e.hormone 2010

environmental signaling: exploring multiple dimensions

new orleans october 19-23 2010

Speaker's Abstracts
(alphabetical)

Rodney R. Dietert, Department of Microbiology and Immunology, Cornell University

Fetal-Neonatal Origins of Lifelong Patterns of Disease: Immune-Related Examples

Early-life environment is known to alter the trajectory of physiological maturation by disrupting key maturational events and/or creating specific epigenetic patterns than can affect one or more generations. These early events create a template that establishes the risk for numerous later-life diseases. But while these diseases are most often studied and are medically managed individually, that is not how they are organized across a life course. Instead, each disease or condition represents part of a broader pattern of largely chronic conditions whose risk is interconnected among the susceptible pediatric population. These patterns of environmentally-influenced diseases are: 1) underpinned by dysfunction, 2) usually emerge during childhood and 3) continue to unfold with each passing decade. Because these patterns of disease are more the rule than the exception, they provide a useful calibration unit for life course-based environmental health protection. This presentation will use immune- and inflammation-related examples of patterns of disease along with known environmental risk factors to illustrate one approach toward a more comprehensive and holistic management of environmentally-influenced health risks.

Allyse Ferrara, Gary LaFleur, and Quenton Fontenot, Department of Biological Sciences, Nicholls State University

Environmental Signaling in Coastal Fishes

The ecologically diverse and productive habitats of southeast Louisiana, which were built by historical deltaic processes of the Mississippi River, received the majority of oil washed ashore from the Deepwater Horizon spill. A continuum of habitats form the estuaries this region: upland and ridge, cypress-tupelo swamps, fresh, intermediate, brackish, and saline marshes, beaches and barrier islands. Nationally, the estuaries of southeast Louisiana, particularly the Barataria and Terrebonne Estuaries, experience the fastest rates of erosion, subsidence, and land loss due to natural and anthropogenic causes. Alteration of hydrology for navigation, flood control, and extraction of gas and oil has suppressed or eliminated environmental signals important to the flora and fauna of the estuaries, the most important of which was the seasonal floodpulse. Historically, distributaries of the Mississippi River carried a spring floodpulse of freshwater, nutrients and sediments into the estuaries. Many organisms of these floodplains have adapted to spring time high water levels, which facilitate access to spawning and feeding grounds. To prevent catastrophic flooding, connections between distributaries and the Mississippi River were blocked by levees leading to the unintentional consequence of preventing the floodpulse from entering the estuaries. Since then, water levels in the upper Barataria Estuary only fluctuate with precipitation, wind direction and tides. The seasonal floodpulse was an important environmental signal and spawning cue particularly in the upper estuaries. Fishes, such as bowfin *Amia calva*, that use

floodplain habitats for spawning may fail to reproduce in dry years. Similarly, in the hydrologically altered upper estuaries, female spotted gar *Lepisosteus oculatus* may spawn incompletely. Despite altered hydrology, estuarine habitats remain physically connected, which facilitates movements of organisms, nutrients, and energy throughout the estuary. Despite salinity differences, bay anchovies *Anchoa mitchilli*, gizzard shad *Dorosoma cepidianum*, yellow bass *Morone mississippiensis*, redfish *Sciaenops ocellatus*, blue crabs *Callinectes sapidus*, brown shrimp *Farfantepenaeus aztecus* and many other species move among estuarine habitats. Small-scale diversion projects (<2,832 m^3s or 100,000 cfs) have reconnected lower portions of the Barataria Estuary with the Mississippi River to re-introduce freshwater and potentially simulate parts of the seasonal floodpulse. Unfortunately, in attempt to prevent Deepwater Horizon oil from reaching interior marshes, diversion projects were operated at full capacity beyond the historic seasonal timing of the floodpulse, resulting in mass mortality of oysters *Crassostrea virginica*. Additional small-scale diversion projects are needed to at least partially restore, the floodpulse throughout the continuum of estuarine habitats. Future management of the Barataria Estuary, including hydrologic modifications, should restore annual variation in timing, duration and magnitude of the historic floodpulse and thereby preserve the continuum of estuarine habitats.

