
University of California, San Diego
Superfund Basic Research Program
Annual Project and Core Updates
December 15, 2003

1. Highlights for 2003

The UCSD Superfund Basic Research Program is a multidisciplinary program that focuses on defining or characterizing important cellular events associated with the etiology of a toxicant response resulting from toxicant exposure. The approaches that our program has undertaken to study toxicant exposure has resulted in the development of tools and biological reagents that will aid in bioremediation and detection of hazardous contaminants in the environment. Our work focuses on understanding the role of crucial signaling pathways and targeted genetic changes that may be tied to the initiation of a toxic episode, as well as taking advantage of these discoveries to develop models that can be used for the biological detection of toxicants. I have outlined two notable advances that have evolved over the past year, one that has originated from one of our biomedical research programs and the other from one of our non-biomedical programs.

Dr. Palmer Taylor-Biomedical

Project 7 Highlights:

The use of organophosphate based pesticides in California is of serious concern because of its extensive use and the potential of human exposure. Dr. Taylor's laboratory has been developing detection systems to quantitate exposure to organophosphates.

Dr. Taylor's laboratory has been developing technology for a fluorescence sensor for detection of organophosphate interactions with acetylcholinesterase. The work focused on defining fluctuations in the molecule around the omega loop in the mammalian enzyme. This study employs an anilino sulfonate label at positions 84 and 81 in the omega loop and 124 at the gorge rim. The findings reveal characteristic motions of the side chains and how they are influenced by ligand binding at the active center and the gorge rim. A second study employing fluorescein iodoacetate and fluorescein maleimide examines whether molecular motion is linked as we extend fluorophore from the active center gorge surface to the outer perimeter of the molecule. This study relates molecular motion seen by decay of fluorescence anisotropy with crystallographic thermal factors.

Analysis of organophosphate conjugation to acetylcholinesterase led to further experiments detailing the kinetics of inactivation, spontaneous hydrolysis and ageing. The fundamental aspects of the analysis uses MALDI mass spectrometry. Since acetylcholinesterase is homologous to neuroligin, a potential candidate target gene for autism, we have characterized the structure of neuroligin analytically by mass spectrometry, and complements analysis of the neuroligin-neurexin interaction by surface plasmon resonance and by biochemical and cell biological techniques. Considerable controversy surrounds whether environmental factors or improved diagnosis account for the increased incidence of autism, however a genetic

component in the disease is incontrovertible. Genetic studies make the neuroligin family an attractive disease target. A molecular target of this uniquely human disease has been a prevailing enigma in the study of the autism spectrum disorders.

These detection systems, along with the development of unique transgenic mice to analyze for acetylcholinesterase sensitivity, are being developed as a tool for detection of organophosphates in the environment. This information is in the early stages of patent development. The UCSD Superfund program feels that this work is pivotal since it will establish not only an analytical tool but also a biological tool to access organophosphate exposure. The state of California is one of the heaviest users of insecticides, and organophosphate runoff and potential exposure to workers as well as surrounding communities is of significant concern. This work is laying the foundation for the development of tools that can be implemented for future risk assessment.

1. Shi, J., Tai, K., McCammon, J.A., Taylor, P., and Johnson, D.A. Nanosecond Dynamics of the Mouse Acetylcholinesterase Cys69-Cys96 Omega Loop, *J. Biol. Chem.*, **15**, 278(33), 30905, 30911 (2003).
2. Jennings, L.L., Malecki, M., Komives, E.A., and Taylor, P. Direct Analysis of the Kinetic Profiles of Organophosphate-Acetylcholinesterase Adducts by MALDI-TOF mass spectrometry, *Biochemistry*, **40** (37), 11083-11091 (2003).
3. Hoffman, R.C., Jennings, L.L., Tsigelny, I.F., Comoletti, D., Flynn, R.E., Sudhof, T.C., and Taylor, P., Structural characterization of recombinant soluble rat neuroligin 1: secondary structure and glycosylation mapping by mass spectrometry, *Biochemistry*, **In Press**.

Dr. Juliann Schroeder

Project 8 Highlights:

The Schroeder lab discovered that the biological heavy metal chelators, named phytochelatins, are transported from roots to leaves of plants and that transgenic phytochelatin expression enhances accumulation of cadmium in plant leaves (Gong, Lee and Schroeder, 2003). Phytochelatin Synthases (PCS) mediate cellular heavy metal resistance in plants, fungi and worms. However, phytochelatins were generally considered to function as intracellular heavy metal detoxification mechanisms and long distance transport of phytochelatins has been hypothesized not to occur during heavy metal detoxification, although this would play an important role for bioremediation. In this study Schroeder and colleagues transgenically targeted the wheat *TaPCS1* cDNA to *Arabidopsis* roots with the *Adh* promoter (*Adh::TaPCS1/cad1-3*) or ectopically expressed with the *CaMV* 35S promoter (*35S::TaPCS1/cad1-3*) in the phytochelatin deficient mutant *cad1-3* (*atpcs1*). Both the root specific PCS expression and ectopic PCS overexpression complemented the cadmium, mercury and arsenic sensitivities of the *cad1-3* mutant. In roots and shoots of *cad1-3*, fluorescence HPLC analyses showed that under Cd^{2+} stress, no phytochelatins were detectable. However, in plants expressing the phytochelatin synthase enzyme only in roots, the enzymatic products, phytochelatins, were detected in roots and interestingly also in rosette leaves and stems. ICP-AES time course analyses of Cd^{2+} accumulation showed that either root-specific or ectopic expression of *TaPCS1* significantly enhanced long distance Cd^{2+} transport and cadmium accumulation in stems and rosette leaves. Transgenic expression of *TaPCS1* reduced Cd^{2+}

accumulation in roots compared to the *cad1-3* mutant. The reduced Cd²⁺ accumulation in roots and the enhanced root-to-shoot Cd²⁺ transport in transgenic plants were abrogated by the glutathione synthesis inhibitor BSO. The presented findings show that (1) Transgenic expression of TaPCS1 suppresses the heavy metal sensitivity of *cad1-3* to arsenic, mercury and cadmium. (2) Phytochelatins can be transported from roots to shoots. (3) Targeted or ectopic expression of the *TaPCS1* gene increases long distance root to shoot Cd²⁺ transport and reduces Cd²⁺ accumulation in roots in a phytochelatin-dependent manner. The newly obtained data suggest that targeted transgenic expression of phytochelatin synthase genes could contribute to engineering plants for enhanced accumulation of heavy metals in aerial parts of plants for bioremediation of contaminated soils and waters.

