

## Animal Posters

### A-2000

Hepatocyte Preservation as Spheroids. LIA H. CAMPBELL, Elizabeth Greene, and Kelvin G. M. Brockbank. Cell & Tissue Systems, Inc, North Charleston, SC 29406. Email: [lcampbell@celltissuesystems.com](mailto:lcampbell@celltissuesystems.com)

With the continued push to reduce the number of animals used in research the development of cell-based assay systems has become more important. Hepatocytes are particularly relevant because of their important role in drug metabolism; however, their specific functions are lost in culture relatively quickly. Cryopreservation of hepatocytes is a logical alternative except after thawing, they do not attach well and their functions are further compromised. This has led researchers to investigate other cell and tissue models for evaluating hepatocyte/liver function. Liver slices are considered an equivalent in vitro model to in vivo liver function, but their time in culture is very short. More recently, the use of liver spheroids has been investigated. Liver spheroids are aggregates of hepatocytes formed in culture. Spheroids resemble an intact liver morphologically containing tight junctions between cells and the presence of liver specific structures such as bile canaliculi. Further, liver specific functions, specifically cytochrome p450 activities have been measured up to 21 days in culture. These results indicate that spheroids could be a suitable alternative to hepatocytes in culture for drug screening, toxicology and/or environmental screening. What is not known is whether spheroids can be adequately cryopreserved for use at a later time and no extensive studies have been done to date evaluating cryopreservation. In this study, the feasibility of cryopreserving liver spheroids was investigated. Hepatocytes were harvested from rat donors and spheroids were allowed to form in culture before being cryopreserved by freezing or by vitrification, preservation without the formation of ice. Metabolic activity and liver specific functions were measured after thawing and compared with fresh controls, hepatocytes cryopreserved in suspension and cell monolayers. Experiments are in progress and it is anticipated that vitrification will produce better spheroid integrity and function than conventional cryopreservation by freezing.

### A-2001

In Vitro Evaluation of Pertussis Toxin Composite Nanofibers as a Non-invasive Whooping Cough Vaccine. T. GAWADE<sup>1</sup>, K. M. Sawicka<sup>2</sup>, E. J. Roemer<sup>3,4</sup>, and S. R. Simon<sup>2,3,4</sup>. <sup>1</sup>Department of Biology, <sup>2</sup>Department of Biomedical Engineering, <sup>3</sup>Department of Pathology, and <sup>4</sup>Department of Biochemistry, Stony Brook University, Stony Brook, NY 11794. Email: [ksawicka@notes.cc.sunysb.edu](mailto:ksawicka@notes.cc.sunysb.edu)

Global resurgence of pertussis (whooping cough) has raised questions about the current preventative and treatment technologies. As a highly contagious disease caused by the fastidious gram negative coccobacillus, *Bordetella pertussis*, whooping cough is primarily considered a pediatric illness. Recent trends show a shift in epidemiology towards adolescents and adults, who can infect the most vulnerable population of neonates and infants. The most frequent misconception about the disease is that protection provided by childhood immunization is life long. In fact, adolescents become susceptible to whooping cough approximately 6 to 10 years after childhood vaccination. A novel non-invasive whooping cough vaccine has been developed by immobilization of Pertussis Toxin (PT) in electrospun polymer (Polyvinylpyrrolidone, PVP) nanofibers. Our in vitro experiments utilized a sensitive assay for PT based on Chinese Hamster Ovary (CHO) cells to examine the bioavailability of PT in novel electrospun coatings. Under standard culture conditions CHO cells form an attached monolayer. In the presence of intact PT, CHO cells lose contact inhibition and undergo characteristic clumping. We qualitatively measured clumping of CHO cells in the presence of native PT, PT-PVP electrospinning solution, and in dissolved PT-PVP electrospun coating. Microscopic observation showed clumping of cells with sensitivity down to 2.5 ng/mL of PT in dissolved electrospun coating at 48 hours. The results of the in vitro assays confirm retention of non-denatured, biologically active Pertussis Toxin in electrospun nanofibers. The superior surface area to volume ratios of non woven electrospun mats provide an improved platform for the development of a non invasive whooping cough vaccine.

