## LONG-TERM GOAL 1
## Use of Mechanistic Information in Risk Assessment


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## Overview

Assessments of health risk from exposures to environmental agents have been performed quite differently depending upon whether the adverse health outcome is cancer or a non-cancer (e.g., neurological, reproductive, developmental) one. To a great extent this practice has been based upon a limited understanding of the modes of action of toxic substances involved in the production of the different classes of adverse health outcomes. The considerable increase in knowledge of the key events involved in the induction of different disease types is changing this knowledge base and the implication of this is discussed later.

Historically, cancer was thought to be a direct consequence of the interaction of a carcinogen with DNA that led to the production of a heritable change in a single cell that with clonal expansion and additional genetic alterations eventually resulted in the production of a tumor. On the basis of this generalized mode of action, it was thought that the dose-response relationship for tumor induction would not show a threshold but would be linear at low doses. Thus, the EPA science policy was established that cancer risk should be estimated by a linear, non-threshold dose-response method.

In contrast, a threshold dose-response curve form has been generally assumed for non-cancer effects based on considerations of compensatory homeostasis and adaptive mechanisms. An implication of a threshold is that, for a particular endpoint, a range of exposures can be tolerated up to some defined level without producing an adverse effect. The dose level at which an increase in response is observed will vary from individual to individual and from chemical to chemical. The risks of non-cancer effects are generally calculated on a daily level of exposure that would not be estimated to pose an appreciable risk of an adverse health outcome over a lifetime.

The use of different approaches for risk assessments for cancer and non-cancer effects has been questioned (NRC, 1994). A significant reason for this is that it is now

understood that carcinogens interact with a number of cellular targets in addition to DNA (e.g. receptors and cellular proteins). This interaction can alter biochemical and biological processes that can eventually result in tumor formation (e.g. enhanced cell killing and regenerative cell proliferation, mitogenesis, altered gene expression). Many of these key events may have threshold levels of response (See US EPA Draft Final Guidelines for Carcinogen Risk Assessment, 2003a). In a similar vein, it has been hypothesized that not all non-cancer responses can be characterized by a threshold response. Of particular importance to these deliberations is that the enhanced understanding of the underlying mechanisms of carcinogenesis and other health effects suggest that common precursor (key) events many underlie some proportion of cancer and non-cancer endpoints. Examples can be found in the cases of receptor-medicated responses, regenerative cell proliferation, altered cell signaling and altered programmed cell death (apoptosis) responses. Knowledge of these plausible similarities in key events leading to some cancer and non-cancer outcomes could aid in the development of a more harmonized approach for conducting cancer and non-cancer risk assessments.

What has become increasingly apparent is that a more complete understanding of the key events involved in the development of the range of cancer and non-cancer outcomes is essential for the development of more harmonized approaches for the assessment of risks. In this context, EPA's *Human Health Research Strategy* (US EPA, 2003b) describes harmonization as the development of a consistent set of principles and guidelines for drawing inferences from scientific information. The need for harmonization is not for the development of a single methodology for all situations, but rather it is the need for consistent application of the pertinent information on toxicity, dosimetry, mode of action and exposure in all risk assessments regardless of adverse health outcome and chemical pollutant. Research in this area is designed to address the development of such a set of guiding principles. One approach to the harmonization of cancer and non-cancer risk assessments is to utilize mechanistic data in a common manner thereby reducing uncertainty in all risk assessments. For that reason, ORD has proposed to focus on elucidating key events in the toxicity pathways from source to response for a range of adverse health outcomes.

The Long-Term Goal for research in this area is that risk assessors and risk managers will use ORD's methods and models to decrease uncertainty in risk assessment, which in turn reduces risk of humans exposed to environmental stressors. The key questions guiding research in this area are:

- What mode/mechanisms of action (MOA) are important for understanding the impact of environmental stressors on human health?
- What are the attributes (e.g., shape of the dose-response, species specificity) of the MOA that impact risk assessment?
- How do we measure, model and/or predict the key attributes of the MOA that could impact risk assessment?
- How do we incorporate mechanistic data and computational tools into risk assessment?

The following examples serve to highlight the contributions that have been made, the current progress in relatively new research endeavors, and the expectations from new initiatives. What can be seen is that real headway has been and is being made in the use of mechanistic data to reduce uncertainty in risk assessments particularly through the development of harmonized approaches.

## Selected Examples of the Development of Mechanistic Data and Its Use or Potential Use in Risk Assessment Harmonization

### Risk Assessment for Dioxins – Ah Receptor Mode of Action

ORD in collaboration with a wide range of other stakeholders from government, academia and industry have conducted a major research effort over a decade to better characterize the risks from exposure to dioxins. The research demonstrated that binding to the aryl hydrocarbon (Ah) receptor provided a common mode of action for the range of toxicological endpoints. Physiologically based pharmacokinetic modeling emphasized this key role of the Ah receptor in the dose dependent behavior of dioxins, and that this mode of action shows concordance across a range of species including rodents and humans. In this regard, the ORD research led to the proposition that steady-state body burdens should be used as the dose metric for cross-species extrapolation. This approach has been widely adopted. In addition, based in part on mode-of-action approaches, dose

response modeling was conducted for cancer and non-cancer responses. For cancer responses a linear extrapolation was recommended. This involved modeling the available data and then calculating a point of departure from which a linear extrapolation to the origin is generated. Linear extrapolation also appeared to be optimal for non-cancer endpoints, based on biochemical and toxicological endpoints.

This research area highlights the value of a mode of action approach for assessing exposure, dose to target tissues, and adverse outcomes at qualitative and quantitative levels.

**Adverse Reproductive Outcomes and Luteinizing Hormone (LH) Disruptions**

A concentrated and focused effort has been conducted on the role of disruption of LH hormone production as a common mode-of-action for a range of adverse reproductive outcomes. In particular, a group of chlortriazines that are used as pesticides have been used as model compounds for these studies. It was demonstrated that this class of chemicals can target the hypothalamic-pituitary-gonadal axis and thereby block the LH surge. The end result is that there is a risk to subsequent fertilization. Given that there are significant reproductive homologies between rodents and humans, this mode of action data can be used to predict adverse outcomes in humans from chlortriazine exposures. The results of this investigation were used by OPPTS in their risk assessment for atrazine (a member of the chlortriazine class of pesticides).