Several lines of research are emanating from these findings. This includes determining which vascular transport pathways can mediate long distance phytochelatin transport; xylem or phloem and what are the molecular mechanisms underlying vascular phytochelatin loading.

Clearly this work will be exploited in the future to determine if these transgenic plants can be used to bioaccumulate heavy metals. Dr. Schroeder is working closely with the UCSD Superfund Outreach Program and Dr. Keith Pezzoli to move these advances into practical applications at hazardous waste sites in San Diego. His laboratory is using microarrays and promoter luciferase reporter transgenics to construct heavy metal reporter lines which can signal exposure to specific heavy metals as well as their transfer through the plant. Patents on these new discoveries are being processed at UCSD.

1. Gong, J-M., D.A. Lee and J.I. Schroeder. Long-distance root-to-shoot transport of phytochelatins and cadmium in *Arabidopsis*. ***Proc. Natl. Acad. Sci. (USA)* 100**:10118-10123 (2003).
2. Lee, D.A., A. Chen and J.I. Schroeder. *Ars1*, an *Arabidopsis* mutant exhibiting increased tolerance to arsenate and increased phosphate uptake ***Plant J.* 35**:637-646 (2003).
3. Thomine, S., F. Lelievre, E. Debarbieux, J.I. Schroeder and H. Barbier-Brygoo. AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. ***Plant J.* 34**:685-695 (2003).

This work has been advertised at the following meetings.

- International PanAmerican Plant Transport Meeting, Cuernavaca Mexico 5/ 2003.
- Superfund Conference UC Berkeley 10/2003.
- American Chemical Society Meeting 3/2003 (PI invited talk held by graduate student: David Lee).
- American Society for Plant Biology Annual Meeting, 7/2003 (by post doc Dr. J.M. Gong).
- Future talk: 7th International Conference on Plasma Membrane Redox Systems 4/2004.

2. Progress Updates

Project Cores

Dr. Michael Karin

Project 1

Our major goal in this project is to generate mouse models that exhibit altered (increased or decreased) sensitivity to environmental toxins present at Superfund sites. We have decided to focus our research on toxicants, such as arsenite (As^{3+}), carbon tetrachloride (CCl_4) or toluene, whose mechanism of toxicity involves the generation of oxidative stress and reactive oxygen species (ROS). In the past we have shown that transcription factor NF- κ B, whose activity was suggested to be regulated by ROS, is an important inhibitor of programmed cell death (apoptosis). Based on this prior knowledge and the importance of apoptosis for general mechanisms of toxicity we decided to focus our efforts on the NF- κ B system. We found that activation of NF- κ B is mediated by a protein kinase complex called IKK, which is composed of two catalytic subunits IKK α and IKK β and a regulatory subunit IKK γ . We have previously shown that IKK β is most important for prevention of apoptosis and deletion of the gene (*Ikk β*) which codes for this subunit results in embryonic lethality due to massive TNF α -induced liver apoptosis. Hence, to generate mice with different levels of NF- κ B activity as models for altered susceptibility to Superfund toxicants we decided to target the *Ikk β* gene. To avoid the embryonic lethality associated with inactivation of *Ikk β* in all cells we generated a conditional, so called "floxed", *Ikk β^f* allele, a segment of which can be deleted upon expression of the CRE recombinase only in cells that express that enzyme. To test the functionality of this system we generated *villin-CRE* transgenic mice that express the CRE recombinase in intestinal epithelial cells (enterocytes). We found that CRE-mediated deletion of the *Ikk β^f* allele is very efficient and that mice lacking IKK β only in enterocytes are healthy, viable and exhibit defective activation of NF- κ B only in this cell type. Most importantly, mice lacking IKK β in enterocytes were found to exhibit excessive damage to the intestinal mucosa, due to increased apoptosis, after gut ischemia-reperfusion or after exposure to ionizing radiation. On the other hand, the ablation of IKK β in enterocytes results in a dramatic (80%) decrease in susceptibility to colitis associated cancer caused by administration of the alkylating agent azoxymethane and the irritant dextran sulfate. The decrease in tumor incidence appears to be caused by the increased elimination through apoptosis of cells that have experienced azoxymethane-induced DNA damage.

We have also used an *albumin-CRE* transgene to delete IKK β in liver cells (hepatocytes). The resulting mice *Ikk $\beta^{\Delta\text{hep}}$* are viable and healthy although they display a complete IKK β deficiency in all hepatocytes and exhibit a major decrease in NF- κ B activation in these cells. Unexpectedly, *Ikk $\beta^{\Delta\text{hep}}$* mice do not exhibit increased sensitivity to bacterial endotoxin (LPS), an efficient inducer of soluble TNF α production by macrophages. These mice, however, are extremely sensitive to the lectin concanavalin A (ConA), which causes massive T cell activation and appearance of NKT cells, within the liver that express TNF α on their surface. As it turns out, cell surface bound TNF α is far more cytotoxic than soluble TNF α . The increased sensitivity of IKK β -deficient hepatocytes to surface bound TNF α was found to be associated with increased activation of the proapoptotic protein kinase JNK. We found that in contrast to IKK β , reduced

JNK activity decreases the sensitivity to ConA-induced fulminant hepatitis and liver damage. Thus IKK β and JNK act as opposing regulators of liver cell survival.

Importantly, we found that decreased IKK β activity increases the production of ROS whereas decreased JNK activity decreases ROS production. The production of ROS plays a central role in the hepatotoxic response, because administration of an anti-oxidant BHA was found to protect against ConA-induced hepatitis and increase the survival of Con-treated *Ikk β ^{hep}* mice. We also found that treatment of cells with arsenite (As³⁺), which we have previously shown to function as an IKK β inhibitor, increases the production of ROS through a similar mechanism. Increased ROS production is likely to play a major role in arsenite toxicity.