**A-2002**

In Vitro Spontaneous Transformation of Rat BDE1 Cholangiocytes Compared with Oncogenic *erbB-2/neu* Transformants. A. E. SIRICA, T. Asano, Z. Zhang, A. Mahatme, and D. J. Ward. Department of Pathology, Virginia Commonwealth University School of Medicine, Richmond, VA 23298-0297. Email: asirica@hsc.vcu.edu

Cholangiocarcinoma is a highly malignant epithelial cancer of the biliary tract whose molecular pathogenesis is still poorly understood. Development of novel in vitro models of cholangiocyte neoplastic transformation can provide powerful new systems for investigating critical molecular mechanisms regulating cholangiocarcinoma growth and progression and for preclinical therapeutic studies. We have recently shown that when immortalized, non-tumorigenic rat cholangiocytes, designated BDE1 cells, (Yang, L., et al., *Gastroenterology* 1993; 104: 840–852) were stably transfected with a constitutively expressed rat *erbB-2/neu* oncogene, they became highly tumorigenic (BDENEu cells) and recapitulated key features of human cholangiocarcinoma (Lai, G.-H., et al., *Gastroenterology* 2005; 129: 2047–2057). Now, by employing selective growth conditions (Hooth, M.J., et al., *Am. J. Pathol.* 1998; 153: 1913–1921), we have been able to achieve in vitro spontaneous neoplastic transformation of BDE1 cells (BDEsp cells). Both cyclooxygenase-2 (COX-2) mRNA and protein, together with activated Akt protein were determined to be significantly overexpressed in BDEsp cells when compared with parent BDE1 cells, and at levels that were found to be higher than those overexpressed by malignant BDENEu cells. In contrast to BDENEu cells, BDEsp cells did not overexpress either *ErbB-2/neu* mRNA or activated protein, nor did they exhibit enhanced phospho-p42/44 MAP kinase expression relative to that of untransformed BDE1 cells. BDEsp cells were further observed to be of intermediate tumorigenicity compared with BDENEu cells, giving rise to slow growing, well differentiated ductal carcinomas, formed at 60% incidence when transplanted into the liver of isogenic rats. BDENEu cells, under identical conditions, yielded rapidly growing, moderately differentiated ductal carcinomas that formed at 100% incidence in liver. These results support a new and unique model of cholangiocarcinoma progression based on spontaneous versus genetic transformation. Supported by CA 39225 and CA 83650 to A.E.S.

**A-2003**

In Vitro Effect of Carotenoids on Breast Cancer Cells. P. OLVERA-CALTZONTZIN and T. Garcia-Gasca. Universidad Autonoma de Queretaro, Facultad de Ciencias Naturales, Queretaro, MEXICO. Email: palomeiros@yahoo.com

Breast cancer is one of the first causes of mortality in women on reproductive age worldwide, and ductal breast cancer is the most common (80% of deaths). Some factors are associated with breast cancer risk, including nutritional factors, particularly antioxidant status. Between antioxidant compounds, carotenoids are abundant on fruits and vegetables.  $\beta$ -carotene is the most common carotenoid; however, lycopene from tomatoes is now consumed in approximately the same amount as  $\beta$ -carotene. Epidemiological studies have shown an inverse relationship between carotenoid intake and cancer but, for breast cancer, results are inconsistent. The goal of this work was to determine the effect of  $\beta$ -carotene and lycopene on proliferation and survival of ductal (ZR-75-1) and glandular (MCF-7) breast cancer cells. Dose-response curves showed that lycopene increased survival and proliferation of both cell lines, the effect was more pronounced on MCF-7 than on ZR-75-1 cells. On MCF-7 cells, the highest concentration tested of  $\beta$ -carotene ( $1 \times 10^{-4}$  M) exhibited cytostatic effect, and lower concentrations stimulated cell proliferation. On the other hand, there was an inhibitory effect of  $\beta$ -carotene on ZR-75-1 cells, proliferation and survival were inhibited nearly 50% when cells were treated with  $1 \times 10^{-4}$  M of  $\beta$ -carotene ( $p \leq 0.05$ ) and lower concentrations stimulated cell growth. Our results show that some carotenoids, but not all, can affect cell growth depending on carotenoid type and concentration. Also, we are demonstrating that ductal breast cancer cells are less resistant than glandular cells. Future studies will evaluate the possible mechanism of action of carotenoids on ductal breast cancer cells.

