Exploration of biomarker candidates for environmental risk assessment- Monitoring protein expression and modification on exposure of HL60 cells to benzene metabolites-

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The use of biomarkers in environmental risk assessment and in the formulation of environmental regulations is lacking when in fact, they have the potential to act as rapid cost-effective integrative measures that indicate adverse conditions at a biologically relevant level. Every year, about 1,000 new synthetic organic chemical compounds are manufactured and made commercially available to the public worldwide. The risk posed by these chemical substances and an estimated 60,000 to 100,000 currently present in the global environment needs to be evaluated. However, conventional methods of toxicity testing are costly, time consuming and often inconclusive. In addition, these tests include controversial animal testing that has faced much criticism from animal rights groups. A possible solution is the development and use of short term *in vitro* tests. The objective of this research was to search for possible biomarker candidates of benzene toxicity.

Benzene is a natural component of crude oil and is mainly produced by petroleum distillation. It has been classified by the International Agency for Research on Cancer (IARC), as a Group 1 carcinogen. It causes hematopoietic toxicity and is a known human leukemogen. Due to its excellent solvent properties, benzene is used in a wide range of industries. Upon inhalation, benzene undergoes biotransformation in the liver in an effort to make it more hydrophilic and thus more easily excreted from the body. Ironically, benzene toxicity is actually expressed through these metabolites.

In a method known as Double Labeling using Isotope Amino Acids and Affinity (DLIAA), which combines Stable Isotope Labeling by Amino acids in Cell Culture (SILAC) and Isotope-coded Affinity Tag Assay (ICAT) mass spectrometry based proteomic analyses methods, changes in cysteine oxidative stress in HL60 cells were monitored upon exposure to 1,4-benzoquinone, one of benzene's most toxic metabolites. Lymphocyte cytosolic Protein 1 (LCP1), a 70-kDa actin regulating protein, was found to have its cysteine oxidation state up regulated significantly (Fig.1). LCP1 is usually expressed in cells of hematopoietic origin but has been reported in many malignant cells of non-hematopoietic lineage. Over-expression of LCP1 is said to increase F-actin bundling activity and is linked to loss of cell-cell adhesion molecules and enhance invasiveness. LCP1 cysteine thiol oxidation on exposure to 1,4-benzoquinone is significant and measurable. Furthermore, since LCP1 is expressed in blood cells, samples for monitoring benzene exposure would be convenient to obtain. Further research is needed to verify sensitivity and specificity of LCP1 cysteine thiol oxidation as a biomarker for chemicals with toxicity similar to 1,4-benzoquinone and for carcinogens in general.

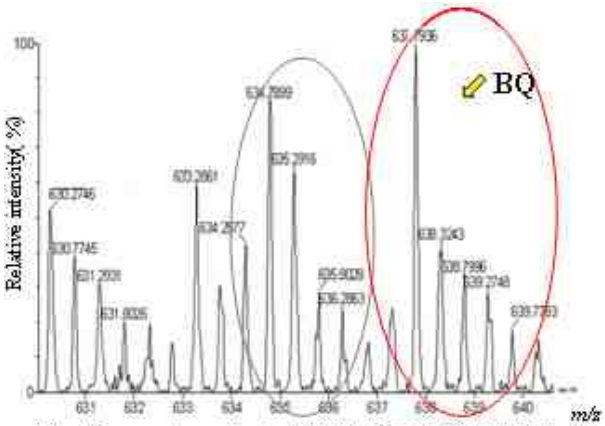

Fig. 1 Mass spectrum, showing upregulation of LCP1 cysteine oxidation in 1,4-benzoquinone treated cells.