We have initiated experiments to examine the susceptibility of *Ikk β ^{hep}* mice to liver carcinogenesis in response to administration of various chemicals. Unlike the intestinal-specific deletion of IKK β , the initial results reveal that the liver-specific deletion results in marked increase in susceptibility to chemical carcinogenesis. Studies during the coming year will focus on understanding the mechanistic basis for this increase, as well as on testing the susceptibility of *Ikk β ^{hep}* mice to a variety of Superfund site chemicals

Dr. Paul Russell

Project 2

It is well known that many of the most potent Superfund chemicals and environmental pollutants impart their toxicity by causing oxidative stress. Our project uses yeast genetics and post-genome technologies (e.g. whole genome microarrays and proteomic mass spectrometry) to understand how eukaryotic organisms respond to oxidative stress. Our goal is to make fundamental discoveries that will be broadly applicable to human health and bioremediation. Our studies are carried out with the fission yeast *Schizosaccharomyces pombe*. This model organism has played a leading role in discovering biological regulatory mechanisms concerned with cell cycle control, DNA damage and checkpoint responses, and mechanisms of cell division, to name only a few subjects. In this project we are extending the usefulness of fission yeast to understand how cells respond to oxidative stress. We have previously shown that oxidative stress activates a MAP kinase (MAPK) known as Spc1 or Sty1. This kinase is the homolog of human p38, a stress-activated kinase studied by our colleagues (see Dr. Karin's project). Typical of MAPK modules in all eukaryotic organisms, Spc1 is controlled by an upstream cascade of MAPK kinase and MAPK kinase kinases. Spc1 controls a heterodimeric transcription factor Atf1-Pcr1 that is the fission yeast equivalent of the human AP-1 related transcription factors. The stress-specific control of AP-1 is rather poorly understood, therefore we undertook studies to determine how Atf1-Pcr1 activity is regulated by oxidative stress. We used a marker insertion strategy to make a library of fission yeast mutants and we then tested these mutants in oxidative stress survival assays.

Dr. Roger Tsien

Project 3

GFP-based indicators of redox status: The redox equilibrium of cells is perturbed by oxidative stress, which is widely suspected to be important in the action of many Superfund pollutants. Redox Green Fluorescent Proteins (roGFPs) are new protein sensors (from the lab of our

collaborator, S. James Remington, U. Oregon) that allow real time visualization of their oxidation state, which reflects the redox status of the cell. Ratios of fluorescence from excitation at two wavelengths indicate the extent of oxidation and thus the cell's redox potential. These indicators are genetically encoded therefore they can be targeted to specific proteins or cell organelles of interest and may be expressed in a wide variety of cells and organisms. Dr. Tsien's laboratory has been evaluating and two roGFPs with physiologically or toxicologically relevant oxidants both *in vitro* and in living mammalian cells.

In HeLa cells, roGFP1 and 2 were respectively 16% and 12% oxidized under basal conditions. They are quite responsive to exogenous oxidants such as hydrogen peroxide, redox-cycling quinones, and permeant dithiols. Also, they show marked oxidation in phagocytes, a type of immune cell that produces reactive oxidants as a primary response to bacterial infection. These cells contain high quantities of an enzyme called NADPH oxidase, a complex capable of producing superoxide. Using a human cell line (HL60), stimulation with phorbol myristic acid (a superoxide inducer) caused an increase in the fluorescence ratio of the roGFPs consistent with a major shift in redox potential towards more oxidizing conditions. However, no responses were seen under other conditions that might have been expected to perturb the redox equilibrium, such as simple exposure of the cells to 100% oxygen gas. Also, growth factors did not cause any detectable changes in roGFPs, contrary to literature reports that they stimulate endogenous production of hydrogen peroxide, a compound capable of oxidizing the roGFPs directly. We have attempted to increase the indicators' sensitivity to minor redox fluctuations. Positively charged amino acids were placed next to the redox sensitive cysteine residues in three single mutants (N149K, A206K, and F223K) and two double mutants (N149K/F223K and A206K/F223K). All five proteins were found to be more sensitive to oxidation by hydrogen peroxide, but none responded to increasing oxygen pressure or growth factors. Subcellular targeting of any of the roGFPs (plasma membrane, mitochondria or nucleus) has not yet improved the reactivity to oxygen saturation /deprivation, or growth factor stimulation. Further investigation of new mutants that express selenocysteine, which is even more sensitive to oxidation, is currently ongoing.

A paper describing this work has been submitted to the Journal of Biological Chemistry. This work was presented as a poster/talk at the Gordon Conference entitled "Oxidative Stress and Disease" (2003).

Dr. Robert Tukey

Project 4

Our laboratory investigates the impact of chemicals found at hazardous waste sites on the control and regulation of the Ah or dioxin receptor (AhR), in addition to developing biological models to assess the presence of these contaminants in environmental samples. Two major ubiquitous toxicants, arsenic and polycyclic aromatic hydrocarbons (PAH), are being studied. PAHs are ubiquitous toxicants that are metabolized by microsomal oxidases and epoxide hydrolases to form ultimate carcinogens. We have demonstrated that metabolic intermediates of benzo[a]pyrene (B[a]P), mainly B[a]P-7,8-dihydrodiol (B[a]P-diol) are potent activators of the Ah receptor leading to induction of CYP1A1 and metabolism to the carcinogen B[a]P-7,8-dihydrodiol 9,10-epoxide (BPDE-2). In cells that contain a functional AhR, the addition of B[a]P-diol leads to nuclear stress, mitochondrial damage and the initiation of apoptosis. While B[a]P-diol serves as a ligand for the AhR initiating metabolism to BPDE-2, we have discovered

that the initiation of apoptosis is tightly controlled by MAP kinase p38. Activation of p38 is best known for regulation of gene transcription and apoptosis. Since the initiation of apoptosis by B[a]P-diol is dependent upon both AhR and p38, we speculate that the role of p38 must be influencing apoptosis prior to mitochondrial damage, most likely by influencing those genes that are necessary for programmed cell death.

Arsenic is a serious contaminant that is generated by anthropogenic sources, which on its own can initiate cell cycle arrest and apoptosis. In combination with other contaminants, such as PAHs or halogenated aromatic hydrocarbons like the dioxins, we have investigated the actions of arsenic on AhR targeted gene expression. When cells are treated with arsenic and TCDD, they arrest in an arsenic dependent fashion in G2/M which corresponds to reduction in TCDD dependent induction of CYP1A1. Arsenic dependent inhibition of CYP1A1 induction by TCDD is not dependent upon alterations in activation of the AhR, as determined by ligand binding, nuclear transport or DNA binding to enhancer sequences specific for binding the activated AhR. The inhibition of CYP1A1 induction by arsenic is a property of cell cycle control, since treatment of cells with vinblastine and arrest in G2/M duplicates the actions of arsenic treatment. It appears that the impact of arsenic on TCDD or PAH induction of CYP1A1 by the AhR is closely linked to cell cycle control.