**A-2004**

Prostaglandins A<sub>1</sub> and E<sub>1</sub> Influence Gene Expression in an Established Insect Cell Line (BCIRL-HzAM1). D. Stanley<sup>1</sup>, C. GOODMAN<sup>1</sup>, Q. Song<sup>2</sup>, S. An<sup>2</sup>, and A. McIntosh<sup>1</sup>. <sup>1</sup>USDA, ARS, BCIRL, 1503 S. Providence Rd., Columbia, MO 65203, and <sup>2</sup> Plant Sciences Division, University of Missouri, Columbia, MO 65211. Email: stanleyd@missouri.edu

In work to determine the biochemical mechanisms of prostaglandin (PG) action in insect cells, we posed the hypothesis that prostaglandins (PGs) influence gene expression. In separate experiments, we exposed the BCIRL-HzAM1 cell line (derived from pupal ovarian tissue of the cotton bollworm, *Helicoverpa zea*) to PGA<sub>1</sub> (interacts with perinuclear receptors) and PGE<sub>1</sub> (acts via G protein coupled receptors). After either 12 or 24 hr exposures to the PGs, control and treated cells were subjected to 2D electrophoresis. Changes in protein expression were recorded by densitometry, showing quantitative changes in >30 proteins. Selected proteins were analyzed by MS/MS (MALDI

TOF/TOF). The derived sequences were used in bioinformatic analyses to identify the proteins. Quantitative changes in a subset of proteins were confirmed by RT-PCR. PG treatments influenced expression of genes encoding proteins involved in stress responses or detoxification reactions (e.g., heat shock proteins, Mn superoxide dismutase and catalase). Changes were also recorded in expression of genes encoding proteins acting in cell structure (e.g., actin-depolymerizing factor 1), metabolism (e.g., glyceraldehyde-3-phosphate dehydrogenase) and responses to external stimuli (e.g., tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein). These findings support our hypothesis that both  $PGA_1$  and  $PGE_1$  influence gene expression in insect cells, as seen in mammalian cells. These two PGs presumably act via different intracellular signal transduction pathways.

#### A-2005

In Vitro Generation of Amoebocytes: A New Source for *Limulus* Amoebocyte Lysate Production. A. G. BRANNAN, K. C. Dee, A. Buchmann, and W. W. Weiner. Department of Applied Biology and Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803. Email: amberbrannan@alumni.rose-hulman.edu

Lipopolysaccharides, a major constituent of the Gram-negative bacterial cell wall, are endotoxins. Exposure of endotoxins to the mammalian bloodstream results in a massive immune response usually ending with fatal shock. Therefore, endotoxin detection tests are required for most injectable drugs, invasive medical devices, and vaccines that come into contact with the bloodstream. This testing uses *Limulus* amoebocyte lysate (LAL). The amoebocytes (AmbCs) required for the LAL test are currently obtained via cardiac puncture of horseshoe crabs, and the declining horseshoe crab population may impact the \$60 million LAL industry. Potential alternative sources of LAL include an in vitro organ culture system recently developed in India. A similar system using the American horseshoe crab *Limulus polyphemus* – one of the two main species currently used in the LAL industry – could offer a much more convenient system to produce lysate with uniform sensitivity. This research developed a system for in vitro AmbC production via organ-cultured horseshoe crab gill lamellae. Endotoxin-free solutions and equipment have been economically produced, methods for surgical removal and disinfection of gill lamellae have been developed, conditions to simulate the in vivo environment have been determined, long-term organ culture of lamellae has been achieved, and these lamellae have produced apparent AmbCs. After stringent comparisons of traditional LAL tests to results obtained

using these AmbCs, this method could supplement and/or replace the current labor-intensive method of obtaining AmbCs.