**Estimation of Cancer Risk at Low Concentrations of Arsenic – Multiple Modes of Action**

The Maximum Contaminant Level (MCL) for arsenic in drinking water has been established recently as 10 ppm. This level is established based on mode of action data available at the time and human tumor data from areas around the world where levels of arsenic in wells used for drinking water are very high. The research program within ORD has concentrated on research that will help establish if this MCL is appropriately protective of the public. This need will be met by predicting the form of the tumor dose-response curve at low concentrations, below that at which any increased tumor frequency resulting from arsenic exposures can be detected. The approach to be used will, in part,

establish the mode(s) of action for arsenic and predict how this will impact tumor responses at low concentrations. To date, ORD research has demonstrated that metabolism of arsenic to its methylated forms is critical for toxicity. The tissue specific levels of the various toxic metabolites have been estimated by PBPK modeling methods. In addition, it has been demonstrated that methylated metabolites of arsenic can induce genetic alterations via the production of reactive oxygen species. These three observations will be critical in the estimation of the form of the dose-response curve at low concentrations and the frequency of tumors at these low concentrations for human exposure.

**Role of Oxidative Stress in the Development of Adverse Health Outcomes.**

In the search for approaches that will provide mechanistic underpinnings to harmonization of risk assessment approaches for cancer and non-cancer endpoints, the need is to identify modes of action (or key events) that could be initiators of this range of adverse health outcomes. The production of oxidative stress in cells and tissues is being investigated for its potential to be such a key event. Oxidative stress has been implicated as being involved in the formation of a broad range of adverse outcomes, but a mechanistic role is yet to be established. The diseases include: asthma, atherosclerosis, cancer, infertility and neurodegenerative disorders. ORD research has demonstrated for a number of chemicals that oxidative stress can be induced in the target tissues for many of these diseases. Such studies have been enhanced by the development of ORD of the use of $0^{18}$-labeling for measuring the levels of oxygen radicals in exposed tissues. For example, the levels of oxidative stress can be manipulated by dietary antioxidants in rodents and humans. In addition, some chronic disease conditions like asthma could involve the lowering of antioxidant defenses. These research efforts are in an early stage of development.

Additional studies that have been initiated recently involve the search for biomarkers of oxidative stress that can be used as predictive tools for the assessment of adverse outcomes. For example, single nucleotide polymorphisms (SNPs) in 4 oxidative damage related genes are being examined by ORD scientists in women in China exposed to polyaromatic hydrocarbons. These SNPs appear to be related to an increase in lung

cancer in these women. This type of study provides the necessary link between oxidative stress and a specific disease. These types of molecular epidemiologic studies are being expanded to other exposed populations, e.g., individuals with high levels of arsenic in their drinking water. These examples serve to strengthen the value of the approaches that link mode of action to adverse health outcomes both in the framework of risk assessment and disease prediction.

**P450 – Mediated Metabolism as a Mode of Action: Toxicity of Conazole**

This research area is a relatively recent addition to ORD's program on the use of mechanistic information in risk assessment. The overall aim is to establish the mode of action whereby specific members of the conazole family of chemicals can induce liver or thyroid cancer and/or reproductive effects whereas others do not. The original hypothesis was that chemical specific metabolism differences and differences in tissue-specific metabolism could be the underlying mechanism that could explain the spectrum of effects.

Initial results have shown that metabolism of specific conazoles explains part of their tissue specificity but is not sufficient to explain the patterns of thyroid tumor development. Thus, mode-of-action studies need to expand beyond considerations only of metabolism. This is significant because metabolic considerations were proposed as the mechanistic support for the risk assessment for conazoles. Gene expression analyses together with computational approaches will be used to identify pathways that are possibly involved in conazole toxicity.

The studies serve to exemplify how mechanistic data can be used to support a specific approach in a risk assessment or to negate a particular approach. In addition, they point out how current genomic approaches can be incorporated into a key event approach for risk assessment and the harmonization of risk assessments.

**Risk Assessment Applications of Mode of Action**

It can be confidently stated that EPA has been an international leader in the development of guidance documents that address a wide variety of risk assessment issues for a range of adverse effects. For example, the EPA has developed new cancer risk

assessment guidelines that incorporate explicit consideration of all biologically relevant information, including data on the mode of action (or key events) by which a particular chemical causes cancer in laboratory animals and/or humans. The use of these types of mechanistic data has already been incorporated into completed or current risk assessments. These include, for example, risk assessments for chloroform, ETBE, vinyl acetate and formaldehyde; non-linear low dose-response curve analysis was conducted or is being considered for tumor induction for these 4 chemicals. Thus, it is abundantly clear that the mechanistic data that have been obtained in support of cancer risk assessments have been utilized. The continuing goal is to extend these types of applications to non-cancer endpoints. An ongoing effort being conducted by the International Life Sciences Institute (ILSI) is developing case studies for cancer and non-cancer endpoints that assess the relevance of key events in the development of different classes of adverse health outcomes. Data generated by ORD Scientists is being used extensively for this effort and ORD Scientists are playing a key role in the deliberations.

As a parallel effort, ORD scientists have initiated computational approaches to dose-response modeling through the development of specific software that takes into account MOA data for cancer and non-cancer outcomes. The benchmark dose software (BMDS) for example, has been utilized quite extensively for the development of cancer slope factors, reference doses (RfDs) and reference concentrations (RfCs) that are used as benchmarks or standards to protect the public from potentially harmful effects of chemical exposures. A wide range of BMDS users (~2000) are registered from industry, academia and government covering some 80 countries.

Not only do ORD scientists developed primary data for risk assessments, they have provided support to multi-laboratory, multi-agency risk assessments based on data developed entirely outside the Agency. An excellent example of this can be found in the integrated characterization of human health and ecotoxicological risk for perchlorate that was based on the proposed mode of action. This activity involved each center and laboratory within ORD, the program and regional offices, and NIEHS and NIOSH. In this case, the harmonization of cancer and non-cancer approaches as well as the development of human health and ecotoxicological risk estimates illustrates the utility of

mode of action modeling. In addition, this extensive effort was accomplished in a short period of time, emphasizing the timeliness of response within ORD.