In an effort to extend these studies in vivo, rodent models of human AhR response are being exploited. Transgenic mice carrying the human CYP1A1 promoter driving the luciferase reporter gene as well as the entire CYP1A1 gene have been constructed. The genes are inducible when mice are exposed to environmental chemicals such as PAHs or TCDD. Expression is found predominantly in liver, with cell specific expression occurring in hepatocytes. Other tissues such as the small intestine also elicit significant induction patterns. Two significant observations were noted. First, there was no extrahepatic expression of the human genes in lung and kidney where rodent Cyp1a1 is induced. Second, we found significant baseline expression as well as inducible expression in adult brain. The induction in adult brain is developmentally controlled, since limited induction occurs in neonatal brain. This is in contrast to full induction patterns seen in neonatal liver, indicating that the developmental patterns of induction in brain are tissue specific. These data indicate that the human CYP1A1 gene may be regulated in a fashion that is different than that observed for control and expression of rodent Cyp1a1.

Dr. Michael Kelner

Project 5

Our project focuses on the regulation of microsomal glutathione transferase (MGST) enzymes and their role in the nephrotoxicity and carcinogenicity of vicinal haloalkene environmental toxins. The MGST enzymes are believed to play a role in the nephrocarcinogenicity toxicity of vicinal haloalkenes by converting these compounds to a cysteine-containing nephrotoxic moiety. During the past year, Dr. Kelner's laboratory has demonstrated that HNF elements account for the high expression of microsomal glutathione transferase –1 (MGST1) observed in liver. This basal expression of MGST1 protein expression is also dependent upon SP1, but not SP3, which is in contrast to the regulation of other genes belonging to the MAPEG super gene family (MAPEG = Membrane Associated Proteins involved in Eicosanoid and Glutathione Metabolism). Other members of the MAPEG family, such as prostaglandin E synthase-1 (PGES-1) appear to be dependent upon SP3 protein expression in conjunction with SP1 regulation.

In addition, studies on the tissue specificity of human and murine MGST genes have been completed. The tissue-specificity of MGST1 was found to be markedly different than anticipated from past studies. While MGST1 is considered to be a liver-specific protein, our studies indicate MGST1 is highly also expressed in organs with high oxidative stress such as endocrine glands. The MGST2 gene is expressed in a variety of internal organs with high activity in the pancreas. The MGST3 gene is expressed at high amounts in the human heart and also in exocrine organs with high peroxidase activity and therefore exposed to constant oxidative stress (e.g. thyroid or salivary glands).

Previously it had been theorized that these genes were housekeeping genes and would not respond to environmental stress. However, our studies this past year demonstrated that the promoter (5'-nontranslated) regions of both the human and mouse MGST1 gene are capable of responding transcriptionally to oxidative stress induced by peroxides or paraquat. Other studies demonstrate that the mRNA of MGST1, MGST2 and MGST3 are increased in response to oxidative stress, and this response occurs within 2 hours. The response detected is actually higher than that noted for "classical antioxidant enzymes" such as superoxide dismutase-2 (SOD2), or glutathione peroxidase-4 (GPX4). Together these studies indicate that MGST's are not housekeeping genes, as previously proposed, but play an active role in determining how an organism responds to environmental stress.

We have also expressed active recombinant MGST1 protein in E coli. Studies are underway to determine if MGST1 can be used as a bioremediator, perhaps by expressing in plants, to markedly reduce the biological half-life of HCBD at Superfund sites.

Dr. Christopher Glass

Project 6

The overall goals of this project are to investigate the roles of nuclear receptors as targets of environmental pollutants that have the potential to disrupt endocrine functions. Nuclear receptors are a class of proteins that act to turn genes on or off in a manner that is regulated by small molecules. A good example of this class of proteins is the estrogen receptor, which in response the binding of estrogen controls the actions of many genes, including genes that are required for sexual development and function. The function of the estrogen receptor can be altered by a variety of environmental pollutants, including polychlorinated biphenyls (PCBs), raising the possibility that exposure to these compounds could disrupt numerous estrogen receptor-dependent process. A second important example is the peroxisome proliferator-activated receptor gamma (PPAR γ). PPAR γ is a nuclear receptor that has regulates fat cell development, glucose metabolism and inflammation. It has recently been shown that PPAR γ and the related receptor PPAR δ can be regulated by phthalates that are used in industry as plasticizers. This observation raises the possibility that high levels of exposure to phthalates, which are nearly ubiquitous in the environment, could alter PPAR γ function, resulting in effects on inflammation and metabolism.

Our first specific aim is to develop methods that provide information on the activities of these receptors in cells and in whole animals. We are using techniques of molecular biology to develop genetically engineered mice that will provide readouts of exposure to compounds that alter the function of these receptors. This work is in progress, but is expected to lead to new ways of measuring the biological impact of exposure to environmental pollutants.

We are also interested in evaluating the potential effects of endocrine disruptors on transcriptional responses of estrogen receptors and PPARs in macrophages. Macrophages are a form of white blood cell that play essential roles in immunity to a variety of pathogens. To this end, we have been working to establish models that will allow us to determine the functions of specific receptors in this cell type. One approach is to develop animals and primary cells that lack a specific receptor of instance. In the case of PPAR γ , knocking the gene out throughout the animal results in embryonic lethality, preventing studies of adult animals. In collaboration with Dr. Frank Gonzales' laboratory at the NIH, we developed methods to selectively knock out the PPAR γ gene in macrophages. Using PPAR γ -deficient macrophages and recently established technologies for measuring gene expression on a genome-wide level, we identified the genes that are turned off and on in macrophages by PPAR γ . Surprisingly, we found that in contrast to other cell types in which PPAR γ is an important activator of gene expression, such as adipocytes. Very few genes were turned on by rosiglitazone in macrophages. Most of these genes play roles in regulating lipid metabolism. Intriguingly, the activity of PPAR γ in these cells was inhibit the the expression of genes that are activated by inflammatory stimuli. The targets of inhibition include critical regulators of immunity, supporting a role of PPAR γ in mediating protective effects of PPAR γ agonists in models of inflammatory disease. These studies were published in the past year in the Proceedings of the National Academy of Sciences. The development of PPAR γ -deficient macrophages will enable a critical assessment of whether PPAR γ is an important target of pthalates and other environmental pollutants in this cell type.