Tyrone Hayes, University of California, Berkeley

ATRAZINE: Under review again

Atrazine is an endocrine disruptor that causes testicular lesions and impairs development and function of the testes in every vertebrate class examined. Atrazine also impairs mammary development in rodents, increases mammary tumor incidence in adult rodents, and induces prostate cancer and prostate disease in rodents and has been associated with these same conditions in humans. Although several mechanisms have been identified, the up-regulation of aromatase and increased estrogen production has been observed in every vertebrate examined and this mechanism is consistent with many of atrazine's effects. The EPA has now completed five special reviews of atrazine under FIFRA, yet none of these reviews have addressed atrazine as an endocrine disruptor and considered data from across vertebrates together. The current consideration of atrazine's reproductive effects on rodents by the EPA has been challenged by the manufacturer (Syngenta) claiming that data are not reproducible or flawed. In another case, an observed decrease in luteinizing hormone in atrazine-exposed rodents, Sygenta's scientists declare that this effect is not "adverse". Although a great deal of attention and media focuses on chemical spills, long-term exposure to chemicals like atrazine may have even more potential to do harm. Perhaps the EPA's reviews should be broadened to address all of the data available rather than asking narrow questions.

Caren C. Helbing, Department of Biochemistry and Microbiology,
University of Victoria, Victoria, British Columbia

Endocrine disruption of amphibian metamorphosis

Thyroid hormones are important regulators of cellular metabolism and control growth and development in humans and other vertebrates. It is estimated that about one in three people have some type of thyroid disease and over half do not know it! Alterations in thyroid hormone action, particularly at important developmental stages such as fetal development, can have serious health implications. An area of increasing concern is the effects that environmental contaminants and personal care products may have on thyroid hormone function. With over 80,000 man-made chemicals in production and complex mixtures coming from sewage treatment facilities, a formidable task is to evaluate their potential health and ecosystem impacts. Frogs are like "wet canaries in the coalmine" acting as important sentinel species. Of the many fundamental biological processes that they share with humans, thyroid hormone signalling pathways are of particular note. The mechanisms of thyroid hormone action are highly conserved yet the tadpole requires thyroid hormone to undergo metamorphosis into a frog. This fact makes them ideal for the detection of disruption of thyroid hormone action. We have been working on establishing molecular tools and assays for amphibia and have uncovered novel endocrine disrupting effects of nanometals at low, environmentally-relevant concentrations.

Ann M. Hirsch, Department of Molecular, Cell and Developmental Biology and Molecular Biology Institute, University of California, Los Angeles

Signals and symbiosis between bacteria and legumes

Sinorhizobium meliloti is a Gram-negative soil bacterium that fixes nitrogen during its symbiotic association with its host legume, which may be either *Medicago*, *Melilotus*, or *Trigonella*. Establishment of a successful symbiosis requires the coordinated action of both partners, which is communicated by various levels of signaling. The first round of signaling starts with the secretion of plant compounds, generally flavonoids, that induce the rhizobial nodulation (*nod*) genes. The combination of host-specific and common *nod* gene expression results in the synthesis of a short-chain lipochito-oligosaccharide (LCO) with various substitutions on the reducing and non-reducing ends known as Nod factor. This signal molecule is postulated to bind to Nod factor receptors in the legume plant Binding subsequently triggers a signal transduction cascade that involves calcium spiking, root hair curling, and the elicitation of cell divisions resulting in the formation of the root nodule. Interestingly, the flavonoid luteolin, a potent *nod*-gene inducer, is not required for *S. meliloti* biofilm formation although the common *nod* genes, which when expressed, result in the synthesis of the LCO backbone, are needed. This finding suggests that some other factor triggers common *nod* gene expression, which is needed for the formation of mature three-dimensional biofilms. Although quorum sensing (QS) would seem to be involved in the synthesis of Nod factor, we and others have found that common *nod* genes do not appear to be QS-regulated. However, quorum sensing is critical for successful root infection by rhizobia because it affects motility, chemotaxis, and the production of EPSII

and EPSI, among other functions. We also found that mutations in either the SinI or SinR QS genes delays *S. meliloti* biofilm formation and alters biofilm architecture compared to wild-type. Furthermore, signal molecules from legume plants in addition to *nod*-gene inducing flavonoids also modulate the symbiotic interaction.

