SUMMARY

Due to very promising physiochemical, mechanical and electrical properties, in the 1950s a new industry was born – nanotechnology – the industry of engineered nanomaterials. Graphene oxide (GO) is one of the most attractive graphene derivatives, and it is widely used in many fields of human life. The growing use of nanomaterials (including graphene oxide) in recent years bring to light many important and worrisome questions regarding its possible toxicity to human, animals and other organisms in various ecosystems. The release of GO into ecosystems during its production, processing, and application undoubtedly increases environmental risk. Thus a thorough understanding of the biological consequences of nanomaterials presence is imperative.

This project was created because there is still a little information describing reproduction processes influenced by nanoparticles, especially on the molecular level. Moreover, it is extremely important to search for any substance, that can reduce nanoparticle (in case of this study – GO) toxicity, or at least prevent from their negative influence. The studies on reproduction and nanoparticles toxicity are relatively scarce. To better understand the environmental risks of GO and to give environmentally responsible advice on its future applications, studies on its adverse effects on reproduction and toxic mechanisms, including molecular level, are essential.

The first aim of this project was to estimate if vitellogenin protein expression in the fat body of Acheta domesticus changes after chronic graphene oxide treatment in food and to assess how graphene oxide affects wild- and long-living strains of house cricket that were exposed to long-term GO stress. The second aim was to evaluate the potentially protective role of ascorbic acid as a powerful antioxidant against graphene oxide.

The first chapter of this manuscript described the effects of long-term GO intoxication in two strains of crickets selected for longevity. Even at low concentrations, GO contributed to changes in the antioxidative enzyme activity - catalase, the viability of cells, DNA damage, and the pattern of vitellogenin protein production. Changes in vitellogenin expression profiles can cause reproduction disorders. Eliminating graphene oxide from the A. domesticus diet improved the cellular parameters, especially in the more specialized, long-living strain. This can be perceived as a remedial effect.

In the second chapter the possible remedial effect of vitamin C on graphene oxide-intoxicated two strains: wild- and long-living of Acheta domesticus is presented. The administration of GO with food resulted in difficulty in maintaining homeostasis. It impaired the cells' viability, antioxidative enzyme catalase activity, vitellogenin protein expression pattern and contributed to greater DNA damage. The presented study showed that vitamin C treatment for 15 days after long GO intoxication had no evident remedial effect on crickets. Moreover, in DNA quality, combined toxicity of GO and vitamin C was suggested, which resulted in more significant DNA damage. It is worth mentioning that the best recovery effectswere visible after the simple removal of GO from food. The insects were able to most of theadverse GO effects during the 15 days after GO removal.

The third chapter of this project (chapter three) was designed to investigate the effects of GO intoxication in the food served over three generations of house crickets. The total contentof vitellogenin protein and the expression of vitellogenin precursor and subunits in the fat body were measured. The vitellogenin expression pattern was changed after multigenerational, chronic GO treatment. Females from the 1st generation of A. domesticus were most affected by GO consumption. The 2nd generation of crickets allocated the energy to the reproduction process to keep a high reproduction rate. Thus, the vitellogenin expression was increased. It seemed that the 3rd generation was able to cope better with the harmful effects of the GO mode of action showing balanced vitellogenin protein expression. The epigenetic

memory mechanism inherited by subsequent generations may be used to fight the toxicity of graphene oxide. Also, particular attention should be paid to the vitellogenin subunits expression. The process of vitellogenin post-translational modifications may be interrupted, and the quaternary structure of vitellogenin may be impaired.
Concluding, long-term graphene oxide intoxication can cause negative effects in the body of Acheta domesticus. Even in low concentrations, GO caused reproduction disorders at the molecular level of vitellogenin protein expression. Vitamin C, which is known for its antioxidative properties, had no remedial effects on the health status of GO-treated organisms. It seems, that the best recovery effect can be achieved when the graphene oxide nanoparticles are simply removed from the food.