Dr. Palmer Taylor

Project 7

The analysis of trypsinized acetylcholinesterase (AChE) by MALDI-TOF MS has demonstrated that distinct chemical modifications resulting from enzyme inhibition mediated by organophosphate (OP) compounds can be detected with exquisite sensitivity and their relative abundances determined. In experiments conducted with recombinant mAChE inhibited by paraoxon, the relative abundances of AChE peptides containing a modified active center serine strongly correlate with the fractional inhibition of the enzyme, achieving a wide detection range of phosphorylated to non-phosphorylated enzyme. Fractionation of trypsinized AChE by reverse phase chromatography gives an enrichment of the peptide containing the active center serine that enhances the detection sensitivity to sub-picomole levels. A comprehensive analysis has elucidated the specific modifications of AChE inhibited by OPs that form diisopropyl, diethyl, and dimethyl phosphoryl enzyme adducts. The relative abundance of phosphoryl AChE conjugates that occur with time following inhibition by OPs has been observed and gives a measure of the propensity of the inhibited enzyme to spontaneously reactivate or undergo the aging process. Mass spectrometric analysis of AChE allows for the direct observation of the abundances of reactivated enzyme and aged conjugate following inhibition by OPs. This approach to determine the kinetic profiles of OP-modified AChE offers advantages compared to classical methods, which have relied on indirect measures of catalytic activity following nucleophilic reactivation of the inhibited enzyme. The ability to quantitate the complement of OP-AChE adducts and hydrolytic products has enabled us to resolve kinetic profiles for the separate processes of inactivation, aging and reactivation of AChE as an isolated enzyme and to demonstrate its potential for exposure detection in intact tissue. Moreover, the simultaneous measurement of the complement of the three species should provide an indication of the duration of exposure to the OP.

The study of OP-AChE conjugates has been extended to an *in vivo* system, where endogenous AChE isolated from mouse brain tissue has been analyzed to detect phosphoryl enzyme adducts that result from exposure of mice to OPs. The abundance of unmodified enzyme and phosphoryl AChE conjugates evident after treatment with different doses of metrifonate confirm that subtle differences in AChE modifications can be detected. The significant advance is that native and modified AChE from a single mouse brain has been isolated and characterized. Substantial variability of AChE activity levels in various tissues of animal species within defined populations has been reported. The detection sensitivity of MALDI-TOF MS analysis is sufficient to resolve discrete modifications of AChE. This has facilitated the analysis of brain enzyme isolated by a strategy that does not require pooling of tissue or purification of enzyme from multiple animals. We have developed several lines of knockout mice with the help of the transgenic mouse core unit. We have deleted alternatively spliced exons from the acetylcholinesterase (AChE) gene. While invariantly spliced exon 1,2,3, and 4 encode the core of the catalytically active enzyme, the alternatively spliced exons are responsible for anchoring the enzyme in specific locations in divergent tissue types. Our first successful knockout, that which deletes exon 5, reported to be used as an anchor only in the hematopoietic system, has given us, through deduction and comparison with the wild type animal, new and exciting information about the role of AChE in blood where its ability to break down organophosphates provides a first defense against pesticides and other environmental pollutants. The exon 5 knockout has shown us that there are several AChE anchors used in blood. The glycopospholipid attachment that exon 5 encodes anchors AChE on the surface of both red cells (multiple reports covering many species) and platelets (until now only inferred). Comparison of wild type platelets and platelets from exon 5 deleted mice shows quite nicely that there is no AChE on the platelet surface when the AChE gene has been altered. Surprisingly though, there is still AChE associated with the platelets. This AChE is released when platelets are activated, and through analysis on sucrose density gradients is identified as the "read-through" form of acetylcholinesterase, there is no splicing to either exon 5 or exon 6. One can certainly envision multiple roles for AChE that is available when platelets are activated. Platelets are known to be activated by a number of different pathways, each responsive to different proteins or ligands. It will be our intention to look at the effect that different cholinesterase agonists and antagonists have on platelets in an attempt to understand why the platelets carry acetylcholinesterase. Other acetylcholinesterase knockouts mice are well on their way. We have also deleted exon 6 (which encodes the AChE form that is widely expressed in brain and muscle; and both exons 5 and 6 that leaves only the read-through form of AChE. We feel that these deletions will help to elucidate not only the role of AChE in various tissues, but perhaps also will show how the unanchored form (read-through AChE) is regulated and functions in the body's response to environmental stress.

Dr. Juliann Schroeder

Project 8

Arsenic is one of the most toxic pollutants at contaminated Superfund sites, yet little is known about the mechanisms by which certain plants survive exposure to high arsenic levels. To gain insight into the mechanisms of arsenic tolerance in plants, Dr. David Lee in the Schroeder lab developed a genetic screen to isolate *Arabidopsis thaliana* mutants with altered tolerance to arsenic. The Schroeder lab identified and characterized a mutant, *ars1*, with increased tolerance to arsenate (Lee, Chen and Schroeder, 2003). *ars1* germinates and develops under arsenic stress conditions that completely inhibit growth of wild type plants and

shows a semi-dominant arsenic resistance phenotype. *ars1* accumulates similar levels of arsenic as wild type plants, suggesting that *ars1* plants have an increased ability to detoxify arsenate. However, *ars1* plants produce phytochelatin levels similar to wild type and the enhanced resistance of *ars1* is not abolished by the gamma-glutamylcysteine synthetase inhibitor BSO. Furthermore, *ars1* plants do not show resistance to arsenite or other toxic metals such as cadmium and chromium and the resistance is therefore arsenate specific. Interestingly, *ars1* plants do show a higher rate of phosphate uptake than wild type plants, and plants grown with an excess of phosphate show increased tolerance to arsenate. Traditional models of arsenate tolerance in plants are based on the suppression of phosphate uptake pathways, and consequently the reduced uptake of arsenate. Our data suggest that arsenate tolerance in *ars1* could be due to a new mechanism mediated by increased phosphate uptake in *ars1* (Lee, Chen and Schroeder, 2003). A new model discussing how increased phosphate uptake could contribute to arsenate tolerance was proposed.

In additional research a detailed report was published showing that the metal transporter AtNRAMP3, previously isolated in the Schroeder laboratory, is targeted to the vacuolar membrane in plants and that AtNRAMP5 plays a role in shuttling metals between the vacuoles and cytoplasm of plant cells.

Dr. Brad Tebo

Project 9

Heavy metals are found as contaminants in many surface and subsurface waters as a result mostly of industrial activity but also due to natural processes (arsenic is an example of a naturally occurring metal contaminant). Bacteria are able to catalyze the transformation of some toxic metals to less toxic and/or less mobile states. We are currently pursuing two strategies to harness the natural activity of bacteria to detoxify heavy metals. One involves the direct microbial reduction of Cr(VI) (a toxic and soluble metal) to Cr(III) (a less toxic and insoluble metal). The other involves the bacterial oxidation of Mn(II) to produce high surface area, highly reactive manganese oxides with tremendous capacity for scavenging heavy metals and degrading organic compounds.