#### A-2006

Achieve New Levels of Expression in Transfected Tumor Cells. LINDA B. JACOBSEN and Susan Calvin. Transfection Laboratory, Roche Applied Science, Indianapolis, IN 46256. Email: lbjacobsen@gmail.com

Some tumor cells are notoriously difficult to transfect. FuGENE<sup>®</sup> HD Transfection Reagent was shown to effectively transfect many key tumor cell lines: HeLa, MCF7, HepG2, PC3, Panc-1, SK-Mel-28, Caco-2, N87, STSTAR, T98G, U87 and others (all cells for studies were obtained from ATCC). Higher levels of expression were obtained with this reagent compared to other commercially available reagents in standard medium as well as 100% serum conditions. In our laboratory, HeLa and MCF7 cells were studied in detail and expression was measured using several reporter genes. Over 90% of the HeLa cells were transfected using GFP as a reporter gene whether cells were grown in standard medium or in 100% serum. Expression levels of MCF7 cells grown in either standard medium or 100% serum were also comparable. Despite the higher levels of expression obtained, transcriptional profiling showed that significantly fewer transcripts were up or down regulated following transfection with FuGENE<sup>®</sup> HD Transfection Reagent, than with another leading transfection reagent tested in the same experiment. In this experiment it was found that large numbers of transcripts were also altered by vectors without the gene of interest. The conclusion from these studies in HeLa and MCF7 cells was that FuGENE<sup>®</sup> HD Transfection Reagent was able to effectively transfect many tumor cells while resulting in a significantly lower number of differentially expressed transcripts suggesting that more meaningful physiological results could be obtained using this reagent. FuGENE is a trademark of Fugent LLC, USA.

#### A-2007

Rapid Identification and Authentication of Animal Cell Culture Based on PCR Size Differences. MERRY LIU<sup>1</sup>, Hsi Liu<sup>2</sup>, Seh-Ching Lin<sup>1</sup>, Angel Hughes<sup>1</sup>, and Abbas Vafai<sup>1</sup>. <sup>1</sup>Division of Scientific Resources, <sup>2</sup>Division of STD prevention, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30329. Email: mliu@cdc.gov

Correct origins of animal cell lines are important in many studies and need to be monitored and verified at regular intervals. Previously we used PCR with primers targeted at the aldolase gene family to correctly identify the origins of 13 animal cell lines. The PCR method, however, was not able to separate cells derived from closely related species such as human and primates, and mouse and rats. Additional DNA sequencing was needed to differentiate these cell lines. In this study, we developed new primers based on similarities in the aldolase gene family. Combining with our previous primer sets we were able to distinguish closely related species. Specifically, a new reverse primer (ALD4) was used with forward primer (ALD1) to separate human cell lines from monkey cell lines and a newly designed forward primer (ALD3) paired with reverse primer (ALD2) separated rat from mouse cell lines. Thus, by using two separate PCR reactions we were able to verify the origins of the cell lines of 13 species. These new primer combinations reduced the time required to perform authentication tests for animal cell lines and are cost-effective.

#### A-2008

Inflammation of A549 Epithelial Cells- an In Vitro Comparison of Various Mineral Oxides' Relative Cytotoxicities and Their Impact Upon ROS Release. P. G. CLAIRE<sup>1</sup>, A. K. Bushkanets<sup>2</sup>, Y. Fukuda<sup>3</sup>, S. R. Simon<sup>2,4</sup>, and E. J. Roemer<sup>3,4</sup>. <sup>1</sup>Department of Biology, <sup>2</sup>Department of Biochemistry, <sup>3</sup>Department of Pharmacology, <sup>4</sup>Department of Pathology, SUNY Stony Brook, Stony Brook, NY 11794. Email: eroemer@tesla.noc.sunysb.edu

