542 DEXAMETHASONE TREATMENT DECREASES HEPATIC ARACHIDONIC ACID EPOXYGENASE ACTIVITIES IN RATS AS A RESULT OF DECREASED CYP2C23 EXPRESSION.

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A sensitive and specific ELISA for 14, 15-dihydroxyeicosatrienoic acid (DHET) has been developed to facilitate investigation of the physiological and pathophysiological roles of epoxyeicosatrienoic acids (EETs) and DHETs, metabolites of arachidonic acid (AA) epoxygenase and soluble epoxide hydrolase (sEH), respectively. Male Sprague-Dawley rats were treated with corn oil or dexamethasone (DEX) (10 mg/kg for 4 days, i.p.) and hepatic microsomal AA epoxygenase activity was measured by ELISA after chemical hydration of 14, 15-EET to 14, 15-DHET. DEX treatment dramatically decreased hepatic microsomal AA epoxygenase activity. This result was confirmed by measurement of [14-C]EET formation activity of microsomal AA epoxygenase after incubation of microsomes with [14-C]AA and NADPH. The [14-C]EET was separated from other metabolites by HPLC. In rats, AA can be biotransformed to EET by the activities of several cytochromes P450, including CYP2C11, CYP2C23, CYP2J3 and CYP2J4. However, Western blot analyses revealed that, among these CYPs, only CYP2C23 levels were dramatically decreased by DEX treatment. These results suggest that decreased AA epoxygenase activities in liver obtained from rats after treatment with DEX was a result of decreased CYP2C23 expression. Contrary to decreased EET formation activities, free + esterified EET levels did not decrease after 4 days of DEX treatment. This result suggests that a long-term DEX treatment is necessary to affect the large EET pool in liver. Supported by NIEHS SBIR Phase II contract ES05459 (H.K. and J.H.C.), NIEHS grant ES07462 (X.D.) and NIEHS Center grant P30 ES06639.

543 EFFECTS OF BYSTANDER CELLS: IMPLICATIONS FOR LOW-DOSE EXTRAPOLATION OF CHEMICAL AND RADIATION-INDUCED CANCER RISK.

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Estimation of cancer risk at low doses of carcinogens has been dominated by the conceptualization of single cells as the target. Research has shown that some of the effects of ionizing radiation (including mutation) are seen not only in the cell that was hit by radiation, but in the neighboring cells (referred to as bystander effects). The fate of both hit and non-hit cells are diverse, ranging from cell death, cell cycle arrest and reproductive failure, mutation, or the development of an unstable genome. This symposium will focus on how bystander effects and other indirect effects contribute to two phenomena related to cancer, the adaptive response and genomic instability. The first presentation will provide an overview of bystander phenomena that have been observed in radiation biology and provide some insight into how they may be mediated. This will be followed by a demonstration of one mechanism by which selection pressures can give rise to an unstable genotype (via signals generated from mismatch repair). The subsequent talk will discuss the indirect mechanisms implicated in arsenic carcinogenesis and their implications for risk of cancer at low doses. The nature of dose-response curves that are plausibly generated through interactions between direct and indirect mechanisms of damage and adaptation will be discussed in the fourth presentation. The final presentation will discuss a general theory of carcinogenesis that includes genetic and epigenetic contributions to carcinogenesis in the context of the overall structure of the tissue. The symposium will provide a forum in which the necessity of addressing these phenomena in risk assessment and the difficulties in doing so can be discussed. The symposium should be of considerable interest to researchers interested in how basic biological responses observed at low doses should influence estimates of carcinogenic risk.