Taisen Iguchi, National Institutes of Natural Science, National Institute for Basic Biology, Okazaki Institute for Integrative Bioscience

Endocrine Disruption in Invertebrates: Sex determination of *Daphnia magna*

Sex-determining mechanisms are diverse among animal lineages and can be broadly divided into two major categories: genetic and environmental. In contrast to genetic sex determination (GSD), little is known about the molecular mechanisms underlying environmental sex determination (ESD). The *Doublesex* (*Dsx*) genes play an important role in controlling sexual dimorphism in a variety of insects and also in vertebrates to a lesser degree. All these organisms use GSD. We identified two *Dsx* genes from *Daphnia magna,* a freshwater branchiopod crustacean that parthenogenetically produces males in response to environmental cues. One of these genes, designated *DapmaDsx1*, is responsible for the production of male traits when expressed during environmental sex determination. The domain organization of DapmaDsx1 protein was similar to that of Dsx from insect species, which were sister groups of the branchiopod crustaceans. The molecular basis for sexually dimorphic expression of *DapmaDsx1* is different from that of insect species studied to date. Rather than sex-specificity being regulated at the level of pre-mRNA splicing in the coding region, *DapmaDsx1* exhibits sexually dimorphic differences in the steady state levels of their transcripts. During embryogenesis, expression of *DapmaDsx1* was increased only in males and its transcripts were primarily detected in male specific structures. Knock-down of *DapmaDsx1* in male embryos resulted in the production of female traits including ovarian maturation whereas ectopic expression of *DapmaDsx1* in female embryos resulted in the development of a male phenotype. This establishes *DapmaDsx1* as a key regulator of the male phenotype in *Daphnia*. Our findings reveal how ESD is implemented by selective expression of a fundamental genetic component that is evolutionarily conserved in animals using GSD. We infer that there is an ancient, previously unidentified link between genetic and environmental sex determination.
(Supported by a Grant-in-Aid from the Ministry of Education, Culture, Sport, Science and Technology (B), a Grant from Ministry of the Environment, Japan, and a grant for the Long-Range Research Initiative (LRI) from the Japan Chemical Industry Association.)

Richard Judson, EPA/ORD/NCCT

Analysis of Eight Oil Spill Dispersants Using Rapid, In Vitro Tests for Endocrine and Other Biological Activity

The Deepwater Horizon oil spill has led to the use of >1 M gallons of oil spill dispersants, which are mixtures of surfactants and solvents. Because of this large scale use there is a critical need to understand the potential for toxicity of the currently used dispersant and potential alternatives, especially given the limited toxicity testing information that is available. In particular, some dispersants contain nonylphenol ethoxylates (NPEs), which can degrade to nonylphenol (NP), a known endocrine disruptor. Given the urgent need to generate toxicity data, we carried out a series of in vitro high-throughput assays on eight commercial dispersants. These assays focused on the estrogen and androgen receptors (ER and AR), but also included a larger battery of assays probing other biological pathways. Cytotoxicity in mammalian cells was also quantified. No activity was seen in any AR assay. Two dispersants showed a weak ER signal in one assay (EC50 of 16 ppm for Nokomis 3-F4 and 25 ppm for ZI-400). NPs and NPEs also had a weak signal in this same ER assay. Note that Corexit 9500, the currently used product, does not contain NPEs and did not show any ER activity. Cytotoxicity values for six of the dispersants were statistically indistinguishable, with median LC50 values ~100 ppm. Two dispersants, JD 2000, SAF-RON GOLD, were significantly less cytotoxic than the others with LC50 values approaching or exceeding 1000 ppm.

