Development of invertebrate models for human health risk assessment in the context of pollution

Devalckeneer Aude, Conotte Raphaël, Rochez Emeline, Colet Jean-Marie

Faculty of Medicine and Pharmacy

Human Biology and Toxicology

University of Mons (UMONS) – 20, Place du parc 7000 Mons, Belgium

Introduction

Laboratory studies on mammals are being increasingly the subject of new legislations, it becomes important to implement alternative solutions that respond to the law of the 3Rs (Reduce, Replace, Refine) [1]. Omics approaches allowing to reduce the number of organisms as well as a refinement of experiments by using non-invasive methods. However, mammals species remains largely exploited in biomedical research and in toxicology studies in particular. Invertebrate animal models which are already used in the environmental risk assessment [2] could be transposed from ecotoxicology to the evaluation of health risks. Indeed, many similarities exist between the detoxification systems and the involved pathways.

The invertebrate models chosen are terrestrial snails (*Helix aspersa*) and earthworms (*Eisenia fetida*) because of their easy use, their abundance, their quick reproductibility and their bioaccumulation characteristics. The study is based on predictive biomarkers allowing to integrate different absorption pathways as well as being a global reflect of exposition. Furthermore, they consider the most sensitive population and can alert at reversible stage of risk. Thereby, these kind of biomarkers could be important for TRV (Toxicological reference value) determination The use of mutiple bio-indicators allows to follow the evolution of the effects across seasons and the combination of several biomarkers assures us to cover the most important toxic effects.

The aim of this study is to use invertebrate models in human health risk assement by using mutiple bioindicators and combining a bench of predictive biomarkers.

Materials and methods

Preliminary studies by ^{1}H-NMR was carried out on different organs (lung, kidney, digestive gland and heart) of *Helix aspersa* as well as on whole body and heamolymph of *Eisenia fetida*. After using CME extraction, the measurments were performed on a NMR Avance 500 Brucker spectrometer to have an overview of metabolism.

Enzymatic activities as GST (Glutathion-S-transferase) and AChE (acetylcholine esterase) were analysed with spectrophotometer in whole body of *Eisenia fetida* [3] and in digestive gland of *Helix aspersa* [4]. GST measurements were performed at 380 nm with CDNB (1-chloro-2,4-dinitrobenzene) as substrate and presence of GSH (reduced glutathione) while AChE activities were measured at 450 nm in presence of DTNB (dithiobis-nitrobenzoic acid) and AcSchI (acetylthiocholine iodide) as substrate. All the measures were taken during 5 minutes long.