544 BYSTANDERS, ADAPTIVE RESPONSES AND GENOMIC INSTABILITY - POTENTIAL MODIFIERS OF LOW-DOSE CANCER RESPONSES.

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There has been a concerted effort in the field of radiation biology to better understand cellular responses that could have an impact on the estimation of cancer risk (and possibly other health risks) at low dose levels.It is unclear which of these responses are produced from chemical exposures. Bystander effects are a response in cells that are known not to have been traversed by a particle track.Low LET responses have been described.Such responses include induction of gene expression, mutagenic responses and cell transformation.Thus, the concept of effects only in "hit" cells does not hold and cells at risk per unit dose takes on a new definition. The bystander effects are the result of different cellular processes; cell-cell communication and diffusion mediated effects. Adaptive responses for a variety of cellular endpoints have been described for radiation and chemical exposures.The response is lower when a small adaptive dose is given prior to a much larger challenge dose than when the challenge dose alone is given.Thus, there is reduction of response per unit dose under adaptive conditions.However, adaptive responses are not universal and there is considerable interindividual variation. A number of studies have shown that genomic instability can occur at times quite far removed from a radiation exposure.This process also calls into question the concept of response per unit dose.Genomic instability is a hallmark of most tumor types.It remains to be established if it is induced or selected by exposure to radiation or chemicals. Adaptive responses and genomic instability can be induced in bystander cells. This further complicates the considerations of dose and response.There is the potential for all these cellular responses to modify the dose response for cancer at low exposure levels.The need is to further study the underlying mechanisms for these responses for radiation and to establish if similar effects can be operative for chemical exposures.(This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

545 THE SELECTION OF MISMATCH REPAIR DEFECTS: THINKING ABOUT EXPOSURE AND RISK ASSESSMENT.

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Defects in human homologs of the bacterial DNA Mismatch Repair (MMR) proteins MutS and MutL have been found to be causative of Hereditary Non-Polyposis Colorectal Cancer (HNPCC). However, of the 9 known human MutS homologs (MSH) and MutL homologs (MLH), the majority of HNPCC mutations are confined to 2 genes, hMSH2 and hMLH1; in spite of the fact that hMSH2 and hMLH1 function as heterodimers with other MMR MSH and MLH partners. We have found that the primary human mismatch/lesion MSH recognition complex (hMSH2-hMSH6) functions as an adenosine nucleotide molecular switch. Mismatch/lesions provoke ADP→ATP exchange by MSH proteins that results in a large conformational transition and the formation of a hydrolysis-independent sliding clamp on the DNA. Our results suggest that stochastic loading of multiple ATP-bound MSH "signaling" sliding clamps, leads to "threshold" activation of the repair machinery. These studies support a new model for MRR in which MSH molecular switches signals the timing of downstream events in a manner that is similar to cellular signal transduction by G proteins. Recent studies on the mechanism of MMR signal transduction will be presented The recognition that MSH and MLH proteins constitute signaling molecules suggested the possibility of signaling interfaces and downstream effectors that are outside of the fundamental MMR process. It has always been assumed that elevated mutation rates, associated with an MMR defect, are the root cause of HNPCC (known as the "Mutator Hypothesis"). Identification of a role for the MMR machinery in signaling damage-induced apoptosis introduced the possibility that defects in the MMR process may not the cause of HNPCC. These and other data have led us to propose that there is a selection for MMR-defects that involves cellular survival following overwhelming DNA damage. Our results are consistent with an additional role for MMR in sensing and signaling cellular DNA damage. These studies have broad implications when considering the risk assessment following toxic exposures of populations containing variable levels MMR damage recognition machinery.

546 PROPOSED MECHANISMS FOR ARSENIC CARCINOGENECITY: IMPLICATIONS FOR THE SHAPE OF THE DOSE-RESPONSE CURVE.

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Epidemiological studies have established that inorganic arsenic is a significant human carcinogen that causes tumors predominantly in the skin and bladder following oral exposure and in the lung following inhalation. Despite numerous experimental studies and proposed hypotheses, there is no consensus on arsenic's mechanism of action. It does not behave as most classical chemical carcinogens, including other metals such as cadmium or chromium. In this respect, it does not induce bacterial or mammalian cell mutations at relevant concentrations nor does it produce tumors in standard one- or two-stage animal bioassays. Recent advances have allowed development of atypical rat or mouse models for arsenic carcinogenesis. These, in conjunction with *in vitro* studies, have suggested that arsenic may be inducing carcinogenesis by one or more mechanisms including it's ability to cause global hypomethylation leading to heritable changes in gene expression, act as a 'comutagen' by inhibiting DNA repair, to induce chronic growth signaling through