Dr. Paul L. Klerks, Department of Biology, University of Louisiana at Lafayette

Oil and Dispersants in Freshwater Ecosystems

Oil entering marshes may affect water-column and bottom-dwelling organisms, and severity and duration of effects may be influenced by the use of dispersants. Laboratory microcosms established with freshwater marsh soil were used to test: 1) toxicity and temporal changes in toxicity of oil and oil spill chemical additives, 2) effects of the chemical additives on hydrocarbon disappearance and biodegradation, and 3) the relationship between hydrocarbon levels and measured toxicity. Oils tested were diesel fuel and South Louisiana Crude (SLC); chemical additives tested were cleaner and dispersant; bioassays used microcosms water (tested with Dapnia and juvenile fish) or microcosms soil (tested mostly with insect larvae). Diesel was more toxic than SCL; chemical additives showed some toxicity by themselves and enhanced oil toxicity; toxicity was especially severe for the benthic organisms; and toxicity declined over time. Hydrocarbon disappearance and biodegradation were unaltered by the chemical additives. The various hydrocarbon measurements were generally poor predictors for the toxicity observed for the microcosms. The implications for dispersant use at the Deepwater Horizon oil leak will also be discussed, as well as its potential for causing endocrine disruption.

Gerald A. LeBlanc, Department of Environmental & Molecular Toxicology, North Carolina State University

Environmental Signaling and Sex Determination

Environmental signals have a significant role in determining the sex of offspring in many species. Typically, the signals and mechanisms of sex determination are poorly understood. Water fleas (Daphnia, Cladocera, Crustacea) are an ideal model to evaluate the mechanisms of environmental sex determination due to a wealth of information available on the biology, genetics, and ecology of these organisms. Efforts in our lab have revealed that daphnids produce male offspring in response to two concurrent environmental signals: high population density and a rapid, precipitous drop in food quantity. In response, maternal daphnids produce a terpenoid hormone, probably methyl farnesoate, that programs oocytes in late stages of maturation to develop into males. Some environmental chemicals are potent mimics of this hormone and can produce all male populations at environmentally-relevant concentrations. In an effort to identify a putative methyl farnesoate receptor, we have identified 25 nuclear receptors in daphnids. Functional analysis of the receptors has revealed that methyl farnesoate binds the retinoid X receptor (RXR) and influences signaling mediated by RXR-containing heterodimeric receptor complexes. We also have discovered that methyl farnesoate activates gene transcription via a novel receptor designated HR97g. We hypothesize that a methyl farnesoate/receptor complex programs oocytes to development into males by orchestrating targeted methylation of the genome. By manipulating the timing of the programming event, we have shown that bilateral gynandromorphic individuals can be produced, presumably by the epigenetic programming of a single cell in a two-cell staged embryo. Further studies are underway to definitively characterize the nuclear signaling processes responsible for male sex determination.

De-Kun Li, Division of Research, Kaiser Permanente Northern California

BPA Exposure and Male Sexual Function

Animal studies have suggested that bisphenol-A (BPA) is a potential human endocrine disrupter; but evidence from human studies is needed. We conducted an occupational cohort study to examine the effect of occupational exposure to BPA on the risk of male sexual dysfunction. Current workers from BPA-exposed and control factories were

recruited. The exposed workers were exposed to very high BPA levels in their workplace. Male sexual function was ascertained through in-person interviews using a standard male sexual function inventory. BPA-exposed workers had consistently higher risk of male sexual dysfunction across all domains of male sexual function than the unexposed workers. After controlling for matching variables and potential confounders, exposed workers had a significantly increased risk of reduced sexual desire [odds ratios (OR) = 3.9, 95% confidence interval: 1.8–8.6), erectile difficulty (OR = 4.5, 95% CI 2.1–9.8), ejaculation difficulty (OR = 7.1, 95% CI 2.9–17.6), and reduced satisfaction with sex life (OR = 3.9, 95% CI 2.3–6.6). A dose–response relationship was observed with an increasing level of cumulative BPA exposure associated with a higher risk of sexual dysfunction. Furthermore, compared with the unexposed workers, BPA-exposed workers reported significantly higher frequencies of reduced sexual function within 1 year of employment in the BPA-exposed factories. Our findings provide the first evidence that exposure to BPA in the workplace could have an adverse effect on male sexual dysfunction.