Whole body and haemolymph

Dissection of Digestive Gland, Kidney, Heart and Lung

Homogenization
CME extraction
Centrifugation

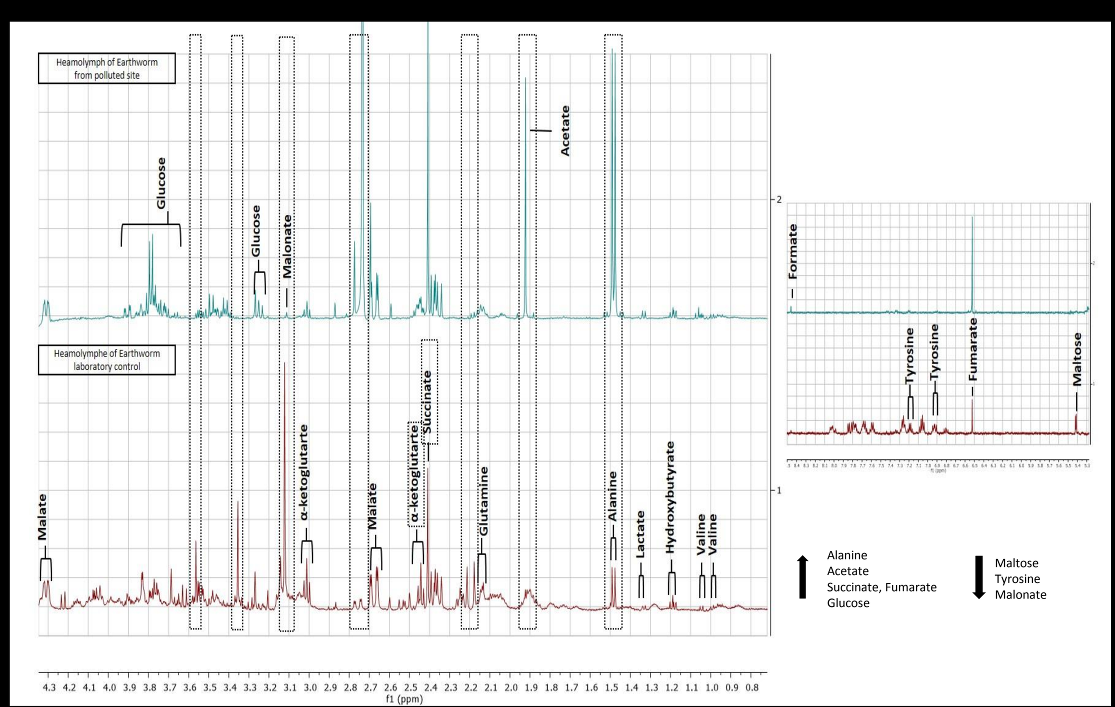


Figure 1 : Comparison of ^{1}H-NMR spectra (500 MHz) of heamolymphe in *Eisenia fetida* between laboratory control and earthworm from the polluted site (Toxicology Laboratory – UMONS)

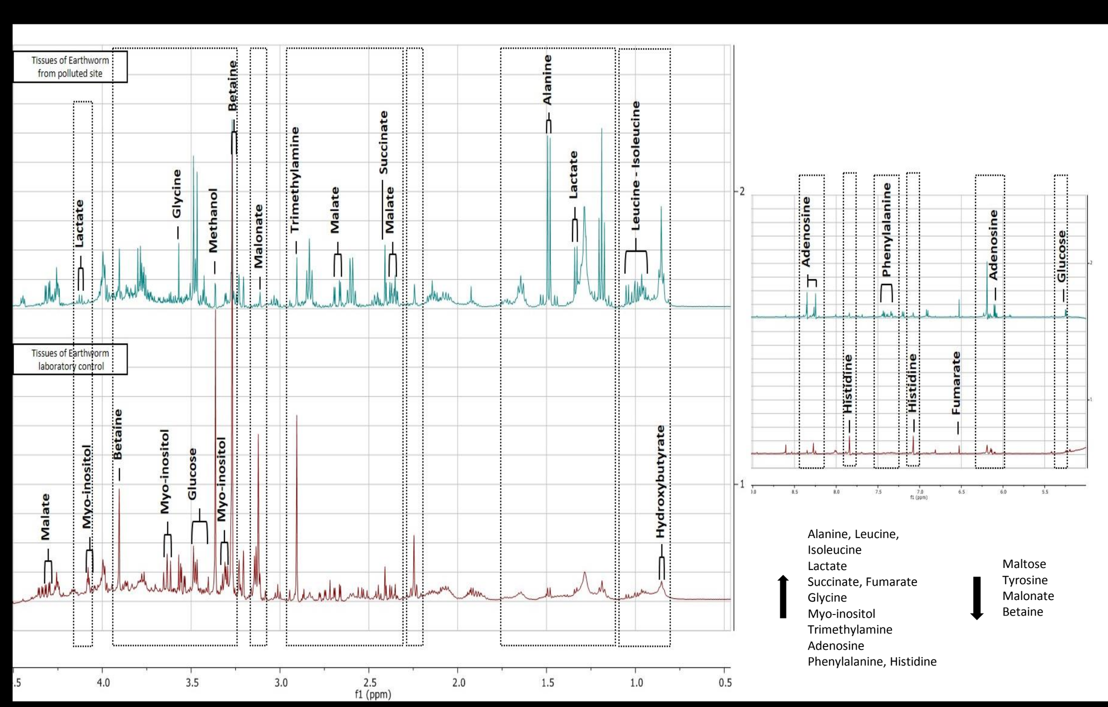


Figure 2 : Comparison of ^{1}H-NMR spectra (500 MHz) of the aqueous phase in tissues in *Eisenia fetida* between laboratory control and earthworm from the polluted site (Toxicology Laboratory – UMONS)