Exposure to minerals via inhalation has long been implicated in the development of a variety of human inflammatory pulmonary diseases. Suspected mechanisms for lung pathologies include the production of reactive oxygen species (ROS) or cell death in response to the toxicity of the inhaled material. This particular study evaluates the inflammatory response of the human lung epithelial cell line, A549, to various mineral oxides. The use of concomitant cytotoxicity assays allowed for rough quantification of ROS release per cell. The mineral samples examined included pyrite (iron disulfide, FeS<sub>2</sub>), nickel disulfide (NiS<sub>2</sub>), titanium dioxide (TiO<sub>2</sub>), olivine (forsterite, Mg<sub>2</sub>SiO<sub>4</sub>) and quartz (SiO<sub>2</sub>). ROS generation was measured via Dichlorodihydrofluorescein (DCFH). This nonspecific fluorescent probe yields fluorescent DCF when cleaved intracellularly by oxidants. Depending upon the mineral being examined, one of two cytotoxicity assays: MTS or calcein AM was chosen. MTS is a less than satisfactory choice with nickel and calcein AM was

incompatible with titanium dioxide. The MTS assay uses a soluble tetrazolium salt that is cleaved in the mitochondria of metabolically active cells to form a soluble formazan product. Absorbance measurements allowed quantification of the product. Calcein AM is a membrane-permeant reagent. Once inside the cells, it is hydrolyzed by endogenous esterase into fluorescent calcein, which is retained in the cytoplasm. Unlike the indirect measure provided by MTS, calcein AM measures cell number directly, independent of metabolic activity levels. The data were analyzed first as percent of control; then the DCFH values were divided by the cytotoxicity values to correct for the approximate number of viable cells in a sample set, yielding a measurement of ROS per cell. At present, this study has yielded a qualitative ranking of reactivity and toxicity of the mineral oxides: with nickel and olivine as the most reactive and quartz and titanium dioxide as the least.

#### A-2009

In Vitro Evaluation of Reactive Oxygen Species Generation in Two Human Cell Lines Exposed to Hurricane Katrina Flood Sediment Samples Containing Varying Pyrite Content. Y. FUKUDA<sup>1</sup>, P. G. Claire<sup>2</sup>, S. R. Simon<sup>3,4</sup>, and E. J. Roemer<sup>3,4</sup>. <sup>1</sup>Department of Pharmacology, <sup>2</sup>Department of Biology, <sup>3</sup>Department of Biochemistry, <sup>4</sup>Department of Pathology, SUNY Stony Brook, Stony Brook, NY 11794. Email: eroemer@tesla.noc.sunysb.edu

Exposure of cells and tissues to reactive minerals can lead to inflammation and ultimately cause life threatening diseases. Humans can be at risk of inhalation hazards from minerals such as iron pyrite (FeS<sub>2</sub>), which is found in environmental sources, including post flooding mud deposits. Sixteen samples of mud left in New Orleans after hurricane Katrina were provided by the US Geological Survey and were evaluated in vitro with two human cell lines: the A549 lung epithelium and the monocytic MonoMac 6 (MM6). The introduction of foreign substances into the human airway can result in the generation of reactive oxygen species (ROS), such as peroxides and free radicals. ROS release has negative effects on cell structures and the result is collectively known as oxidative stress. Measurement of cellular ROS production was done with 2',-7'dichlorodihydrofluorescein (DCFH), a broad spectrum nonspecific ROS detector that is oxidized intracellularly to its fluorescent form: 2',-7'dichlorofluorescein (DCF). The DCF produced was measured using a fluorescent plate reader at excitation and emission wavelengths of 485 nm and 530 nm. All assays were done in a 96 well plate format and designed to evaluate the effect of sample concentration on ROS production. Plates contained cell-free blanks,

untreated control cells, positive control cells and cells exposed to mud concentrations of 7.8125, 15.25, 31.25, 62.5, 125, and 250  $\mu\text{g}/\text{mL}$ . Prior to addition to the cells, the mud samples were sonicated to break up large aggregates and clumps and yield a more uniform suspension. Looking at raw ROS data, only four of the samples ranked similarly in their ROS responses in both cell types. Spearman rank correlation was used to determine if pyrite concentration was related to ROS release. The Spearman calculation indicated a positive correlation for A549 cells, but no correlation for MM6 cells. This means that for the epithelial cells, but not the monocytic, pyrite concentration is related to ROS production.