Angel Nadal, Instituto de Bioingeniería, Universidad Miguel Hernandez

BPA and Glucose Homeostasis

Bisphenol-A (BPA) is one of the most widespread endocrine disrupting chemicals (EDCs) used as the base compound in the manufacture of polycarbonate plastics. BPA may be involved the aetiology of many modern diseases, including metabolic syndrome (Diamanti-Kandarakis et al 2009; Gross 2007). In humans, epidemiological evidence associate Bisphenol-A exposure in adults with higher risk of type-2 diabetes and other metabolic disorders (Lang et al, 2008, Myers and vom Saal 2008). However, causal evidence relating type-2 diabetes and BPA are scarce and therefore a matter of great interest at the present moment. In adult male mice, environmental relevant doses of BPA disrupt beta cell function and induce insulin resistance (Alonso-Magdalena et al, 2006 and 2008). Here we studied the action of environmentally relevant doses of BPA on glucose metabolism in mothers during pregnancy and the impact of BPA treatment on these mothers later in life. We also investigated the consequences of in uterus exposure of BPA treatment on glucose homeostasis and pancreatic function on their offspring.

Pregnant mice were treated with either vehicle, BPA 10 or 100 μg/kg/day on days 9-16 of gestation. To directly assess the impact of BPA treatment, glucose metabolism experiments were done on pregnant mice and their offspring.

Exposure to BPA aggravated the physiological insulin resistance presented during pregnancy further impairing glucose tolerance, hyperinsulinemia, hypertriglyceridemia, and hyperleptinemia. It also decreased Akt phosphorylation at the level of skeletal muscle and liver. BPA treatment during gestation had long-term consequences for the mother later in life. Four months after labor, mice were heavier, hyperinsulinemic, hyperleptinemic and developed insulin resistance. Male offspring at six months of age presented glucose intolerance, insulin resistance and altered blood parameters. Islets of Langerhans from

these mice presented altered Ca^{2+} signaling and insulin secretion in response to glucose with no change in beta cell mass.

We conclude that BPA must be considered as a new relevant diabetogenic factor. Our results show that exposure to BPA during gestation elicits important disruption of glucose metabolism in mothers during pregnancy, predispose mothers to obesity and type-2 diabetes later in life and provokes a pre-diabetic state in male offspring when reaching adulthood.

B.W. O'Malley_Department of Molecular and Cellular Biology, Baylor College of Medicine

NRs and Coactivators

Nuclear receptors (NRs) are major regulators of growth, inflammation, and metabolism. NR coactivators, such as the SRC-1/p160 family of molecules, amplify nuclear receptor induction of gene expression by coordinately implementing the downstream subreactions required for efficient NR-dependent transcription, such as chromatin remodeling, initiation, elongation, alternative RNA splicing, and eventually, ubiquitinylation and turnover of the receptor-coactivator transcription complex itself. Thus, coactivators represent transcriptional 'master genes' that can play mediating roles in the development of many inherited and acquired hormone-related human pathologies.

We will present evidence that SRC-family coactivators control multiple facets of growth and carbohydrate metabolism in mammals. SRC-3 controls primary breast tumor growth. SRC-1 is complementary to SRC-3 in oncogenesis; SRC-1 regulates, not primary tumor growth, but distant breast cancer metastasis. Finally, SRC-2 is a metabolic gate-keeper for glucose release from liver and its absence causes a Von Gierke's Disease phenotype in mice. Recent work in mice in our lab has substantiated that SRC-2 also regulates fat absorption from the gut and storage to result in positive energy accretion/balance.

The coactivator class of molecules is already providing important new insights to human diseases and promises to map future experimental blueprints for attacking 'polygenic diseases' and to generate novel ideas for proteomic therapeutic interventions. Studies are now underway in our laboratory to discover novel ligands that can directly bind to SRC-family coactivators and inhibit coactivator function by causing premature degradation of the molecules or inhibiting their functional interactions with NRs or other co-coactivators.