### Incorporation of New Technologies into Risk Assessment – Computational Toxicology

The EPA has realized that there is a clear need to develop approaches for incorporating data developed using the newer technologies of genomics (including proteomics, metabolomics and bioinformatics) into the set of types of mechanistic data that can provide support to the risk assessment process. The Agency developed an interim policy to address this proposed need. In addition, two documents were developed that charted the way forward for the Agency and ORD: Potential Implications of Genomics for Regulatory and Risk (http://www.epa.gov/OSA/genomics.htm) and the Framework for a Computational Toxicology Research Program (http://www.epa.gov/computox). Current efforts are underway in the Agency to implement the recommendations in these documents through the activities of workgroups and with the establishment of the National Center for Computational Toxicology. While significant progress has been made on the use of genomics technologies in the conduct of mechanistic research as highlighted in the posters for this review, the direct application to the risk assessment process is part of ongoing research discussions.

## Summary

The preceding set of 6 examples has been selected from a much larger portfolio of studies in support of the use of mechanistic data in risk assessment. They each highlight the types of study that can be designed to address the understanding of the relationship of a particular mode of action with an adverse outcome. In addition, the value of the research to our basic understanding of cellular and tissue toxicity is clearly demonstrated. The detail behind the broad conclusions presented above can be found in the Topic Area Abstracts and the Poster Presentations for this Long Term Goal.

## References

National Research Council (NCR) *Science and Judgment in Risk Assessment*. Washington, DC: National Academy Press, 1994.

US EPA. *Draft Final Guidelines for Carcinogen Risk Assessment*. EPA/630/P-03/001A, 2003a, www.epa.gov/ncca/raf/cancer2003.htm.

US EPA. *Human Health Research Strategy*. Office of Research and Development, Washington, DC, 2003b.

**Poster LTG-01**
**Dose Response Modeling for Dioxins**

**Presenter:** Mike DeVito and Linda Birnbaum (NHEERL)
**Contributors:** Bruce Rodan (NCEA) and Bill Farland (DAA)

**Science Question:**

- What are the shapes of the dose response curves for the biochemical and toxicological effects of dioxins?

**The Research:**

2,3,7,8-Tetrachlorodibenzo-p-dioxin (dioxin) is a potent toxicant that induces a wide spectrum of effects in experimental animals including cancer, developmental and reproductive toxicities and immunotoxicity. The effects of dioxin are mediated by binding and activating the Ah receptor (AhR). The activation of the AhR by TCDD initiates a cascade of events resulting in alterations in growth factors and their receptors, hormones and their receptors, and proteins involved in numerous cellular functions such as cell cycle regulation and intermediary metabolism. Many of these biochemical changes may mediate the toxic effects of TCDD. One can consider the biochemical and toxicological effects of dioxins as a continuum, starting with biochemical changes leading to toxicological events. Hence, understanding the shape of the dose-response relationship for the biochemical effects may provide insight into the shape of the dose-response relationship for toxic responses, particularly in the low-dose region. The USEPA Benchmark Dose Software was used to analyze over 280 dose-response data sets examining biochemical and toxicological effects of dioxin in experimental animals. An important finding in this analysis is that the biochemical effects tend to have lower $ED_{01}$ values (a dose that produces 1% of the maximum response) compared to more complex effects such as immunotoxicity or tissue weight loss. This finding is consistent with the hypothesis that the biochemical responses are precursors to the toxic responses of these chemicals. Another difference between the biochemical and toxicological responses is that the biochemical responses tend to have linear dose-response relationships in the low-dose region. In contrast, the toxicological responses had a tendency to demonstrate more threshold-like dose-response relationships.

Dose-response analysis for carcinogenic effects of dioxins in experimental animals and humans was also examined. A variety of tumor types have been observed following lifetime exposures to experimental animals. Dose-response analysis indicates that the predominant shape of the dose-response curve in the experimental region for these animal cancer results is linear. This does not imply that a nonlinear model such as the quadratic or cubic would not fit these data. In fact, it is unlikely that in any one case a linear model or a quadratic model could be rejected statistically. These studies had only three experimental dose groups; hence, these shape calculations are not based on sufficient doses to guarantee a consistent estimate, and they should be viewed with caution. A similar review of the human data found that several model structures,

including linear, provide adequate descriptions of some of the human data. The experimental animal and epidemiological data are not sufficient to mandate the selection of any particular model shape.

**Impact and Outcome:**

This research reduces uncertainty in risk assessment by:

- Showing that empirical dose-response data from cancer studies—both human epidemiological and bioassays—do not provide consistent or compelling information supportive of either threshold or supralinear models and are insufficient to move from EPA's default linear extrapolation policy in the proposed carcinogen risk assessment guidelines (U.S. EPA, 1996, 1999, 2003). This policy indicates that, for cancer dose-response, the data are to be modeled within the observed range and a point of departure (POD) calculated from which a linear extrapolation to the origin is generated. Based on this analysis, EPA recommends $1 \times 10^{-3}$ pg TEQ/kg body weight/day as an estimator of upper bound cancer risk for both background intakes and incremental intakes above background. This value is approximately 6 times greater than the present slope factor.
- For non-cancer endpoints, dose-response analysis indicated that for a majority of the biochemical responses and a number of toxicological responses a linear dose response model fit the data.
- The margin of exposure between key events potentially on the pathway to cancer and non-cancer effects, and the high percentage of observed linear responses suggest that a proportional model should be used when extrapolating beyond the range of the experimental data.
- Short of extrapolating linearly over one to two orders of magnitude to estimate risk probabilistically for cancer and non-cancer effects in the face of the uncertainties described above, a simple MOE approach may be useful to decision makers when discussing risk management goals.
- Risk management decisions will have to be based on a policy choice, because this analysis does not strongly support either approach.
- The results of the dose-response assessment are presently under review at the National Academy of Science.

**Poster LTG 1-02**
**Use of Mode of Action in Developing Cross-Species Dose Metrics**

**Presenter:** Janet Dilberto (NHEERL)
**Contributors:** Mike DeVito and Linda Birnbaum (NHEERL)

**Science Question**:

- How can we extrapolate the dose that produces a toxic effect in an animal to an equivalent dose in humans for persistent bioaccumulative toxicants?