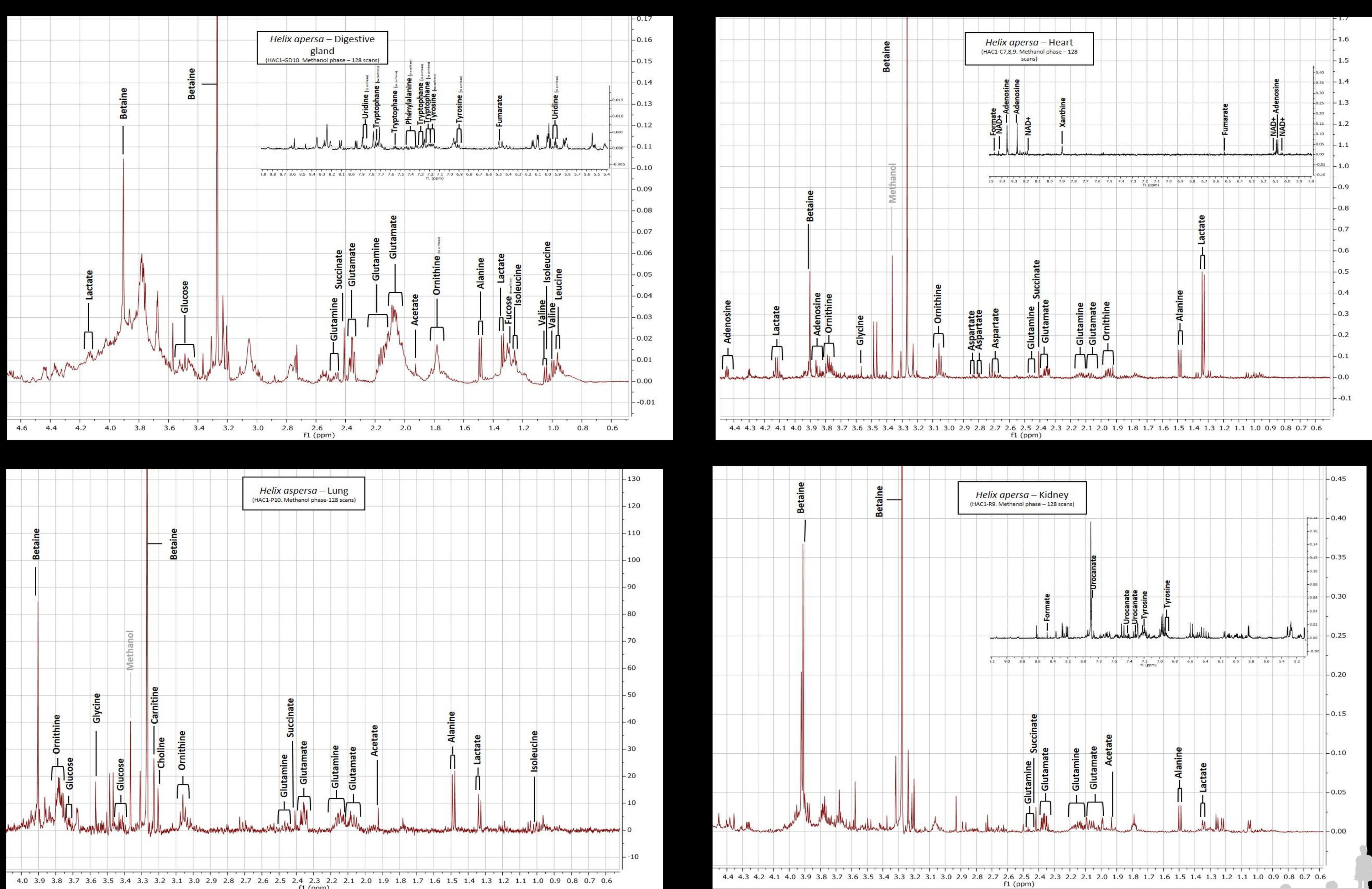


Figure 3 : Comparison of ^{1}H-NMR spectra (500 MHz) of the aqueous phase of organs in *Helix aspersa* (Toxicology Laboratory – UMONS)