EF Orlando, University of Maryland, Animal & Avian Sciences Department

Brain-Pituitary-Gonadal Axis Response to Short-Term Exposure to EDCS in the Adult Fathead Minnow

We seek to further our understanding of how the brain-pituitary-gonadal axis in fish responds to short-term exposures of EDCs. Adult, female fathead minnows were exposed

for 48 hrs to a range of concentrations of the androgen, 17β-trenbolone, and the antiandrogen, flutamide, singly and in a mixture. We similarly exposed adult, male fathead minnows to the estrogen, 17α-ethynylestradiol and the antiestrogen, ZM189-154. From the brain, pituitary, and gonad of individual females, we measured the expression of certain regulatory and steroidogenic pathway genes including gnrh2, fshb, lhb, cyp11, cyp17, cyp19a, and cyp19b; in the brain and gonad of males, we similarly measured gnrh2, cyp19a, cyp19b, cyp11, and cyp17; and certain sex steroid receptors in both sexes via real-time PCR. In these same fish, plasma concentrations of E_2 in females and T in males were measured using RIAs and EIA for 11-KT in males. Aromatase enzyme activity was also quantified in the ovary of the females. Within 48 hrs, we observed a treatment-specific effect on the expression of regulatory and steroidogenic enzyme genes, steroid hormone concentrations, and ovarian aromatase enzyme activity. Our data suggest that short-term exposures may be a useful approach for examining the early response of the brain-pituitary-gonadal response to estrogen- and androgen-receptor mediated effects in fishes. This research was supported by EPA STAR Grant Number: RD-83184801-0.

Catherine R. Propper, Department of Biological Sciences, Northern Arizona University

Environmental Chemicals and their Effects on Amphibians at Organismal and Population Levels

Environmental contaminants impacting endocrine function affect individual organisms' capacity to reproduce and survive. Investigations of endocrine disrupting compounds provide clear effects at the individual organismal level, but can also provide information suggestive of larger scale population impacts. Our work in three amphibian species demonstrates that exposure to individual compounds affects development, reproduction, and behavior, all of which affect fecundity, a key component of population level birth rate. In red-spotted newts, exposure to the pesticide, endosulfan, blocks pheromonal communication and reduces mating success. In bullfrogs, exposure to the surfactant, octylphenol, induces shifts in timing of gonadal differentiation and changes expression of key genes involved in gonadal formation and function. This same compound causes changes in courtship behavior in Western clawed frogs. Furthermore, we demonstrate that exposure to wastewater effluent is associated with shifts in the timing of metamorphosis in another model system, South African clawed frogs, and in gonadal development in wild bullfrog populations. These organismal-level effects need to be scaled into population-level responses in order to understand the magnitude of exposure to environmentally ubiquitous anthropogenic compounds.

Stewart M Rhind, *Macaulay Land Use Research Institute*

Treated sewage sludge on pasture as a source of environmental signals

Sheep grazing pastures fertilized with either sewage sludge or inorganic fertilizer are being used as a large animal model to investigate the effects on animals of prolonged exposure to environmental (low) levels of a mixture of endocrine disrupting compounds (EDCs) (sludge-exposed; treated) or to background levels of these pollutants (inorganic fertilizer; control). It was postulated that exposure to sludge would be associated with increased tissue accumulation of persistent EDCs and would be of concern to human consumers of milk and meat products. Results indicate that tissue accumulation is generally minimal but highly variable, both with individual animal and within and between chemical classes. Accumulation in tissues of fetuses, the most vulnerable stages of development, is generally, but not always less than in maternal tissue but individual fetal and maternal burdens are poorly correlated. Despite the absence of measurable increases in rate of tissue accumulation of EDCs, exposure to sewage sludge is associated with significant perturbations of fetal hypothalamic and pituitary expression of selected neuropeptides, fetal ovarian structure, gene and protein expression, fetal testicular structure and enzyme expression, offspring behaviour and maternal mammary and bone structure. It is concluded that physiological disruption can be induced by exposure to low concentrations of multiple EDCs which result in either small/ transient increases, or no increase, in tissue concentrations and that experimental designs should take account of the potential physiological effects of mixtures of EDCs in which each individual compound is present at an apparently harmless concentration.