**The Research**:

Risk assessments of environmental chemicals typically rely on animal data to estimate the dose that has potential for adverse effects in humans. In order to extrapolate the animal data to humans, a number of default approaches have been developed. The default uncertainty factor for animal to human extrapolation is 10. This factor is divided into a factor of three for pharmacokinetics and three for pharmacodynamics. For persistent bioaccumulative toxicants (PBTs), the evidence suggests pharmacokinetic parameters, such as half-life, do not scale well using the default method. For example, the half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is approximately 70-300 times longer in humans compared to experimental animals. Thus, for PBTs, scaling an animal dose to a human dose requires alternative methods since the default method is inappropriate for this class of chemicals. The development of better methods to extrapolate the animal data to humans requires a measure of dose (dose metric) that incorporates the species differences in the pharmacokinetics and mode of action of the chemical. Research at ORD was designed to examine the utility of a series of dose metrics for use in species extrapolation for TCDD, a prototype PBT. The dose metrics examined included tissue concentrations, body burdens and area under the blood concentration versus time curve (AUC). These metrics were chosen based on the mode of action and pharmacokinetics of TCDD. To evaluate these dose metrics initial efforts in our laboratory used empirical dose response modeling in assessing the dose metrics for species extrapolations. These studies demonstrated that tissue concentrations were good predictors of both biochemical and toxicological responses across exposure paradigms. Our studies on the pharmacokinetics of TCDD demonstrated that TCDD body burdens are proportional to tissue concentrations in two different species. Because tissue concentrations are good predictors of response across exposure regimens for different responses in two different species, this provides support for the use of body burdens as a dose metric for cross-species extrapolations. The responses examined in our studies are reversible (biochemical) or have very short windows of sensitivity (developmental toxicities). In contrast, responses such as cancer require prolonged exposure to occur. It is uncertain whether body burden is the most appropriate dose metric for cancer. Future studies are needed to validate body burden as the most appropriate dose metric for cancer. In addition, extension of this work to other PBTs would aid in testing the use of body burden as a dose metric for all PBTs

**Impact and Outcomes**:

This research is decreasing uncertainty in risk assessment by:

- Supporting EPA's proposed use of steady-state body burdens as the dose metric for cross species extrapolations for dioxins. In risk assessments the EPA uses a default uncertainty factor of 10 for animal to human extrapolations. The use of body burdens as a dose metric has replaced this uncertainty factor, thus increasing the scientific basis of the risk assessment.
- Supporting the use of body burden in dioxin risk assessments by the WHO, the Ministry of the Environment of Japan and the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA).

**Poster LTG 1-03**
**Use of Mode of Action in the Risk Characterization of Dioxins**

**Presenter:** Linda Birnbaum (NHEERL)
**Contributors:** David Cleverly (NCEA), Mike DeVito (NHEERL), Bill Farland (DSA), Brian Gullett NRMRL), Matt Lorber (NCEA), Bruce Rodan (NCEA), John Schaum (NCEA), Linda Tuxen (NCEA), and Dwain Winters (OPPTS)

**Science Question**:

This research addresses two science questions, including the following:

- Can the common mode of action of TCDD and related chemicals be used to reduce uncertainty and better characterize the health risk from these ubiquitous contaminants?
- How can all of the components of the risk assessment paradigm be integrated to provide a comprehensive risk characterization based on this common mode of action?

**The Research:**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD; "dioxin") is the prototype for a family of structurally related chemicals which are environmentally and biologically persistent, cause a common spectrum of responses, and share a common mechanism of action via binding to the aryl hydrocarbon (Ah) receptor. The Ah receptor is a highly conserved protein found throughout the Vertebrate Kingdom. It functions, after being activated by binding to dioxins, by binding to other key regulatory proteins in many cell types and leads to changes in cell growth and differentiation. Human cells and organs respond similarly to animal cells. Although more studies have been conducted with TCDD than with other dioxins, such as some of the polychlorinated dibenzofurans and PCBs, this entire class of compounds have been shown to cause biochemical (e.g., induction of metabolizing enzymes, oxidative stress, effects on hormones, changes in cytokines and growth factors) and toxic responses (e.g., reproductive, neural, dermal, cardiovascular, immune, bone and teeth, toxicity) in both sexes of essentially all species examined at some stage of development or maturation. The developing organism, whether fish, amphibian, bird, or mammal, appears to be uniquely sensitive to the adverse effects of persistent activation of the Ah receptor. Dioxins are also carcinogenic in fish, rats, mice, hamsters, and humans.

Dioxins are largely the product of industrial activities, although small amounts can be generated by natural processes. Source apportionment has demonstrated that regulatory controls on incineration, bleaching of paper and pulp products, biocides and herbicides, and industrial chemicals over the past twenty years have led to decreases in new emissions by over 90%. This is now being reflected in reductions in the body burdens in the general population, as well as decreases in environmental levels in air, soil, sediment,

and wildlife. Approximately 50% of new emissions are due to non-point source and uncontrolled burning.

The major route of exposure to the general population is through microcontamination of the food supply. Because dioxins are so resistant to degradation, both environmentally and biologically, and they are highly lipophilic, they concentrate in fatty tissues and bioaccumulate up the food chain. Measurements of food levels and intake are consistent with the amounts found in people. Physiologically based pharmacokinetic modeling has emphasized the key role of the Ah receptor in the dose dependent behavior of dioxins and the important role of body fat in the long half-life observed in both people and animals. The recent understanding that dioxins distribute according to the same principles in rodents and humans demonstrates species concordance in both pharmacokinetics, as well as response.

A multiplicity of adverse effects have been demonstrated to occur in non-human primates, rats, and mice at body burdens within a factor of ten of 5 ng/kg, the current average in the US population. This suggests that the margin of exposure is very low for dioxins. In addition, the cancer slope factor, based on both animal and human data, suggests that the excess upperbound risk could be as high as one in a thousand.

**Impact and Outcomes**:

- This highly integrated research effort is largely completed within ORD.
- The risk characterization efforts not only involved all of the ORD labs and centers, but scientists from many other federal, state, and international agencies, academia, industry, and public interest groups.
- The Risk Characterization chapter of the multi-year Dioxin Reassessment is currently undergoing review by the National Research Council.

**Poster LTG1-04**
**Oxidative Stress and Human Health**

**Presenters:** Reeder Sams (NCEA) and Bellina Veronesi (NHEERL)
**Science Questions:**

This research addresses a number of science questions, including the following:

- What is oxidative stress and why is it important for human health?
- Why is oxidative stress of relevance to the EPA?
- How can science based on the characterization of oxidative stress be used in risk assessment?

