

## Background

Pesticides are amongst the most highly regulated chemicals in use, undergoing extensive toxicity testing prior to approval. Nevertheless, concerns persist that long term, low level exposure may have deleterious effects on health. Although there is some epidemiological evidence for this, difficulties in exposure assessment and confounding have prevented any causal relationships to be established.

In support of proposed epidemiological studies on human health effects of pesticide exposure, we will develop and validate suitable biomarkers of exposure and of early indicators of adverse effects, based on mechanistic studies in experimental animals.

## Assessment of exposure

Pesticides or their principal metabolites can be quantified in biofluid, such as urine and plasma, utilising HPLC or MS. We have extensive experience of analysing low molecular weight compounds in exposed populations (Fig. 1). An appreciable limitation of such approaches is the relatively short half-life of most modern pesticides. We will therefore seek alternative matrices for analysis, such as hair, in which retention is for much longer.

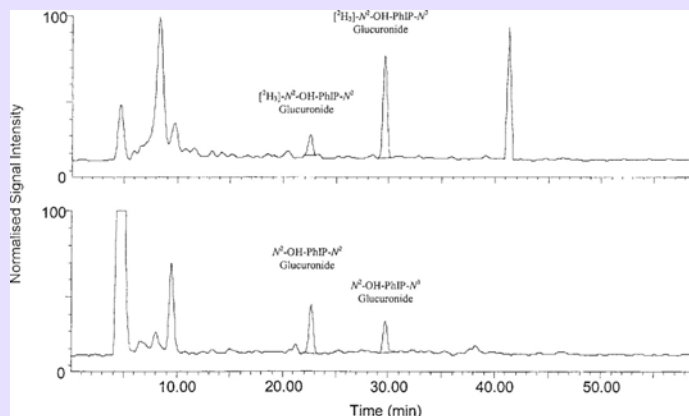


Fig. 1 LC-MS analysis of biomarker of exposure to the dietary carcinogen PhIP, at levels of < 1 ng/ml in human urine

## Identification of candidate biomarkers of early effect

Proteomics can be used to select and characterise potential biomarkers of early effect. In a proof-of-principle study, rats were treated for 3 weeks with the model neurotoxins, acrylamide and methylmercury, at doses below those producing overt toxicity. The protein profiles of serum, urine and CSF samples were determined using SELDI-TOF MS. A combination of three protein ion levels in serum enabled correct classification of treatment group thus: 88% control, 100% acrylamide, 92% methylmercury (Fig. 2).

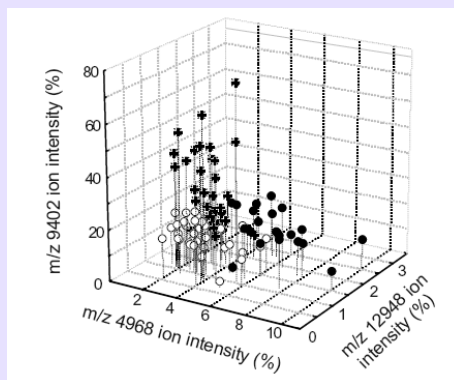


Fig. 2 Discrimination of neurotoxicant treatment group using three serum protein ions. Control (●), acrylamide (○), methylmercury (+)

## Characterisation of candidate biomarkers of early effect

SELDI-TOF MS provides a highly efficient means of comparing protein profiles and identifying protein peaks of interest. Specific proteins can be identified using 1D SDS-PAGE/LC-MS. Using this approach, we have identified the major proteins responding in the estrogen responsive MCF-7 cell line, following exposure to the endocrine active pesticide endosulfan (EDS), as the core histones (Fig. 3).

## Development of immunoassays

Following identification of putative biomarker proteins, immunoassays can be developed using a directed anti-peptide antibody approach. Specificity is assured by selecting unique, accessible peptides from the entire genome (Fig. 4).

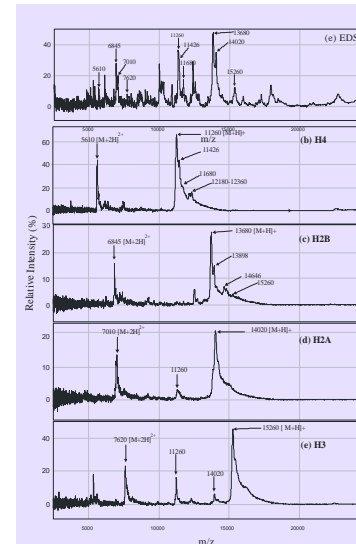


Fig. 3 Identification of protein ions in MCF-7 cells responding to endosulfan exposure, as determined by SELDI-TOF MS, using 1D SDS-PAGE/trypic digestion/LC-MS

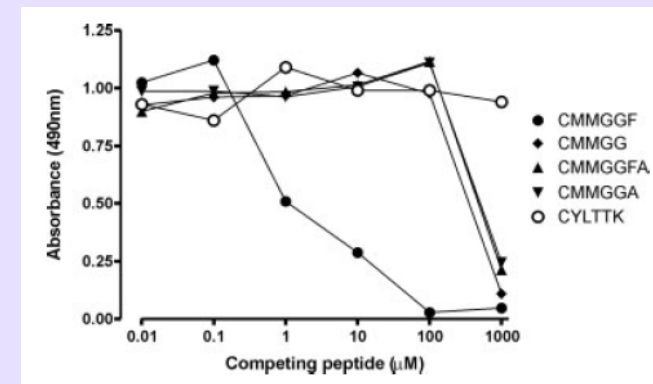


Fig. 4 Anti-peptide antibody targeted to the C-terminus of chaperonin, which does not recognise even closely related peptides to any significant extent.

## Conclusions

We have developed a strategy that should enable biomarkers of early effect to be identified experimentally. Homology comparison with the human genome will establish whether the same or a related protein should be targeted. The sensitivity and specificity of such biomarkers would need to be investigated in appropriately designed studies. Their use would greatly enhance parallel epidemiological studies.