Eric V. Stabb,

Signal molecules in a squid-bacterium light-organ symbiosis

The mutualistic light-organ symbiosis between the bioluminescent bacterium *Vibrio fischeri* and the Hawaiian bobtail squid, *Euprymna scolopes*, is a powerful model for studying natural bacteria-animal interactions. *E. scolopes* hatchlings lack *V. fischeri*, which they must obtain from their surroundings. After infection, the squid carry *V. fischeri*, and only this bacterium, in epithelium-lined crypts of a specialized light-emitting organ. Examples of both intraspecies and interspecies signaling are evident in this symbiosis. For example, morphological changes in the host tissues are triggered by symbionts, and such effects can be mimicked with fragments of bacterial peptidoglycan and lipopolysaccharide, which are examples of microbe-associated molecular patterns (MAMP's) commonly recognized by hosts. The bacteria also use acyl-homoserine lactone pheromones to coordinate expression of bioluminescence upon colonization. This signaling requires a high cell density and is often referred to as "quorum sensing"; however, the pheromones act as more than census-taking molecules for *V. fischeri*.

Paul Straight, Texas A&M University, Biochemistry and Biophysics

Interspecies communication in bacterial development

Bacteria synthesize secondary metabolites that are key to their survival and function within microbial communities in nature. A single species of bacteria may synthesize numerous bioactive compounds. The ecological factors that require a complex and varied portfolio of metabolites are largely unknown. A primary challenge for understanding microbial metabolic exchange is to circumvent the limited relevance of secondary metabolites in isolated culture. We use bacterial co-culture as an innovative strategy to query secondary metabolite function in model interspecies interactions. In co-culture experiments, we observe that secondary metabolites act as chemical cues between species to modulate metabolism and development. The genera Bacillus and Streptomyces are representative of broadly distributed bacteria that produce an array of structurally diverse, bioactive compounds. Our experiments focus on Bacillus subtilis and species of Streptomyces to define the regulatory impacts of metabolic exchange. We report that a reciprocal exchange of antibiotic metabolites promotes balanced growth of two species, indicating a role for antibiosis in the stability of bacterial communities. Through expanded use of microbial co-culture, modes of metabolic exchange and chemical signaling that determine microbial community dynamics may be revealed.

Caz Taylor and Erin Grey, Ecology and Evolutionary Biology, Tulane University

Impacts of the Deepwater Horizon oil spill on Blue Crabs

Blue Crabs are an ecologically and economically important species in the Gulf of Mexico. Although mostly an estuarine species, Blue Crabs spend the first 30-60 days of their life cycle in a planktonic larval phase in the ocean. When the Deepwater Horizon rig exploded on April 20, 2010, the spawning season was just starting. The millions of gallons of spilled oil and the dispersant potentially affected an entire year's cohort of Blue Crabs. All summer, we have been sampling post-larval recruitment of crabs in estuaries along the Gulf coast as well as collecting plankton in the ocean at varying distances from the spill site. I will discuss the findings so far from this first field season.

Laura N Vandenberg, Tufts University, Center for Regenerative & Developmental Biology

BPA is a model endocrine disruptor: Lessons from animal studies, epidemiology and public policy

Bisphenol A (BPA) is a widely produced chemical used to manufacture polycarbonate plastics and epoxy resins. Concern about this chemical was initially raised because of its estrogenic activity in vitro and in vivo. Additionally, hundreds of animal studies have been performed to test the safety of this chemical, with a large proportion of these studies examining the effects of low doses. After all of this research and millions of dollars in grant monies spent, there may be only one thing on which scientists, regulatory agencies and industry can agree: there is a plethora of information available on BPA. Where there is a large amount of disagreement is the danger of BPA to humans and the environment.