**The Research*:***

Oxidative stress (OS) can result from an imbalance between the intracellular production of oxidants (e.g., reactive oxygen species, free radicals) or oxidants from exogenous sources and the reduction of these by endogenous scavengers. Excess oxidants damage the cell's lipids, proteins, and nucleic acids and in time, this cumulative damage can initiate or contribute to adverse health effects and disease states such as asthma, atherosclerosis, diabetes, arthritis, cancer, chronic obstructive pulmonary disease, infertility and neurodegenerative diseases. In addition to health status, certain individuals appear to be at greater risk to OS mediated disease due to their age, health status, genetic profile, gender, diet and life style conditions. OS is associated with exposure to various chemical groups and environmental agents such as air pollutants, pesticides, metals, PCBs, water disinfectant by-products, nanoparticles and UVB radiation. ORD scientists are examining the relationships between chemical exposure, OS and adverse health outcomes. For this, they have developed unique methods for measuring OS, linked chemicals that generate OS to damage in various target systems, determined pathways that are affected by OS and related OS mediated genomic changes with pathological damage**.** ORD researchers have described chemically induced OS in biological systems ranging from the molecular level to that of the whole organism to provide comprehensive data sets describing chemical toxicity. Contemporary and conventional endpoints of OS have been used in these interrelated models to generate data sets on the dose-response relationship between chemicals and OS (i.e., toxicodynamics and toxicokinetics), their mode of action, their application to species extrapolation, and their influence on susceptible populations. Although historically, most ORD research has focused on the OS properties of air pollutants (e.g., ozone, particulate matter), current research efforts are examining other chemical classes to provide ORD with more complete data sets on a chemical's toxicity.

**Impact and Outcomes:**

- EPA-ORD researchers have demonstrated that OS is a common mode of action for numerous chemicals of Agency interest.

- This information is likely to be a significant component in the assessment of the effects of chemical mixtures.
- These studies provide unique data sets that have been used by several program offices (Office of Water, Office of Air, and Office of Pesticides) and ORD in their "weight of evidence" decisions for risk assessments. Such data can support more accurate risk assessment for environmental chemicals.
- Using mechanistically based data instead of default assumptions will reduce uncertainty and strengthen the risk assessment process by providing appropriate protection of human health together with the associated economic benefits.
- Generation of these scientifically defensible risk assessments provides informed decision making capability to support EPA regulatory policies.

**Poster LTG1-05**
**Oxidative Stress as a Common Pathway Linking Exposure to Toxicity**

**Presenters:** Gary E. Hatch and Urmila Kodavanti (NHEERL)
**Contributors:** Prasada Kodavanti, Bellina Veronesi, Andrew Ghio, Michael Madden, David DeMarini (NHEERL)

**Science Questions**:

This research addresses the two following science questions:

- Is oxidative stress (OS) a common mode-of-action for environmentally induced toxicity?
- Is disease-associated OS a common underlying condition explaining increased risk from certain environmental exposures?

**The Research:**

Accumulating evidence supports the unifying concept that measurement of OS could provide a broad measure of toxicity useful for risk assessments and for identification of susceptible populations. Research conducted in ORD has demonstrated that both air and water pollutants (ozone, nitrogen oxides, chlorine, bromate) are oxidants which react directly with biological molecules. Other pollutants (airborne particulate matter [PM], many volatile organic compounds, biogenic agents, arsenic and asbestos) may act more indirectly by increasing oxidant release by inflammatory cells. While the precise measurement of OS in the body has been a challenge, ORD researchers have pioneered its measurement using oxygen-18 labeling. This approach allowed direct comparison of the degree of OS induced in rats and humans undergoing comparable environmental exposures; thereby allowing pharmacodynamic information to be incorporated into dose-response analysis. ORD investigators have also shown that 1) the level of OS can be manipulated by dietary antioxidants in rodents and humans, 2) that metabonomic measurements of antioxidant substances can be used as an index of OS susceptibility, and 3) that some chronic disease conditions, such as asthma, might involve the lowering of antioxidant defenses. Animal models with increased susceptibility to PM have been shown to have genetic deficits in antioxidant defenses. ORD studies of the role of OS in the carcinogenicity of asbestos, arsenic and bromate provide evidence that OS is important in tumor promotion. Recently, ORD scientists have confirmed that OS is involved in effects on cell signaling following exposure to complex mixtures such as PM. Gene and protein expression analysis has also been used to identify signaling events which are mediated by OS. Overall, these novel approaches have strengthened the evidence for involvement of OS in adverse health outcomes. Further research efforts that will provide high throughput gene expression and protein (i.e., signaling) data should be

useful for improving our understanding of OS mechanisms, and in providing biological plausibility for the effects of a variety of pollutants and mixtures.

**Impact and Outcomes:**

- OS has been accepted as a major mechanism by which PM induces its toxic effects.
- OS as a mechanism has been used in the risk assessment of criteria pollutants (ozone and nitrogen oxides) and hazardous air pollutants (phosgene, carbon tetrachloride, chlorine, acrolein) are regulated based on their ability to induce OS.
- Some of the most reliable extrapolations of toxicity from acute to chronic and from animals to humans involve the use of OS measurements.
- ORD studies have also significantly contributed to the National Ambient Air Quality Criteria documents for PM and ozone.
- There is increasing recognition that many different types of environmental stressors may act through the OS MOA.
- Current research supports that the OS MOA may be useful in cumulative risk assessment of air pollutants and other environmental stressors having a common MOA.
- Further validation and acceptance of methods and theories relating to the role of OS in both cancer and non-cancer effects should simplify the task of assessing risk from pollutant mixtures.

**Poster LTG1-06**
**Research to Improve the Predictive Value of Markers of Oxidative Stress**

**Presenters:** Jane Gallagher and Prasada Kodavanti (NHEERL)
**Contributors:** Bellina Veronesi, Tony Huang, Gary Hatch, Joyce Royland and David DeMarini (NHEERL); Reeder Sams (NCEA)

**Science Question:**

This research addresses two science questions, including the following:

- Can we develop specific, reliable noninvasive methods for measuring oxidative stress in humans to be used in conjunction with more classical indicators of health status?
- Can we develop a predictive *in vitro* screening model that relates oxidative stress changes in cells to responses in more complex multi-organ systems?