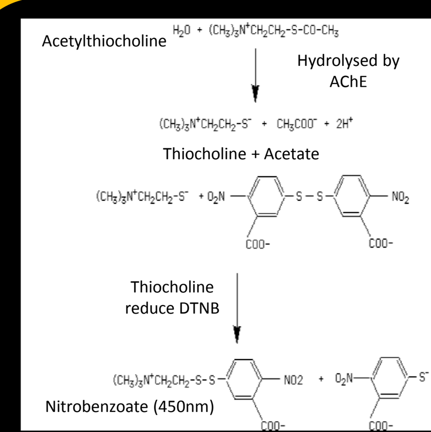
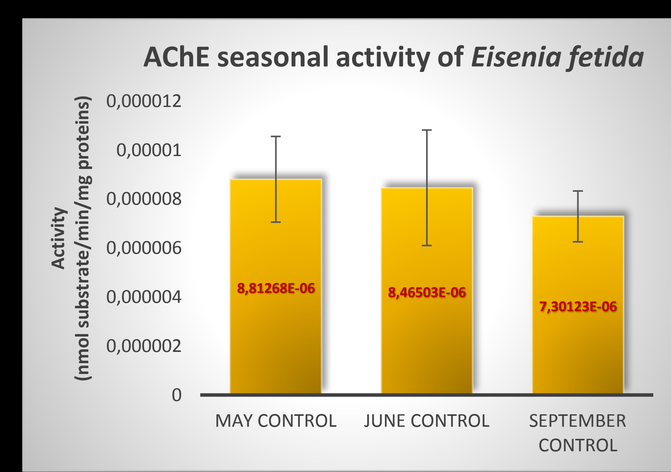


Figure 4 : Reaction catalysed by AChE



All the enzymatic activities are specific activities expressed in amount of proteins existing in sample determined by a Bradford assay as described by *Habig and al. (1974)* for GST [5] and by *Ellman and al. (1961)* for AChE [6].

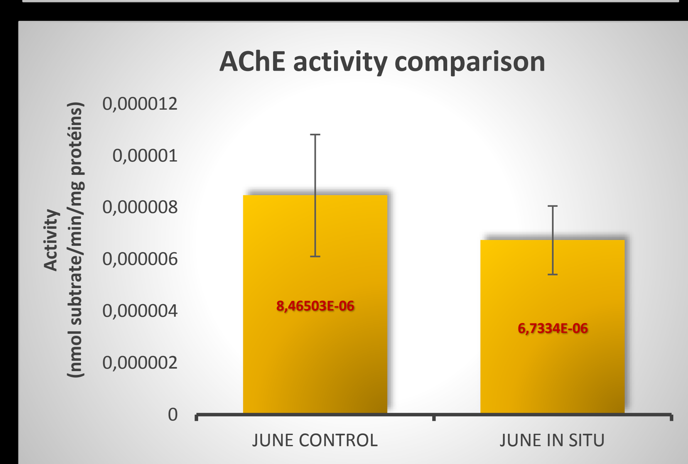
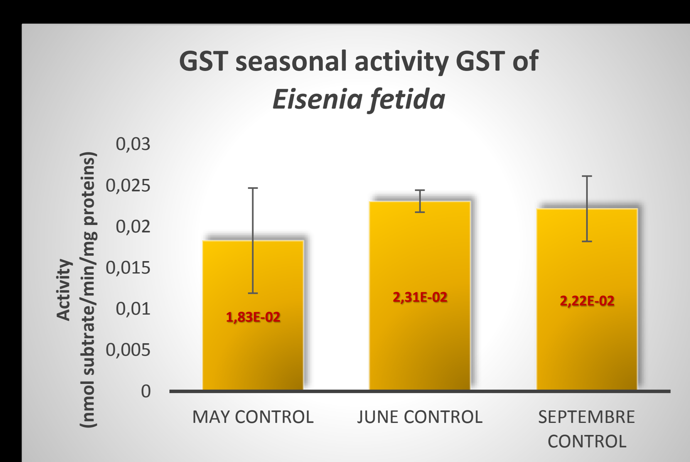


Figure 5 : Preminiary assays of AChE activity in *Eisenia fetida*

GST and AChE activites of individual controls were assayed at different month to show the seasonal variation of basal activity. A comparison between laboratory population of *Eisenia fetida* and species from the polluted site have shown small variations. However, experiments need to be reiterated on a larger number of individuals in order to be statistically reliable



