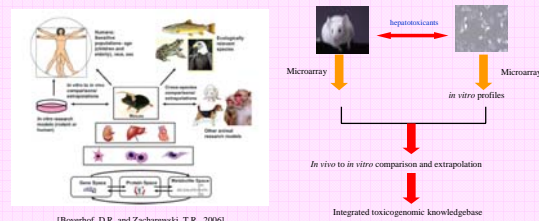


**Abstract**—We investigated the global gene expression from both mouse liver and mouse hepatic cell line treated with hepatotoxic chemicals, acetaminophen (APAP) and thioacetamide (TAA), respectively. They were tested to compare between *in vivo* and *in vitro* profiles, and to assess the feasibility of the extrapolation between two systems. For *in vivo* study, mice were orally treated with APAP or TAA and sacrificed at 6 and 24 h. For *in vitro* study, APAP or TAA were administered to a mouse hepatic cell line, BNL CL.2 and sampling was carried out at 6 and 24 h. Hepatotoxicity was assessed by analyzing hepatic enzymes and histopathological examination (*in vivo*) or LDH (lactate dehydrogenase) assay (*in vitro*). Global gene expression was assessed using microarray. In high dose APAP- or TAA-treated mice group, there was centrilobular necrosis at 24 h after treatment with the elevation of serum ALT (alanine aminotransferase, AST (aspartate aminotransferase) and LDH. However, there was no histopathological changes and serum biochemistry at low doses. In high dose treated cell line, cellular toxicity was evident at 24 h after treatment with the elevation of LDH, while no alteration at low doses. Statistical analysis of global gene expression identified that there were 436 differentially expressed genes (*in vivo*) and 500 genes (*in vitro*) by APAP, while there were 776 genes (*in vivo*) and 1734 genes (*in vitro*) by TAA. Hierarchical, *k*-means and SOM (self organizing maps) clustering analysis showed that there were similar patterns between *in vivo* and *in vitro*. Pathway analysis for differentially expressed genes identified that there were 4 (*in vivo*) and 11 (*in vitro*) pathways by APAP, and indicated glutathione metabolism pathway as common pathway. There were 11 (*in vivo*) and 22 (*in vitro*) pathways by TAA, and indicated pathways for glutathione metabolism, bile acid biosynthesis and nitrogen metabolism etc as common pathways. Taken together, gene expression patterns and clustering profiles are similar between *in vivo* and *in vitro* systems for both hepatotoxicants. Our results suggest it may be feasible to develop toxicogenomics biomarkers or profiles by comparing *in vivo* and *in vitro* genomic profiles specific to these hepatotoxic chemicals for application to prediction of liver toxicity.

**Purpose-**

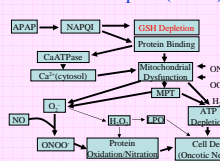
- It is needed to develop rapid and sensitive system(s) that can estimate and evaluate toxic compounds
- Alternative *in vitro* tests should be developed in toxicogenomic researches.
- We investigated the global gene expression from both mouse liver and mouse hepatic cell line treated with hepatotoxic chemicals, acetaminophen (APAP) and thioacetamide (TAA), respectively. They were tested to compare between *in vivo* and *in vitro* profiles, and to assess the feasibility of the extrapolation between two systems.



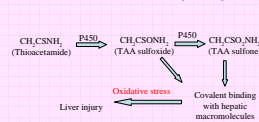
[Boverhof, D.R. and Zacharewski, T.R., 2006]

**Materials and methods**

**Acetaminophen (APAP)**



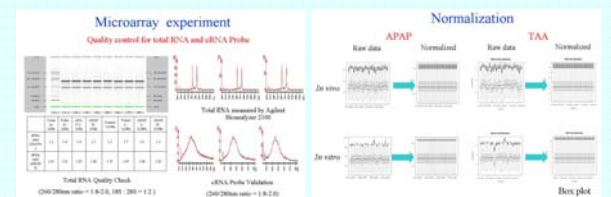
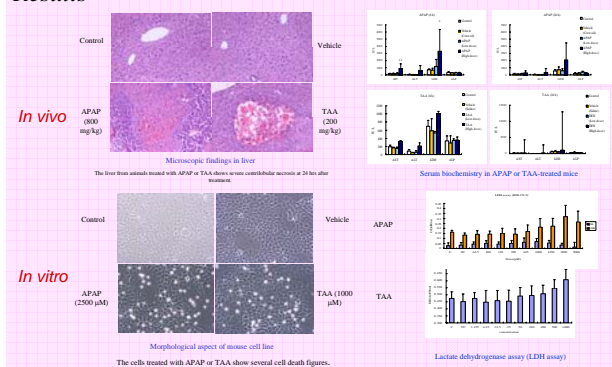
**Thioacetamide (TAA)**



- \* In vivo assay**
  - ICR mice, 6 week-old
  - APAP 80 or 800 mg/kg, oral
  - TAA 20 or 200 mg/kg, i.p.
  - Sacrifice at 6 h and 24 h
  - Serum biochemistry (AST, ALT, LDH, ALP)
  - Histopathological examination of livers
  - RNA isolation and cRNA synthesis
  - Microarray using ABI chip
  - Global gene analyses
- \* In vitro assay**
  - Mouse hepatic cell line: BNL CL.2
  - APAP 250 or 2500 μM
  - TAA 100 or 1000 μM
  - Incubate at 6 h and 24 h
  - Lactate dehydrogenase assay (LDH assay)
  - RNA isolation and cRNA synthesis
  - Microarray using ABI chip
  - Global gene analyses