persistent activation of the MAPKinase pathway and induce oxidative damage through formation of dimethylarsenic radicals. Such mechanisms suggest the likelihood that the dose-response for arsenic may be non-linear in the low dose region and evoke the possibility that such events as genomic instability and by-stander effects may be involved.

547 BIOLOGICAL IMPLICATIONS OF ADAPTIVE RESPONSES AND BYSTANDER EFFECTS FOR INDIVIDUAL AND POPULATION DOSE-RESPONSE CURVES.

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The view that the dose-response for a carcinogenic stressor must be low dose linear when the stressor adds to an existing carcinogenic process has long held sway (Crump et al., Cancer Research 36:2973, 1977). As our understanding of biological complexity has increased, however, alternative possibilities have arisen. Interactions between the stressor and adaptive responses such as cell cycle checkpoint control and induction of DNA repair may lead to nonmonotonic dose response. In addition, data from radiation biology suggest that interactions between hit and bystander cells can modulate responses to radiation. The potential effects of adaptive responses can be readily illustrated with computational models. For example, a model describing a DNA-damaging agent and having the rate of DNA repair an inducible function of the level of DNA damage can generate a spectrum of dose-response shapes for DNA adduct burden. These shapes can vary from monotonically increasing to threshold to J-shaped depending on the potency with which the additional DNA damage induces repair and the degree to which repair can be induced. Another mechanism that can give rise to nonmonotonic dose-response involves low dose DNA damage that invokes cell cycle checkpoint control and high dose cytotoxicity, leading to a J-shape for the rate of cell division. The common theme in these mechanisms is the ability of the body to react to the environmental stress in a way that modulates the shape of the dose-response curve, responses that theoretical arguments for low dose linearity did not, at the time, have data to describe. Adaptive responses also have important implications at the population level. Cell cycle checkpoint controls and induction of DNA repair capacity are genetically mediated processes. Heterogeneity at the relevant loci could define segments of the population with different capacities for adaptive response and quite different dose-response behaviors in the low dose region. Study of these factors will inform considerations of uncertainty factors applied to intrahuman variability.

548 IMPLICATIONS OF EPIGENETIC EFFECTS FOR MODELING DOSE-RESPONSE.

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Dose-response analysis for cancer risks can be improved by the use of a broad array of data and biologically-based mechanistic models. Much of the mechanistic modeling around cancer risks has focused on the classical multistage model of carcinogenesis. The activity in this model is dominated by genetic events and then independent growth of individual cells. However, much of the recent work on carcinogenesis over the past few years has demonstrated that tissue structure and paracrine signalling pathways can play an important role in the development of tumors. There are two models in the literature that have addressed this issue; a model focusing on tissue structure and another on movement of clones of cells rather than individual cells. This talk will outline a general theory for carcinogenesis which includes both epigenetic and genetic events and the overall structure of the tissue. The existing models in the literature will be assessed against this broader based model and the implications for dose-response will be discussed.

549 GENOMICS AND PROTEOMICS IN REPRODUCTIVE AND DEVELOPMENTAL TOXICITY.

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The focus of this symposium is to provide examples where proteomic and/or genomic data have been applied to better understand mechanisms of reproductive and developmental processes/toxicities. Unlike many biochemical studies previously used to characterize the effects of toxicants which often generate apical endpoints, the promise of genomic and proteomic analyses is to understand the underlying mechanisms that cause the specific cellular responses that mediate or are associated with the effects. A major challenge is determining the relationship of genetic/proteomic alterations, particularly the temporal relationship (primary or secondary effect) and generalized nature and quantity of the genes affected, to the end toxicity. Thus, the role and function of the gene products in normal as well as abnormal physiology must be addressed. The use of genomics and proteomics to determine general alterations in genes/proteins have been conducted for a few years now. The intent of this symposium is to provide a forum where the genes/proteins altered by toxicant exposure are consistent to what is known with respect to the altered biology, and likely play a role in the mechanism of toxicity.