Scientists and environmental advocates have begun to ask, is BPA a model endocrine disruptor? Can the lessons learned from BPA – both scientific and regulatory – be applied to other environmental chemicals? Here, I will give a brief history of BPA, including its use in consumer products, remarkable findings from animal studies, recent discoveries from epidemiology studies, and the failure of regulatory agencies around the world to appropriately synthesize the available data. I propose that yes, BPA is a model endocrine disruptor. As such, it has given scientists a snapshot of the damage environmental chemicals can have on developing organisms, including humans. Additionally, as members of a larger society, it has shown us how the regulatory process can go awry.

Wei Xu
Department of Oncology, University of Wisconsin, Madison

Small molecule screening of estrogenic ligands identifies ER dimer- selective compounds with putative therapeutic efficacy

Estrogen receptor (ER) dimerization is prerequisite for its activation of target gene transcription. Since the two forms of ER, ERα and ERβ, exhibit opposing functions in cell proliferation, the ability of ligands to induce ERα/β heterodimers versus their respective homodimers is expected to have profound impacts on transcriptional outcomes and cellular growth. However, there is a lack of direct methods to monitor the formation of ERα/β heterodimers *in vivo* and to distinguish the ability of estrogenic ligands to promote ER homo- versus hetero-dimerization. Here we describe the identification of estrogenic small molecule ER ligands by high throughput transcriptional activation assays and the subsequent characterization of these compounds using secondary Bioluminescence Resonance Energy Transfer (BRET) screening assays. While the primary transcriptional activation assays allowed determination of transcriptional activation specific to ERs, it did not allow determination of ER subtype specificity. Thus, we employed the BRET assay for monitoring the formation of ERα/β heterodimers and their respective homodimers in live cells. Using this two-step screening of small molecule libraries, we identified 10 lead bioactive compounds capable of inducing ERβ/β homodimers and ERα/β heterodimers while having minimal activity on pro-proliferative ERα/α homodimers, posing a model that compounds promoting ERα/β heterodimer formation might have therapeutic value. The two lead hits were subsequently pursued for their biological activity in cell-based assays using breast cancer and normal mammary cell lines. Using those compounds, we demonstrated that ERα/β heterodimers are anti-proliferative in cell line models. Thus, our three step screening process of transcriptional transactivation assays, BRET assays, and cell-based biological function assays is applicable to drug screening for dimer-selective SERMs, and this strategy can furthermore be employed to study other nuclear receptor dimers. Furthermore, we applied BRET assay as a primary screening method in a high throughput screen of crude bacterial extracts not previously screened for ER modulatory function. A natural product, actinopolymorphol A, was identified that preferentially induces ERα/β heterodimerization. Actinopolymorphol A represents the first representative a new ER modulatory scaffold.

Daniel Zalko

BPA's halogenated relatives

Bisphenol A (BPA), which is used to produce various plastics and resins, is among the largest selling chemicals worldwide (>3 million tons/year). Several halogenated analogues of BPA also exist. Tetrabromobisphenol A (TBBPA) is used as a flame retardant. Its annual production is estimated above 150 000 metric tons. TBBPA has been demonstrated to

undergo debromination in biota, producing lower molecular weight brominated BPA analogues (mono-, di- and tri-BBPA) and, ultimately, BPA itself. Tetrachlorobisphenol A (TCBPA) has also been reported to be used as a flame retardant, but its production is estimated to no more than 10 000 metric tons/year. However, BPA is a phenolic compound, and phenolic compounds can be readily chlorinated in the environment, as already reported for other endocrine disruptors, such as alkyl-phenols. BPA was demonstrated to be chlorinated into TCBPA as well as intermediary chlorinated BPAs congeners (mono-, di- and tri-CBPA), which occurrence was recently demonstrated in water samples as well as in human tissues.

The estrogenicity of BPA has been demonstrated both *in vitro* and *in vivo*, and is likely involved in the onset of many of its adverse effects. However, BPA halogenated analogues are not equally estrogenic, and also target other nuclear receptors. Moreover, and as already shown for BPA, biotransformation could also modulate the biological activity of bisphenols, through the production of active metabolites. Compared to BPA, little is known regarding the biological activity and the toxicity of halogenated BPAs and their related biotransformation compounds, though both for chlorinated and brominated analogues of BPA, human exposure has been demonstrated.