**The Research:**

Biomarker development has improved our ability to detect early chemical-induced changes at the molecular, cellular, and pre-clinical level that are often predictive of adverse health outcomes. Despite the evidence demonstrating the importance of oxidative stress (OS), systematic validation of biomarkers of OS and antioxidant defenses is limited. ORD researchers are involved in validating a broad array of OS markers as part of NIH's Biomarkers of Oxidative Stress Study (BOSS). Researchers incorporate a battery of OS assessments in animal and humans to document OS-mediated damage to specific proteins, lipids or DNA sites.

ORD researchers involved in epidemiological and clinical studies now typically include more OS measurements together with more established measures of exposure and effect. For example, plasma and blood levels of reactive oxygen species/antioxidants are assessed in healthy individuals, asthmatic children and adults, and other compromised individuals. Plasma and whole blood OS data are currently being analyzed from individuals exposed to arsenic through their drinking water. In another ORD epidemiological study, single nucleotide polymorphisms in four oxidative damage-related genes were associated with elevated incidence of lung cancer in polycyclic aromatic hydrocarbon-exposed women in China. These genes play a role in the generation, prevention or repair of oxidative damage.

ORD scientists are examining OS as a common mode of action for inducing toxicity and carcinogenesis by many hazardous chemicals. Emerging technologies such as genomic and high-through-put data methodologies are being used to describe the role of OS in chemical-induced toxicity. These models measure cellular and genomic endpoints of OS in chemically exposed phagocytic cells representative of key body systems. Test chemicals that elicit threshold responses and OS genomic profiles are then examined in more integrated cell culture systems, laboratory animals, and or animal models of disease to confirm adverse effects. This tiered approach links experimental systems across all

levels of biological organization from the gene to whole animal. This approach is used to test chemicals as diverse as structurally related pesticides, drinking water disinfection by-products, ambient air pollutants, aeroallergens and nanoparticles.

**Impact and Outcomes:**

- This research contributes to the development of a mechanistically-based models in support of scientifically defensible human health risk assessments.
- The identification and validation of biomarkers of OS has led to a better understanding of the consistency of effects between species and more established measures of exposure and effect.
- Coupling sensitive OS measurements with gene expression analysis in experimental systems and human biomonitoring studies will provide data that will be important for human risk assessments.
- OS appears to be a common mode of action for a number of carcinogenic and non-carcinogenic chemicals
- Treatment with antioxidants may mitigate the toxic effects of some environmental stressors.
- Research supports the potential use of rapid *in vitro* screening approaches to identify environmental stressors with an OS MOA.

**Poster LTG1-07**
**Harmonization of Tumor and Reproductive Effects of Atrazine**

**Presenter:** Tammy E. Stoker (NHEERL)
**Contributors:** Susan Laws, Michael G. Narotsky, Jerome M. Goldman and Ralph L. Cooper (NHEERL)

**Science Question**:

- This research addresses how mechanistic and mode of action data can be used in risk assessment to identify and predict adverse reproductive and non-reproductive outcomes in test species and humans.

**The Research**:

The chloro-S-triazine herbicide, atrazine, is one of the most widely used herbicides in the U.S. Earlier work demonstrated that prolonged atrazine exposure resulted in an increased incidence of mammary tumors in the rat suggesting that atrazine was a carcinogen. Studies conducted under this project demonstrated that a central nervous system (CNS) mode of action was responsible for the earlier onset of mammary tumors in the rat, and that this mode of action was not relevant to humans. Specifically, atrazine caused a premature reproductive senescence in the rat typified as constant estrus, a condition that produces the endocrine environment (enhanced estrogen and prolactin stimulation) conducive to tumor growth. As reproductive senescence in the rat is the result of altered CNS regulation of luteinizing hormone (LH) secretion, we hypothesized that atrazine was altering the secretion of pituitary hormones. Our dose-response studies supported this hypothesis by showing that atrazine suppressed both LH and prolactin by disrupting the hypothalamic mechanisms controlling pituitary hormone secretion. As the causative factors associated with reproductive aging in the rat (impaired hypothalamic function) and human (depletion of primary follicles) are dramatically different, the possibility that atrazine would cause of mammary tumors in woman by this mode of action is remote. However, because the hypothalamic regulation of LH secretion in the rat and human is similar, it is likely that the chlorotriazine herbicides could influence the secretion of these important pituitary hormones in humans. In ongoing studies, we are examining other reproductive outcomes that occur in response to the disruption of the pituitary hormone secretion.

**Impact and Outcomes***:*

- This work was instrumental in assessing the cancer risk of atrazine by demonstrating an endocrine mode of action for mammary gland tumors.
- This research was critical to OPPTS's atrazine risk assessment document and at the SAP/SAB review of atrazine. (www.epa.gov/oppsrrd1/reregistration/atrazine/hed_redchap_16apr02.PDF).
- The mode of action studies were instrumental in identifying other potentially adverse reproductive effects, especially in the developing animal, and played a role in applying the Food Quality Protection Act 10x factor (3X retained), setting the aPAD

and cPAD, as well as the setting acute LOAELs for atrazine. (www.epa.gov/oppsrrd1/reregistration/atrazine/ srrd_overview_may02.pdf).

- Currently, data from studies on related chlorotriazines and common metabolites are being incorporated into the atrazine cumulative risk assessment. (http://www.epa.gov/pesticides/cumulative/ triazines/newdocket.htm)
- Studies are in progress examining other classes of environmental chemicals which may alter LH as well as other pituitary hormones.

**Poster LTG1-08**
**Characterizing the Adverse Reproductive Outcomes Following Luteinizing Hormone Disruption**

**Presenter:** Susan C. Laws (NHEERL)
**Contributors:** Tammy E. Stoker, Michael G. Narotsky, Jerome M. Goldman and Ralph L. Cooper (NHEERL)

**Science Question**:

- This research address the range of adverse reproductive outcomes and diseases that can occur following disruption of luteinizing hormone (LH) in an animal model and how can they be applied for understanding the impact of environmental stressors on human health.