#### A-2010

Developing an In Vitro Assay to Evaluate Human Cellular Responses to Reactive Mineral Oxides. A. K. BUSKANETS<sup>1</sup>, P. Claire<sup>2</sup>, Y. Fukuda<sup>3</sup>, S. R. Simon<sup>1,4</sup>, and E. J. Roemer<sup>1,4</sup>. <sup>1</sup>Department of Biochemistry, <sup>2</sup>Department of Biology, <sup>3</sup>Department of Pharmacology, <sup>4</sup>Department of Pathology, SUNY Stony Brook, Stony Brook, NY 11794. Email: eroemer@tesla.noc.sunysb.edu

We are developing in vitro systems to evaluate human cell responses to mineral exposure. Inhalation of minerals can cause inflammation resulting in conditions such as coal workers pneumoconiosis (CWP), an interstitial lung disease that can lead to respiratory failure. Exposure to mineral oxides causes production of reactive oxygen species (ROS) known to incite an inflammatory response in cells and cause apoptosis. To assess the effects of mineral exposure we exposed cells from the human respiratory epithelial line, A549, to equal concentrations of nickel (Ni), olivine (OL), titanium (Ti), pyrite (Py), and quartz (Qt) particles. To accurately gauge the effects of minerals on cells several specific combinations of methods to measure ROS and cytotoxicity were evaluated. ROS was quantified using dichlorodihydrofluorescein (DCFH), a nonspecific fluorescent probe that yields fluorescent DCF upon cleavage by oxidants. To determine if the minerals induced cell death we examined two cytotoxicity assays. The first used MTS, a soluble tetrazolium salt that is cleaved in the mitochondria of metabolically active cells to form an aqueous soluble formazan product which is quantified with absorbance measurements. By measuring metabolic activity this system provides an indirect measurement of the number of viable cells. The second was Calcein AM, a widely used green fluorescent cell marker that is membrane-permeant and can be introduced into cells via incubation. Calcein AM is hydrolyzed inside cells by endogenous esterases into highly negatively charged green fluorescent calcein that is retained in the cytoplasm. Unlike the indirect measure provided by

MTS, Calcein AM measures cell number directly, independent of metabolic activity levels. The adapted assays were improved with applications of different layouts of the 96-well plates to minimize edge effects and achieve the most accurate results. These cytotoxicity and ROS assay methods can be combined as needed and specifically applied for each of the five test minerals, providing a system for generation of data that is both robust and reproducible.

#### A-2011

The Respiratory Syncytial Virus-F Protein Does Not Activate the Neuroimmune System in Mice. E. M. BENDIK<sup>1</sup>, D. R. Johnson<sup>2</sup>, G. G. Freund<sup>2,3</sup>, and S. S. Korban<sup>1</sup>. <sup>1</sup>Dept. of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL; <sup>2</sup>Dept. of Animal Sciences, University of Illinois, Urbana, IL; and <sup>3</sup>Dept. of Pathology, University of Illinois, Urbana, IL. Email: bendik2@uiuc.edu, korban@uiuc.edu, drjohs1@uiuc.edu, freun@uiuc.edu

In our continuing effort to develop an effective oral plant-based *respiratory syncytial virus (RSV)* vaccine, we investigated the sickness behavior of mice challenged with *RSV-F*. Sickness behavior has been defined as a coordinated set of nonspecific behavioral modifications including loss of appetite, libido, motor activity and interest in the physical and social environment that occur in response to infection. *RSV-F* has been shown to be an activator of the innate immune system through ligation of the toll-like receptor 4 (TLR4). Here, we report that in 5 strains of mice susceptible to *RSV* infection, peritoneal administration of *RSV-F* fails to activate the neuroimmune system, as measured by social exploration of a novel juvenile. We intraperitoneally (i.p.) injected male DBA/2J, CBA/J, 129P3/J, AKR/J, and Balb/C mice with long strain UV-inactivated *RSV-F* and measured social exploration for a period of 24 hours. No decrease in social exploratory behavior in any of the strains following i.p. *RSV-F* as compared to saline injected controls was observed. Taken together, these data demonstrate that the TLR4 ligand i.p. *RSV-F* does not activate the neuroimmune system in *RSV* susceptible strains of mice.