**Results**

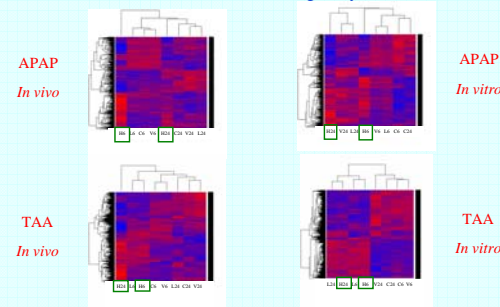


**Differentially expressed genes (DEG)**

	Statistics	<i>In vivo</i>	<i>In vitro</i>
APAP	2-way ANOVA False Discovery Rate (α: 0.1)	436	500
TAA	2-way ANOVA False Discovery Rate (α: 0.1)	776	1734

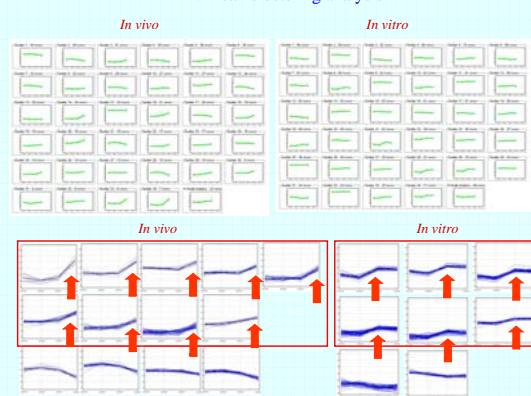
Analyzed by *svs*™ and *R™* (Comprehensive microarray analysis software)

**Hierarchical clustering analysis**



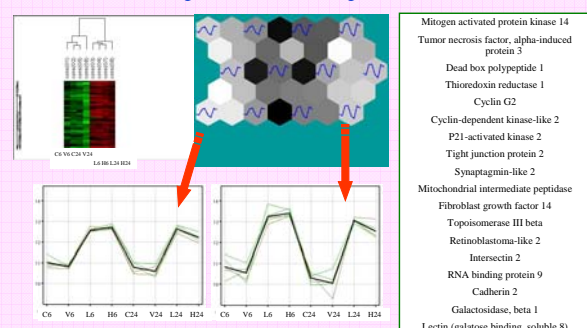
C6: control at 6 hr; V6: vehicle at 6 hr; L6: low dose at 6 hr; H6: high dose at 6 hr  
C24: control at 24 hr; V24: vehicle at 24 hr; L24: low dose at 24 hr; H24: high dose at 24 hr

**k-mean clustering analysis**

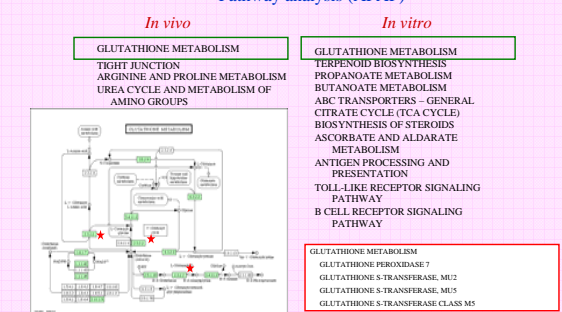


Some clusters represent up-regulation at both 6 and 24 hours after TAA treatment *in vitro*, while at 24 hours only *in vivo*.

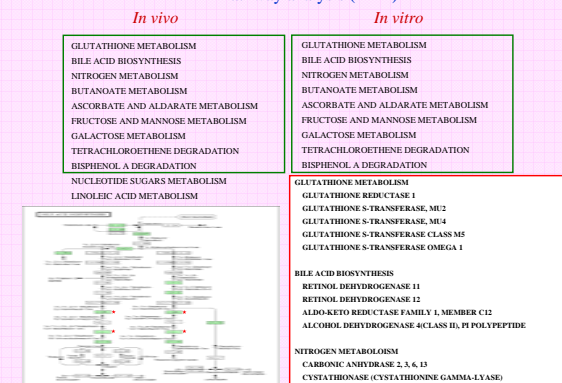
**Hierarchical clustering SOM clustering Gene lists**



**Pathway analysis (APAP)**

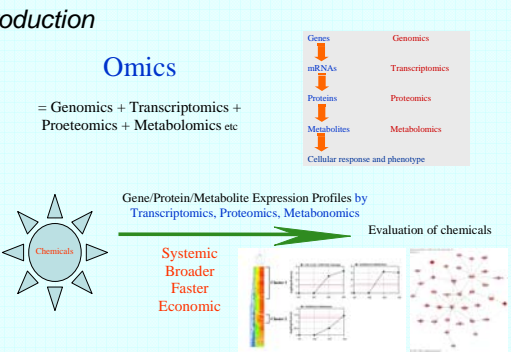


**Pathway analysis (TAA)**



**Conclusions**

1. Gene expression patterns and clustering profiles are similar between *in vivo* and *in vitro* systems for both hepatotoxicants.
2. It suggest it may be feasible to develop toxicogenomics biomarkers or profiles by comparing *in vivo* and *in vitro* genomic profiles specific to these hepatotoxic chemicals for application to prediction of liver toxicity.



**Research Progress**