1- Hexavalent chromium (Cr(VI)) reduction

The research in this area aims at understanding the mechanism of Cr(VI) toxicity as well as that of Cr(VI) reduction. Members of the *Shewanella* family are able to catalyze the reduction of Cr(VI) to Cr(III), however, the reduction capacity is limited and the rate of Cr(VI) reduction plummets after a certain amount of Cr(VI) has been reduced. This may be due to the intracellular accumulation of reduced Cr as we showed in a recent publication (Middleton et al, 2003). However, in the presence of complexing agents, the capacity of cells for Cr(VI) reduction is increased concomitantly with the binding and stabilization in solution of Cr(III). The complexation of Cr(III) increases both the viability of cells and the total capacity of cells for Cr(VI) reduction. We are currently investigating the mechanism by which Cr(III) complexation alleviates toxicity to cells.

A parallel effort involves the purification of the protein(s) responsible for Cr(VI) reduction. The Cr(VI) reducing activity is greatest in the cell membrane and classical protein purification techniques are being used to further purify the enzyme(s). In addition, the Cr(VI) reducing

fraction is also being tested for its ability to reduce other metals such as U(VI), Fe(III) or Mn(IV).

2- Mn(II) oxidation

Our current efforts focus on the purification of Mn(II)-oxidizing proteins from two organisms: a spore forming bacterium, *Bacillus* sp. strain PL-12, and an α -proteobacterium, strain SD-21. The Mn(II)-oxidizing activity is most likely associated with a protein complex and possibly requires multiple factors; consequently, the purification of the whole complex has proven to be an arduous task. Copper and calcium ions and a quinone cofactor, PQQ, have been found to stimulate Mn(II)-oxidizing activity in SD-21 and another Mn(II)-oxidizing. We are continuing to try to get sufficient protein via polyacrylamide gel electrophoresis for mass spectrometric analysis of in-gel trypsin digested active Mn(II)-oxidases to identify which gene products are in the active Mn(II)-oxidizing complex.

In parallel to the protein purification efforts, transposon mutagenesis is being used to produce mutants of SD-21 deficient in the ability to oxidize Mn(II). In addition, we are comparing several strains of Mn(II)-oxidizing *Bacillus* sporeformers for the organization of genes shown to be involved in Mn(II) oxidation: strain SG-1 (our model sporeformer), PL-12, and MB-7. Sequence analysis has shown many of the same genes are present, although one gene, *mnxC*, is absent in both PL-12 and MB-7. In addition, *mnxA* and *mnxB* are inverted in these strains. We are currently trying to assess whether these differences result in different Mn(II)-oxidizing activities.

Administrative Core

In overseeing the UCSD Superfund program, the Administrative Core organizes the monthly scientific activities of the program as well as assuring that the scientific work is communicated nationally. This has entailed scientific exposure at several national meetings (ie. Society of Toxicology) by both students and principle investigators, in addition to active participation of the UCSD Superfund at NIEHS sponsored meetings. Examples include presentations by Dr. Pezzoli at the 2003 Annual Superfund Meeting in New Hampshire and Dr. John Helly at the Emerging Scientific Issues for Superfund meeting that was held at Berkeley, CA. The Administrative Core oversees the Superfund Seminar series which also includes a Distinguished Seminar program. UCSD invited Dr. Stephen Hecht from the University of Minnesota to present his research on nicotine metabolism and the impact of nitrosamines on human health. We also organized an External Review of the Superfund program last October that was reviewed by Drs. Reed, Guengerich, Eric Johnson and Stephen Hecht. With the development of recent advances stemming from laboratory and core based research, we have engaged the assistance of Dr. Donna Shaw from the UCSD Technology Transfer Office to facilitate the writing and processing of new inventions. This is different from previous years where we have relied on the initiative of the individual investigators to contact the Tech Transfer Office for processing of their ideas. Donna will also be participating in our monthly PI meetings to identify potential opportunities for technology transfer of ideas to the private sector.

We also use this core to encourage student and postdoctoral travel to scholarly meetings. Alice Chen, Nan Tang and Jessica Bonzo all presented posters at the Superfund Annual Meeting held in New Hampshire. Alice also participated at the Emerging Scientific Issues for Superfund meeting that was held at Berkeley, CA, and recently presented her research at a Heavy Metals and Plants meeting in Philadelphia last September, while Nan presented her research at the Angiogenesis and Microcirculation Gordon Conference. Dr. Rizlan Bencheikh-Latmani in Dr. Tebo's laboratory presented a poster at the American Society of Microbiology, and Dr. Shujuan Chen and Jessica Bonzo in Dr. Tukey's laboratory presented papers at the annual SOT meeting that was held in Salt Lake City last March.

The City of San Diego and the Environmental Services Department has invited Dr. Tukey to participate on the Technical Advisory Committee (TAC) overseeing one of San Diego's largest landfills-The Mission Bay Landfill. This committee is chaired by Councilmember Donna Frye and will deal with environmental exposure issues associated with the landfill. The Mission Bay Landfill resides on the South side of Mission Bay, which serves as a popular aquatic resort area and has been targeted by community groups as a potential hazard towards human health.

Research Cores

Dr. Elizabeth Komives Mass Spectrometry Core

Besides the MALDI-TOF mass spectrometric detection of AChE alkylation by organophosphonates (described in the Highlights section), we have been working hard on two other areas. First is the detection of small molecules that bind heavy metals. In collaboration with the Schroeder lab, we have been analyzing phytochelatins by LC/MS. These small peptides have repeating glutathione-like sequences and we have shown that they bind a host of different heavy metals including mercury, cadmium, and lead. We are in the process of attempting to measure quantitatively the binding affinity of these different heavy metals to the phytochelatins PC2, PC3, and PC4. These have been synthesized in the Komives lab, and purified in large quantities. The synthetic peptides are useful as standards for quantifying the amounts of different phytochelatins in plant samples, as well as for the metal-binding studies. In a second project related to heavy metal binding, we have been working with the Tebo lab to understand the structure and function of various siderophores that are secreted by marine organisms. These have been analyzed by MALDI-TOF mass spectrometry, and we are currently identifying side chain variations and metal-binding properties.

A second large effort is underway in phosphorylation site analysis. So far, we have been using LC/MS/MS with product ion detection to locate phosphorylation sites in IKK in collaboration with the Karin group. This is a new area for us, and we are currently trying to increase our sensitivity. At present, we are able to map phosphorylation sites on purified proteins, but not on proteins isolated from in gel digests.