#### A-2012

Applications of Neutral Red Uptake Assay for Basal Cytotoxicity Assessment in Response to Viruses, Toxins, and Chemicals. C. J. CAO<sup>1</sup>, A. E. Chamber, M. M. Wade<sup>2</sup>, and M. A. Major<sup>1</sup>. <sup>1</sup>US Army for Health Promotion and Preventive Medicine. <sup>2</sup>US Army, Edgewood Chemical Biological Center; Aberdeen Proving Ground, MD 21010. Email: cheng.cao@us.army.mil

The neutral red uptake (NRU) assay has recently been validated (2002–2005) in a three-phase study sponsored by the NIEHS, EPA and ICCVAM and is now approved as an in vitro method useful in reducing the use of animals, particularly in rodent lethality testing. The studies reported herein seek to improve the utility of this test for in vitro toxicity screening of increasingly diverse agents/samples by extending the use of the NRU assay to measure basal cytotoxicity of biosamples and chemicals with low water solubility. At the time of this submission, viruses, toxins, and explosive compounds have been tested on 3 cell lines. The cytotoxicity of TC-83-T1, Vaccinia-T1 and Tamiami as determined by the NRU assay was comparable with that from conventional plaque tests, and found to be both more practical and more accurate. The toxicity of the explosives TNT and RDX was tested on three cell types using suspensions of the explosives in the culture medium. Basal cytotoxicity was demonstrated for both compounds on all three types of cells. A measure of the cytotoxicity of RDX was found that could not be detected using other in vitro assays. Testing with other types of toxicants showed that the NRU assay was also suitable for determining cytotoxicity of toxins such as the sea nettle toxin. The NRU assay will require further refinement to determine its feasibility and usefulness in rapid compound prioritization and risk assessment. These studies will include testing of more diverse agents in more complex environmental matrixes.

#### A-2013

In Vitro Evaluation of Antihypertensive Mechanism of the Dipeptide Val-Tyr in Rat Aorta Organ Bath Experiments. L. Vercruyse<sup>1,2</sup>, N. Morel<sup>3</sup>, J. Szust<sup>1,2</sup>, J. Van Camp<sup>2</sup>, and G. SMAGGHE<sup>1</sup>. <sup>1</sup>Dept. Crop Protection, <sup>2</sup>Dept. Food Safety and Food Quality, Fac. Bioscience Engineering, Ghent University, B-9000 Ghent, BELGIUM and <sup>3</sup>Lab. Physiologie, Université Catholique de Louvain, B-1200 Bruxelles, BELGIUM. Email: guy.smagghe@ugent.be

Antihypertensive peptides derived from food proteins are biologically active peptides with a potential role in prevention and treatment of hypertension. These peptides are proven to be in vitro angiotensin converting enzyme (ACE) inhibitors, but the actual antihypertensive mechanisms in vivo are still unclear. The aim of this research was to clarify the antihypertensive mechanisms of the dipeptide, Val-Tyr. Val-Tyr was first identified as an in vitro ACE inhibitory peptide derived from sardine muscle hydrolyzed by *Bacillus licheniformis* alkaline protease. The in vivo antihypertensive activity in spontaneously hypertensive rats as well as in human subjects has been reported, although the underlying mechanisms are not fully elucidated. Organ

bath experiments with rat aortic rings were used to investigate five important antihypertensive mechanisms, corresponding with the mode of action of the main antihypertensive drugs used today. We confirmed ACE inhibitory activity in the organ baths. Stimulation of the aortic rings with angiotensin I after incubation of the rings with 5 mM Val-Tyr resulted in a decreased contraction, indicating ACE inhibition. Furthermore, we studied the effect of Val-Tyr on angiotensin II receptors, adrenergic receptors, Ca<sup>2+</sup> channels and NO dependent vasodilation in aortic rings, but no activity towards these four mechanisms was detected. In conclusion, our results indicate that the aorta is a target site of the antihypertensive peptide Val-Tyr. Five important antihypertensive mechanisms were tested, but only ACE inhibition could be detected.