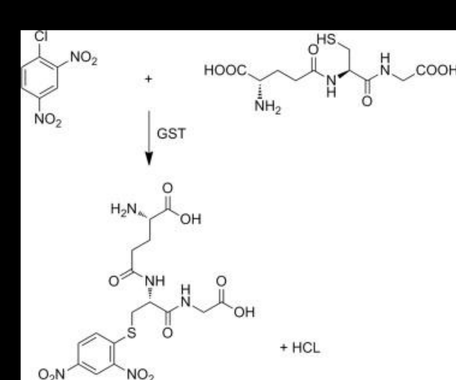


Figure 6 : Reaction catalysed by GST

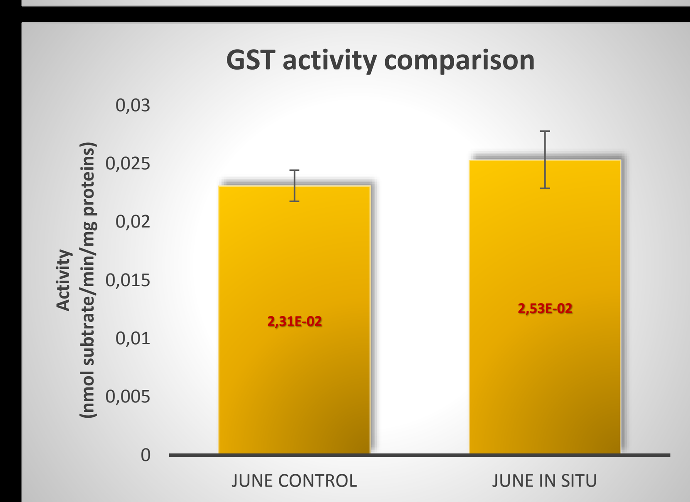


Figure 7 : Preminiary assays of GST activity in *Eisenia fetida*

Perspectives

Following this preliminary study, it is important to use additional biomarkers such as genotoxic evaluation (comet assay) and oxidative stress (Catalase, Acyl-CoA oxydase).to improve the translation from invertebrates to humans.

All the biomarkers used must be compared with mammals in same condition with different kind of pollutants.

.

In addition of these enzymatic activities, the detection of biomarkers usually followed up in pulmonary and cardiac pathologies in biomedical research need to be developed in invertebrate models to make a proper assesment of human health.

Invertebrate models for human health risk assessment ?

The results obtained in invertebrates indicate the involvement of cellular mechanisms similar to those found in mammals, evidencing their conservation through evolution. The use of invertebrate models could therefore be applied to meet the demand of the 3Rs law by replacing vertebrate organisms. Therefore, the association of invertebrates with metabonomic techniques can be a prelude to the implementation of alternative methods. As pulmonate organism, *Helix aspersa* could be a great model for pulmonary effect. allowing the detection of protein and lipid biomarkers of pulmonary disorders. The discovery of the presence of pulmonary surfactant in *Helix aspersa* has been already demonstrated [7]. Therefore, its analysis could allow the detection of protein and lipid biomarkers of pulmonary disorders after exposure to air pollution and may play a role in assessing the risk to humans.

References : [1] Riley et al., *Rapid, responsive, relevant (R3) research : a call for a rapid learning health research enterprise*. Clinical and Translational Medicine. 2013; [2] Cortet et al., The use of invertebrate soil fauna in monitoring pollutant effects. Eur. J. Soil Biol. 35, 115-134. 1999. [3] Velki et al., *Application of microcosmic system for assessment of insecticide effects on biomarker responses in ecologically different earthworm species*. Ecotoxicology and Environmental Safety 104, 110-11. 2014; [4] Ismert et al., *Effects of atmospheric exposure to naphatalene on xenobiotic-metabolising enzymes in the snail Helix aspersa*. Chemosphere 46, 273-280. 2002. [5] Habig et al., *Glutathione-S-Transferase : The first enzymatic step in mercapturic acid formation*. Journal of Biology Chemistry 249, 7130-7139. 1974; [6] Ellman et al., A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochemical Pharmacology 7, 88-95. 1961; [7] Daniels et al., *Surfactant in Gas Mantle of the Snail Helix aspersa*. Physiological and Biochemical Zoology. 1999.