**The Research:**

In addition to a targeted alteration in the cyclic (ovulatory) rise in LH in the female, persistent shifts in the concentrations of this hormone may adversely impact reproductive function and affect other more wide-ranging physiological processes. In mammalian females, both hyper- or hyposecretion of LH can disrupt ovulation or pregnancy. Furthermore, impaired LH secretion is associated with delayed puberty, premature reproductive aging and a variety of other reproductive disorders, including the development of mammary, granulosa cell and Leydig cell tumors in rats. Research in our laboratory has shown that a single dose of the formamadine acaracide, chlordimeform or the dithiocarbamate pesticide, thiram will delay the ovulatory surge of LH for 24 h. This change in LH alters pregnancy outcome (i.e., increased numbers of resorptions and decreased litter size). The embryonic effects are associated with altered oocyte function during fertilization. A primary question in this project is whether or not other environmental stressors that cause changes in basal LH or the ovulatory surge of LH will result in similar adverse outcomes. Thus, this work characterizes the effects of these other compounds on LH secretion, the particular cellular mechanisms involved, and how such effects are related to the timing and/or duration of exposure. The timing studies focus on broader lifetime exposure concerns, such as alterations in the onset of puberty and reproductive aging, as well as specific questions concerning exposure during certain critical periods of the estrous cycle and pregnancy. Current studies are also evaluating the potential adverse effect of cumulative exposures to one or more classes of compounds.

**Impact and Outcomes**:

- These studies contribute to the scientific understanding of the physiological alterations that occur as a result of altered LH secretion, which is critical for the proper interpretation of reproductive toxicology studies for risk assessment.
- Data from the studies evaluating the reproductive risks of atrazine were used to support the Agency's Special Review of this compound (www.epa.gov/oppsrrd1/reregistration/atrazine/hedredchap16apr02. PDF)

and are currently included in the upcoming Cumulative Risk Assessment for the chlorotriazines (www.epa.gov/pesticides/cumulative/triazines/newdocket.htm).

**Poster LTG1-09**
**Identifying How Environmental Chemicals Affect LH Secretion: A Systems Biology Approach**

**Presenter:** Jerome M. Goldman (NHEERL)
**Contributors:** Susan C. Laws, Tammy E. Stoker, Michael G. Narotsky, and Ralph L. Cooper (NHEERL)

**Science Question**:

- This research provides the methods and models to determine the neuronal/molecular targets that can be used for risk assessment of environmental toxicants that alter the hypothalamic/pituitary control of gonadal function.

**The Research**:

Luteinizing hormone (LH) plays a critical role in reproduction in both the male and female. In the female rat (and human) the mid-cycle (or ovulatory) surge of LH is a functional endocrine event that stimulates the final stages of follicular and oocytic maturation that precede ovulation. Moreover, recent evidence has shown that a dysregulation of LH may have a role in the pathogenesis of a number of human diseases, ranging from gonadal hyperplasia and other reproductive tumors to Alzheimer's disease. Our laboratory has already demonstrated that a variety of environmental compounds are able to block the LH surge and that this disruption, or alterations of LH in general, will lead to many adverse reproductive outcomes including impaired pregnancy maintenance, reduced fertility, delayed puberty, premature reproductive senescence and mammary gland tumors. Importantly, the type of adverse reproductive outcome may be the result of the specific neuroendocrine mechanism targeted by the toxicant. Because multiple mechanisms are involved in the neuroendocrine control of LH secretion, and because the specific mechanism involved in a toxicant's effect may determine the specific adverse outcome, we are developing a strategy to more quickly identify the cellular/molecular targets within the brain-pituitary gonadal axis following exposure. This approach utilizes our knowledge of the physiology and timing of the mechanisms that regulate the LH surge and our ability to measure several of the key events involved. To date we have identified a number of compounds (representative of a wide range of chemical classes) that impair the LH surge (and ovulation) including the formamidine (e.g., chlordimeform and amitraz) and dithiocarbamate (i.e., thiram, dimethyldithiocarbamate, metam sodium) pesticides which alter gonadotropin-releasing hormone by disrupting catecholamine neurotransmission . Similar effects on the LH surge have been observed in response to exposure to the chlorotriazine herbicide atrazine and the organophosphate, molinate, but the mechanism(s) are yet to be determined. In this protocol, the potential target neuronal pathways are assessed using ELISAs and HPLC to examine CNS changes. The extent to which systemic factors (local changes in steroidogenesis etc) may be involved in the disruption of the LH surge is also being examined.

**Impact and Outcomes:**

- This research has shown that a variety of chemicals targeting different components of the hypothalamic-pituitary-gonadal axis are able to block the LH surge, an effect that can have adverse consequences for subsequent fertilization.
- By determining the mechanisms involved in a toxicant-induced change in the regulation of this hormone, we can better predict the extent to which the compound represents a threat to human reproductive functions and other pathologic conditions that may be a consequence of LH dysregulation.
- When used in combination with data on tumor development and reproductive function, this mechanistic information, when considered in light of the reproductive homologies between rodents and humans, can be used by risk assessors to both predict and explain the data and provide the necessary information for a sounder basis for risk assessment.

**Poster LTG1-10**
**Research Directed to Improve the Arsenic Cancer Risk Assessment: Defining the Role of Biomethylation and Reactive Oxygen Species in Arsenic Carcinogenesis**

**Presenter:** Stephen Nesnow (NHEERL)
**Contributors:** David Demarini, Susan Hester, Kirk Kitchin, Andrew Kligerman, Leon King, Jeffrey Ross, Sheau-Fung Thai, Witold Winnik, and Doug Wolf (NHEERL); Reeder Sams (NCEA); Ron Mason and Maria Kadiiska, (NIEHS); William Cullen (University of British Columbia)

**Science Question**:

Exposure to arsenic is worldwide due to both natural and man-made processes (refining, pesticides). Naturally-occurring inorganic arsenic is found in drinking water and several pesticides in commerce are methylated arsenicals. Arsenic-associated human diseases include bladder, lung, skin, and liver cancer. At present, the mode(s) of carcinogenic action of arsenicals in humans is unknown. This data gap prevents a scientifically-defensible quantitative human cancer risk assessment from being established for arsenic. Inorganic arsenic is biotransformed through a metabolic cascade to trivalent methylated forms, some which are highly toxic and genotoxic. Some inorganic and methylated arsenicals are carcinogenic in rodents, but their modes of action are unknown. We have evidence that arsenicals induce reactive oxygen species whose formation may explain arsenic's carcinogenic effects both in rodents and humans. We pose the following scientific questions:

- What is the mode of action of arsenic-induced bladder cancer in rodents and humans?

- What is the role of reactive oxygen species and oxidative stress in arsenic's toxicity and cancer?