#### A-2014

Effect of Green Tea and Garlic on Cancer Formation in Mouse Model. S. W. Li<sup>1</sup> and X. J. FAN<sup>2</sup>. <sup>1</sup>Baylor University (Student), Waco, TX 76798, and <sup>2</sup>Department of Hybridoma Lab, Diagnostic Systems Laboratories/Beckman Coulter Inc., 445 Medical Center Blvd., Webster, TX 77598. Email: jfan@beckman.com

I have demonstrated that green tea and garlic extracts were extremely effective in killing gastric cancer cell line and as well as other cancer cell line in vitro. My data strongly supports the idea that green tea and garlic extracts works as an anti-carcinogen and aid in prevention and treatment of gastric cancer also other cancers. However, based on the principle of the drug development, primary work is to perform the cellular study in vitro, then scientists often study cancer in animal models designed to stimulate human cancer. Experimental medications are usually tested in these models to see whether they are safe and effective before they are tested in people. Green tea contains many polyphenolic compounds (catechins), including the most important and dominant catechin, (–) epigallocatechin gallate (EGCG). Garlic contains organosulphur compounds, which are potent antioxidants. Therapeutic actions of green tea and garlic have been attributed primarily to its catechins and organosulphuric compounds, but more specific in vivo studies are needed to better clarify its pharmacological properties. Therefore, I have studied how green tea and garlic extracts effects on gastric cancer cells (AGS) induced tumor on mice. In this project, I have designed three different groups of mice (group 1: feed with green tea extracts; group 2: feed with garlic extracts; group 3: feed with water as a control group) since the mice started to drink water. Gastric cancer cells (AGS) were injected on these three groups of mice (8 weeks old female Balb/c

mice) subcutaneously (s.c.) into the right flank. After two weeks of injection the mice were monitored for tumor appearance and growth. The radius of tumor was measured and tumor size was calculated on each mouse. Mouse serum was taken from the mouse tail before injection of AGS and after 4th and 6th week injection of AGS. Carcinoembryonic antigen (CEA), a tumor associated oncofetal antigen was measured by ELISA. CEA levels are important prognostic indicators and provide valuable information with regard to recurrence after surgical removal of the tumor and effectiveness of therapy. Also, alpha-fetoprotein (AFP) level was determined by ELISA. To demonstrate the mechanism of green tea and garlic extracts works as an anti-carcinogen, my peripheral blood mononuclear cells (PBMC) were cultured with green tea and garlic extracts, and was stimulated the cytokine (TNF-alpha) production. The results illustrated that gastric cancer cells (AGS) were induced rapid formation and growth of tumor when the mice feed with water only. In contrast,

AGS was developed slow-growing tumor and after longer latent period when the mice feed with green tea and garlic extracts. The one mouse from garlic extracts group remained tumor free for the 7 weeks duration of the experiment. The tumor size was significant smaller when the mice feed with green tea and garlic extracts as compared with mice feed with water only. The results demonstrated that there were significant lower in the CEA and AFP level from these mice after being feed with green tea and garlic extracts as compare to the mice feed with water only. From Western blot, there were increased TNF-alpha expression after being induced by green tea and garlic extracts as dose dependent from PBMC. In conclusion: the green tea and garlic extracts were incredibly effective in cause apoptosis on gastric cancer cells or in prevention of tumor grow through amplified expression of cytokine (TNF-alpha) in mouse model. My results powerfully support the role of therapeutic green tea and garlic extracts in the treatment of gastric cancer.