A third large effort is underway in glycosylation site analysis. This effort has resulted in one publication. In this work, the glycosylation sites of neuroligins, a family of transmembrane proteins that function in synapse formation and/or remodeling by interacting with β -neurexins (β -NXs) to form heterotropic cell adhesions. The large N-terminal extracellular domain of NLs,

required for β -NX interactions, has sequence homology to the α/β hydrolase fold superfamily of proteins. Using peptide mapping and mass spectrometric analysis, four disulfide bonds were found. Disulfide pairing of Cys 117 to Cys 153 and Cys 342 to Cys 353 are consistent with disulfide linkages that are conserved amongst the family of α/β hydrolase proteins. The disulfide bond between Cys 172 and Cys 181 occurs within a region of the protein encoded by an alternatively spliced exon. The disulfide pairing of Cys 512 and Cys 546 in NL1 yields a structural motif unique to the NLs, since these residues are highly conserved. All five potential N-glycosylation sequons in NL1 at Asn 109, Asn 303, Asn 343 and Asn 547 and Asn 662 were found to be glycosylated. Analysis of N-linked oligosaccharide content by mass matching paradigms reveals significant microheterogeneous populations of complex glycosyl moieties. In addition, O-linked glycosylation was observed in the predicted stalk region of NL1, prior to the transmembrane spanning domain. From predictions based on sequence homology of NL1 to acetylcholinesterase and the molecular features of NL1 established from mass spectrometric analysis, a novel topology model for NL three dimensional structure has been constructed. This paper has been accepted for publication. Ross C. Hoffman, Lori L. Jennings, Igor Tsigelny, Davide Comoletti, Robyn E. Flynn, (2004) "Structural Characterization of Recombinant Soluble Rat Neuroligin 1: mapping of secondary structure and glycosylation by mass spectrometry." *Biochemistry* (in press).

**Dr. Mark Ellisman
Imaging Core**

The imaging core is part of the National Center for Microscopy and Imaging Research (NCMIR-<http://ncmir.ucsd.edu/>) that provides investigators with access to imaging and analytical resources. The Imaging Core has been working actively with Dr. Roger Tsien to implement new technologies that rely upon properties associated with luminescence imaging. Several approaches are being developed for use with the Superfund program. Dr. Tsien has been pioneering the development of tools that utilize protein fusion technologies to identify expression patterns by fluorescence. By constructing fusion proteins attached to fluorescence GFP proteins or by integrating a small motif (6 to 20 residues) containing the sequence –Cys-Cys-Xaa-Xaa-Cys-Cys, imaging of fixed or live cells with targeted protein expression patterns can be obtained. Most impressive, the use of tetra-cysteine tagged proteins can be employed through FIAsh labeling and fluorescence to monitor the cellular localization of proteins. This technique can be utilized to examine cellular expression of unique proteins even down to the electron microscope level. By combining GFP based technologies with FIAsh labeling, tools are being developed to examine the impact of environmental toxicants on states of the cell that might lead to oxidative stress (Tsien and Karin lab), gene expression of specific cytochrome P450s (Tukey lab), and unique luminescence of proteins being expressed in transgenic plants (Schroeder lab). The imaging core provides expertise and training for Superfund based laboratories to use the host of sophisticated equipment in place at the NCMIR, as well as recombinant DNA tools to construct the appropriate constructs for fusion protein based experiments. Dr. Ellisman has given seminars at Superfund conferences and NIEHS sponsored meetings, and has made the NCMIR available to other Superfund programs throughout the country.

**Dr. Pamela Mellon
Mouse Genetics Core**

The Mouse Core's major function is the creation of novel genetically manipulated mouse models for the Superfund investigators. In addition, we assist with the design, characterization, and maintenance of these important mouse models. The core is heavily used by the laboratories of Drs. Karin, Tukey, Glass, and Taylor. A substantial array of transgenic and knock-out mouse models have been created in the initial years of the Superfund granting period. Highlights include the creation of conditional knock-outs for the IKK β gene, knock-outs of alternatively spliced exons of acetylcholinesterase, a conditional knock-out of PPAR γ , total knock-outs of JNK1 and 2, and transgenic reporter systems for the detection of toxic agents that interact through the Ah receptor and induction of the *CYP1A* genes.

With the Karin lab, the core has produced a conditional floxed allele of the *Ikk β* gene, which codes for the IKK β catalytic subunit of the IKK complex, was created in the core facility. Drs. Karin and Tukey have utilized this model to knock out IKK β in a cell type-specific manner allowing them to determine the role of the canonical NF- κ B signaling pathway in various responses. Thus far, analyses have been carried out in mice in which IKK β is knocked out only in macrophages, enterocytes, or liver. All of these studies have proven extremely interesting and fruitful. Some of these studies will lead to significant new directions in the future. In addition, the core participated in the p38 mitogen-activated protein kinase, JNK1 and JNK2-deficient mice with Dr. Karin. Dr. Karin demonstrated the importance of MAPK deficient animals to understanding the toxicity initiated by exposure to anthrax.

With Dr. Glass, we have been involved in the generation of mouse models for the study of the roles of PPAR γ and endocrine disruptors. This work includes the creation of reporters for PPAR γ regulation of gene expression in vivo. This work is in progress, but is expected to lead to new ways of measuring the biological impact of exposure to environmental pollutants.

With the Taylor Lab, the Core has created selective knock-outs of the alternative exons of the acetylcholinesterase (AChE) gene (exon 5, exon 6 and exon 5 plus 6), achieved both by a neomycin insert and by the CRE-LOX system. While invariantly spliced exons 1, 2, 3, and 4 encode the core of the catalytically active enzyme, the alternatively spliced exons are responsible for anchoring the enzyme in specific locations in divergent tissue types. The knock-out that deletes exon 5 has shown us that there are several AChE anchors used in blood. Comparison of wild type platelets and platelets from exon 5 deleted mice shows quite nicely that there is no AChE on the platelet surface when the AChE gene has been altered. Surprisingly though, there is still AChE associated with the platelets. This AChE is released when platelets are activated. Other acetylcholinesterase knockout mice are well on their way. We have also deleted exon 6 (which encodes the AChE form that is widely expressed in brain and muscle); and both exons 5 and 6 leaving only the read-through form of AChE. Analysis of these mice will help to elucidate not only the role of AChE in various tissues, but perhaps also will show how the unanchored form (read-through AChE) is regulated and functions in the body's response to environmental stress.

