroy soon, and finally were exfoliated. The abruption of their leftovers was sometimes followed with disruption of underlying tissues and bleeding, less often with powerful bleeding. Similar deteriorations usually occurred earlier and manifested brighter in "eastern" allospecies individuals. That may be considered as the consequence of this allospecies range being spread on the territories with higher climate drought comparing to those inhabited by "western" allospecies.

## 4. Conclusions

The indexes of pulmonary and surface diffusive respirations in P. *corneus* s. l. allospecies showed their clear dependence on the environmental pesticide concentration. Toxic-resistance of "eastern" allospecies to the used toxicants appeared lower than in "western" allospecies. That's why the first appeared more sensitive and less endurance to the impact of used toxic agent.

Allospecies of ramshorn can be recommended for use in the system of ecological monitoring as indicator species. As the aim functions, the indexes of pulmonary and surface respirations should be considered.

## ORCID iDs

Yu V Ikonnikova https://orcid.org/0000-0001-6887-0529
O I Uvaieva https://orcid.org/0000-0003-1894-0386
T A Vakaliuk https://orcid.org/0000-0001-6825-4697

## References

[1] Kolesnyk N 2015 *Fisheries science of Ukraine* **4**(34) 31–53 URL https://doi.org/10.15407/fsu2015.04.031
[2] Shefali, Kumar R, Sankhla M S, Kumar R and Sonone S S 2021 *Biointerface Research in Applied Chemistry* **11**(3) 10131–10140 URL https://doi.org/10.33263/BRIAC113.1013110140
[3] Maurya P K, Malik D S and Sharma A 2019 Impacts of pesticide application on aquatic environments and fish diversity *Contaminants in Agriculture and Environment: Health Risks and Remediation* (India: Agro Environ Media) chap 9, pp 111–128 URL https://doi.org/10.26832/AESA-2019-CAE-0162-09
[4] Sytnyk Y, Kolesnik N and Bersan T 2012 *Fisheries science of Ukraine* **3**(21) 8–13 URL https://fsu.ua/index.php/en/2012/3-2012-21/2012-03-008-013
[5] DeLorenzo M E, Scott G I and Ross P E 2001 *Environmental Toxicology and Chemistry* **20**(1) 84–98 URL https://doi.org/10.1002/etc.5620200108
[6] Severo E S, Marins A T, Cerezer C, Costa D, Nunes M, Prestes O D, Zanella R and Loro V L 2020 *Ecotoxicology and Environmental Safety* **190** 110071 ISSN 0147-6513 URL https://doi.org/10.1016/j.ecoenv.2019.110071
[7] Rani R, Sharma P, Kumar R and Hajam Y A 2022 Effects of heavy metals and pesticides on fish *Bacterial Fish Diseases* ed Dar G H, Bhat R A, Qadri H, Al-Ghamdy K M and Hakeem K R (Academic Press) chap 3, pp 59–86 URL https://doi.org/10.1016/B978-0-323-85624-9.00016-6
[8] Stara A, Pagano M, Capillo G, Fabrello J, Sandova M, Vazzana I, Zuskova E, Velisek J, Matozzo V and Faggio C 2020 *Science of the Total Environment* **700** 134914 URL https://doi.org/10.1016/j.scitotenv.2019.134914
[9] Ayad M A, Fdil M A and Mouabad A 2011 *Archives of Environmental Contamination and Toxicology* **60** 462–470 URL https://doi.org/10.1007/s00244-010-9549-7
[10] Helfrich L A, Weigmann D L, Hipkins P A and Stinson E R 2009 Pesticides and aquatic animals: A guide to reducing impacts on aquatic systems URL https://vtechworks.lib.vt.edu/bitstream/handle/10919/48060/420-013_pdf.pdf
[11] Babych Y V, Kyrychuk G, Romaniuk R, Stadnychenko A and Uvayeva O 2023 *Folia Malacologica* **31** 9–18 ISSN 1506-7629 URL https://doi.org/10.12657/folmal.031.002