550 CYBERTERATOLOGY: INVESTIGATING THE PHYSIOLOGICAL STATE OF THE EMBRYO *IN SILICO.*

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Functional genomics and computational biology provide new ways to unravel the complex responses of developing tissues to drugs and chemicals that increase or lower the risk for specific malformations. A major challenge in the application of microarray data to experimental teratology is relating the genome-wide changes in gene expression with cellular changes leading to pathogenesis. To address this issue, we generated a microarray dataset with RNA samples collected from tissues of early mouse embryos following exposure to different teratogens, doses, times, and intervention strategies. Computer analysis of the dataset revealed a higher order structure for genes functioning in the same biological process, sharing spatial-temporal expression domains, or mapping to chromosomal regions of conserved synteny. Many physiological changes predicted by higher order data structure could be confirmed utilizing independent experimental methodology. Some alterations were anticipated (receptor tyrosine kinase signaling pathway) and others not (coenzyme A and biotin-dependent carboxyl group transfers carried out by the mitochondrion). Therefore, a comprehensive gene expression matrix that captures the biological complexity of the embryonic transcriptome and its regulation provides a computational resource for deducing the physiological state of the embryo during development and disease. (Supported by grants AA13205 and ES09120 from the NIH and grant R 827445 from the EPA but does not reflect agency policy).

551 ZEN AND THE ART OF TERATOGENICITY SCREEN DEVELOPMENT: APPLICATIONS OF MECHANISTIC PROBLEM SOLVING TO GENERATION OF TAILORED TERATOGENICITY SCREENS.

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Using problem solving approaches with a combination of *in vivo* and *in vitro* developmental systems, a platform of basic knowledge can be generated which facilitates teratogenicity screen development. Such screens have been utilized to achieve two goals: define the teratogenic mechanism and identify compounds with reduced teratogenic liability. The problem-solving tactics are multi-tiered, where at each tier, increasing knowledge is gained regarding the basis of compound-induced teratogenicity. These steps utilize data from the original *in vivo* embryo-fetal development study and then use a combination of *in vivo* critical drug-sensitivity window identification, applicable *in vitro* systems and finally genetic screens. This knowledge building approach enables a better understanding of the biological insults which result from embryo-fetal exposure to a teratogen as well as optimize selection of the most relevant developmental model systems and genetic pathways to pursue in generating sensitive and predictive gene expression screens. A case study of a compound identified to be a potent skeletal teratogen will be presented to illustrate how the multi-tiered approach has been applied in studying potential teratogenic mechanism(s) and development of screen design for identification of non-teratogenic back-up compounds for future drug development.

552 DISRUPTION OF PROSTATE GROWTH AND DEVELOPMENT BY DIOXIN.

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2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (dioxin) inhibits prostate growth and development in the mouse. The earliest effect is inhibition of the initial step in prostate development: emergence of prostatic buds of basal epithelial cells from the fetal urogenital sinus (UGS). Using wild type and aryl hydrocarbon receptor (AHR) knockout (AHRKO) fetuses exposed to dioxin on GD 13 (5 ug/kg dam) we found that ventral prostatic bud formation was totally blocked and dorsal lateral bud numbers were reduced in wild type but not AHRKO fetuses on GD 18 demonstrating that the effect of dioxin was AHR-dependent. To investigate the gene expression profile induced by dioxin in the urogenital mesenchyme (UGM) and urogenital epithelium (UGE) of the ventral and dorsal regions of the UGS; wild type