With Dr. Tukey, the core has created a number of key transgenic mice carrying genes that express the human *CYP1A1* promoter as well as the entire *CYP1A1* gene. Creation of the

CYP1A1-luciferase mice is turning out to be a valuable tool for the detection of environmental toxicants that activate the Ah receptor. In addition, it is being demonstrated that the human CYP1A1 gene is regulated in a fashion that is quite different than that the regulation of the rodent gene. This may have an important impact on future studies directed at defining the role of the AhR and the *CYP1A1* gene in controlling the initiation of a toxic or carcinogenic episode. In addition, the work has demonstrated that a clear development profile is in place that underlies the expression of the human CYP1A1 gene, possibly through altered interactions with the Ah receptor.

Outreach Core

Drs. Keith Pezzoli & Hyam Leffert

The Outreach Core is continuing to make advances on two fronts: (a) mentoring high school students in UCSD's SBRP labs, and (b) creating advanced information and visualization technologies for linking SBRP science to policy and planning in the San Diego-Tijuana city-region.

a. **Superfund Basic Science Research Program for high school students at the Preuss School and Sweetwater High School.** To date, eighteen students have participated in all academic R01 labs, including labs at the SDSC and the UCSD Biology Department Natural Reserve System. Seven students have received San Diego Science and Engineering Science Fair Awards (some more than once). Our first Education Outreach 'graduate', Josephine Aguilar, from Sweetwater High School, has been accepted into the science program of UCLA. Three students are co-authors on a paper (in revision) to *J. Environmental Toxicology and Health* ["Derivation and Study of Human Epithelial Cell Lines Resistant to Killing by Chromium Trioxide", K-H Son, M. Zhang, Eliana Rucobo, Dwight Nwagwe, Frederick Montgomery, and H. L. Leffert]. This program continues to give students hands on experience in basic science research labs, including work on: heavy metal toxicity [Cr(VI)] in bacterial (Tebo lab) and human cells (Leffert lab); environmental mapping software and websites (with Dr. Ilya Zaslavsky); role of *Nod2* in NF κ B stress response (Karin lab); early growth stage competition between native and non-native plants (with Isabelle Kay); water quality monitoring collaboration with San Diego Bay Keeper (Schroeder lab); and, studies of recombinant acetylcholinesterase (Taylor lab), Ah receptor gene expression (Tukey lab), glutathione conjugation (Kelner lab), endocrine stressors (Glass lab) and yeast stress response genes (Russell lab). See: <<http://superfund.ucsd.edu/outreach/index.html>>.

b. **The Regional Workbench Consortium (RWBC).** Over the past year the Outreach Core has successfully placed our Superfund toxicant research agenda into regional policy, planning, and community decision-making processes. To accomplish this, we have worked closely with researchers, students, community activists and non-profit organizations, and environmental planners, under the aegis of our SBRP's Regional Workbench Consortium (RWBC). The RWBC is a web-based network of academic and community partners working together to link environmental health science and technology to regional policy and planning. We "rolled out" the RWBC during a major conference we organized on May 29, 2003. Nearly 200 participants took part from university, industry, government and community-based organizations <<http://www.regionalworkbench.org/expo/expo2003.php>>. This event (RWBC EXPO 2003) helped us consolidate a number of key partnerships. With our on-line interactive mapping and

visualization tools, we are assisting partners who need access to Superfund-related toxicant and other environmental data on a regional scale. These partners include the San Diego Regional Water Quality Control Board, Conservation Resource Network, Baykeeper, San Diego River Park Foundation, Tijuana River National Estuarine Research Reserve, San Diego Association of Governments, and the City of Carlsbad, among others. An exhibit we created (by invitation) to portray this work won the Grand Showcase Award at the ESRI International Users Conference, July 7-11, 2003. ESRI is the world's largest developer of Geographic Information System/ GIS software; over 15,000 people attended the conference. By invitation, we also presented our SBRP-Regional Workbench and related projects at the NIEHS's SBRP annual conference (November 9-12, 2003).

Training Core

Dr. Laurence Brunton

The UCSD Superfund Basic Research Program is committed to training at both the graduate and postdoctoral level. In 2003, we supported eight graduate students through stipends. As with the other programs and research interests in the SBRP, these students come from across the campus and are represented in several different graduate programs. **Biomedical Sciences Graduate Program;** Jessica Bonzo, Daniel Machemer and Melissa Passino. **Biochemistry Graduate Program;** Colette Dooley. **Scripps Institute of Oceanography Graduate Program;** Greg Dick. **Biology Graduate Program;** Alice Chen, Nan Tang and David Waner. Coming from diverse research backgrounds, the students integrate their interests by attending the monthly Program Meetings (<http://superfund.ucsd.edu/index.html>) in addition to meeting once every two weeks as a group to discuss research interests. The students also were introduced to the technologies based upon GIS mapping along with training by Dr. Keith Pezzoli designed to give hands on training with the Regional Work Bench (<http://superfund.sdsc.edu/gis/course/syllabus.htm>).

We will also be establishing a Superfund Journal Club that will be sponsored at the Scripps Institute of Oceanography, and open to our graduate students, postdoctoral fellows and other interested individuals with an interest in learning about Environmental-Molecular Toxicology. The SBRP Journal club will be part of our academic curriculum and offered for credit for interested graduate students.

3. Patent updates

New information related to patents for UCSD includes newly submitted and pending patents as follows:

Project 1 – Karin

We have disclosed the proprietary mouse strains we have generated to the Tech Transfer Office at UCSD. The disclosures include: *Ikk β* conditional deletion (the *Ikk β* -floxed mouse), mice lacking IKK β in intestinal epithelial cells and mice lacking IKK β in liver cells.

Project 4 – Tukey

The development of new transgenic mice response to Ah receptor ligands have been disclosed to the Tech Transfer Office at UCSD.

Project 7 - Taylor

The University of California, San Diego has taken a patent position on the "Fluorescence Ligand Binding Assay of the Acetylcholine Binding Protein and Analogs of Ligand-Gated Ion Channels," SD2003-085-1.

Project 8 - Schroeder

A submitted patent on the use of phytochelatin synthases was issued by the US patent office: Phytochelatin Synthases and Uses Therefore. P.A. Rea, O.K. Vatamaniuk, S. Mari, Y-P. Lu, J.I. Schroeder, E.J. Kim, S. Clemens, U.S. Patent Number 6,489,537 B1 (patent issued 12/2002).

4. Superfund sites table updates

Our program does not have any SBRP sites – No information or updates to report.

5. Contact information updates

Information to correct was e-mailed to Kathy Ahlmark on December 15, 2003 as a Redline/Strikeout version and as a Final version along with this Update submittal.