Effects of imidacloprid on detoxifying enzyme glutathione S-transferase in *Folsomia candida*

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There are several endpoints in ecotoxicological aspect when testing environmental pollutants. In the present study, we used a so-called surrogate endpoint to understand the effect of pesticide imidacloprid on *Folsomia candida* (Collembola), a standard test organism for estimating the effects of pesticides and environmental pollutants on non-target soil arthropods. The alteration of the specific activity of the detoxifying enzyme glutathione S-transferase from *F. candida* was investigated during imidacloprid exposure comparing to the control group. 100 individuals of the test animals (obtained from laboratory synchronized culture) were introduced to the plastic box lined with 50 g of culture substrate (a mixture of activated charcoal and calcium sulfate) saturated with 5 ml of 2.5, 5.0, and 7.5 mgL^{-1} imidacloprid (imidacloprid concentration in a substrate was 0.25, 0.5, and 0.75 $mgkg^{-1}$) for a period of 48 hrs. Control groups were treated in the same way without insecticide. Animals were collected and put into liquid nitrogen and then kept at -20 ^{o}C until need. Change of steady state levels of *GST* mRNA and GST enzyme activities were investigated. Extracted proteins were separated according to their sizes by SDS-PAGE and the resolved protein bands were detected by silver staining. The size of the glutathione (GSH) pool in the organisms was additionally determined.

Imidacloprid-exposure had a certain degree of effect on Collembola depending on the dose. At the lowest concentration of imidacloprid (2.5 mgL^{-1}) the difference between animals in control and test group could not be observed. At a concentration of 5.0 mgL^{-1} imidacloprid, the test animals became inactive (less mobile) and at 7.5 mg L^{-1} imidacloprid paralysis and dead individuals were found. In case of 2.5 mgL^{-1} imidacloprid treated group, although appearance and activity of test animals were the same as found in control, metabolic alteration was detected. GST activity increased with increasing concentration of the pesticide from an initial activity of 0.12 $\mu mol.min^{-1}.mg^{-1}$protein in the control group up to 0.25 $\mu mol.min^{-1}.mg^{-1}$protein in the sample treated with 5.0 mgL^{-1} imidacloprid and began to sink at the highest concentration. A three fold up regulation of *GST* steady state mRNA levels was detected in the samples treated with 5.0 $mgL^{-}1$ imidacloprid compared to control, while a 2.5 and 2.0 fold up-regulation was found in 2.5 and 7.5 mgL^{-1} imidacloprid treated organisms, respectively. By contrast, the size of the GSH pool decreased with increasing concentration of imidacloprid possibly because it was used to react with toxic parent compound and/or metabolites in detoxification process. The protein fractions supposed to contain enzymes related to glutathione and glutathione metabolism were separated according to their molecular mass on a 4-20% SDS-PAGE gel. The most intense band of GST subunits from each test condition appeared at the molecular mass of 25 kDa. No new GST subunit was found in imidacloprid-treated protein extracts. The results suggest that glutathione S-transferase may be involved in the response of *F. candida* to the toxic stress of imidacloprid. We conclude that, the change of GST activity and GSH amount could be possible indicators (biomarkers) for the ecotoxicological risk assessments of environmental pollutants.