**The Research**:

This broad research effort integrates chemical, biochemical, biological, molecular, and genomic approaches to uncover and delineate the critical events in the induction of bladder cancer in rodents and humans. Two key biomethylated arsenicals, the trivalent methylated [DMA(III)] and the pentavalent methylated [DMA(V)], are key intermediates that we will use to study important events in the bladder cancer process in rodents and in rodent and human urothelial cell lines: DNA damage (clastogenesis), DNA repair, DNA methylation, cytotoxicity, increased cell proliferation, altered cell cycle control, and apoptosis. Our research addresses this problem at the molecular, cellular, and organismal levels. We seek to identify those reactive oxygen species responsible for the critical events involved in the initiation and progression of arsenic carcinogenesis. We plan to examine the alterations in gene expression, cell proliferation, and apoptosis in the transitional epithelium of the urinary rat bladder in a series of studies at exposure concentrations at or near some environmental levels. We will compare these findings to those obtained from the exposure of rat and human urothelial cells in culture to arsenicals

to define the cellular and molecular events associated with arsenic-induced carcinogenicity.

**Impact and Outcomes:**

- This research has made a significant contribution to understanding the mechanism by which environmental stressors such as arsenic produce cancer.
- This research contributes to the Agency's need for knowledge of the MOA of a chemical to perform the extrapolation step of a dose-response assessment, as indicated in the Proposed Guidelines for Carcinogen Risk Assessment
- This research addresses the question concerning linearity or nonlinearity of the dose-response relationship for tumors.
- The results from this research are having a significant impact on the outcome of the assessment of arsenic in drinking water and by pesticide exposure.
- Research on biomethylation of arsenic has influenced the Office of Water and Office of Pesticide Programs in their ongoing reevaluation of the risk to arsenic exposure.
- This collaborative research effort between EPA, NIEHS, and academia has been influenced in its research direction by the needs of OW and OPP.
- Clarifying the mode of action of arsenic carcinogenesis will impact the establishment of both the arsenic unit cancer risk and the Maximum Contaminant Level (MCL) of arsenic in drinking water and the risk assessments of organoarsenicals.

**Poster LTG1-11**
**Internal Dosimetry and Physiological Modeling: The Bridge Between Arsenic Exposure and Health Effects**

**Presenter:** E.M. Kenyon (NHEERL)
**Contributors:** M.F. Hughes and M.V. Evans (NHEERL); J. Blancato (NERL); M.Styblo (University of North Carolina)

**Science Question:**

- Can low dose extrapolation for Arsenic (As) be improved by understanding the *quantitative* and *mechanistic* relationship of As target tissue dosimetry and subsequent development of adverse effects?

**The Research:**

Our experimental framework integrates pharmacokinetic and pharmacodynamic model development with collection of appropriate experimental data for model parameterization and evaluation. The major goal is to reduce uncertainty in arsenic low dose extrapolation. Our modeling approach has three major components: (1) development of models describing As methylation in liver at the subcellular and cellular levels to identify key mechanisms and processes for inclusion in the larger As model, (2) sequential development of whole body individual PBPK submodels for each of the major forms of As, starting with DMA and working backwards to monomethylarsonic acid (MMA), followed by arsenite and arsenate, and (3) correlation of arsenic exposure and target tissue dosimetry with measures of early biological effect. Hepatic methylation model development is supported by pharmacokinetic studies using rat liver cytosol and rat and human hepatocytes. Arsenic PBPK model development is supported by pharmacokinetic studies in mice with model evaluation data for the scaled up human model provided by epidemiologic studies conducted in the Human Studies Division. The model will subsequently be implemented in the ERDEM platform (Exposure Related Dose Estimating Model) developed by ORD researchers. This approach was selected because using the ERDEM platform facilitates linking the As PBPK model with ORD exposure models.

**Impact and Outcomes:**

- The model developed as part of this research can be used by Program Offices in evaluating health risks posed by both inorganic arsenic and organoarsenical pesticides.
- The model has utility for estimating tissue dosimetry in humans resulting from actual exposures, i.e. multi-media, multi-pathway exposure assessment.
- The model can also be used to test assumptions used in default risk assessment methods against experimental data as well as for extrapolation of dose between species and among age classes.

- Research has established that inhibition of the second methylation step by arsenite and binding of arsenite to protein are essential mechanisms to describe arsenic kinetics at the cellular and subcellular levels.
- These mechanisms have been incorporated in the As PBPK model developed for mice and humans. Further refinement and evaluation of this model is underway now that tissue data for several arsenic metabolic species are available from both acute and subacute exposures in mice (i.e., time course data for levels of inorganic As, and mono and dimethylated arsenic in blood, liver, lung, and kidney).
- Research evaluating the relationship of exposure to arsenite with markers of oxidative stress reveals that tissue levels of inorganic arsenic in liver and kidney are a sensitive and specific marker for heme oxygenase induction.
- Oxidative stress is considered one of several possible modes of action for arsenic-induced cancer.

**Poster LTG1-12**
**Metabolism as a Critical Aspect of the Actions of Arsenic as a Toxin and Carcinogen**

**Presenter**: D. J. Thomas (NHEERL)
**Contributors:** M. Hughes, B. Adair, and E. Kenyon (NHEERL); J. Creed (NERL); M. Styblo, S. Waters, Z. Drobna, V. Devesa (University of North Carolina at Chapel Hill)

**Science Question**:

Although epidemiological evidence associates chronic exposure to inorganic arsenic with increased risk of cancer and degenerative diseases, the modes of action of arsenic as a toxin or carcinogen are not well understood. A growing body of evidence suggests that many of the adverse health effects associated with chronic exposure to inorganic arsenic are in fact caused by methylated metabolites which are formed from inorganic arsenic. Two general questions can be framed for these metabolites:

- How is inorganic arsenic converted to methylated products?
- Can the formation of these metabolites be linked to specific adverse effects?

**The Research**:

This research effort is divided into two complementary projects. The first project has elucidated the enzymatic basis for conversion of inorganic arsenic into methylated products. This effort has identified the *AS3MT* gene which encodes an enzyme that catalyzes oxidative methylation of arsenicals and reduction of pentavalent arsenicals to trivalency. Thus, a single gene encodes a protein that catalyzes both reactions needed to convert inorganic arsenic to the methylated products which are the putative mediators of some of the toxic and carcinogenic effects associated with chronic inorganic arsenic exposure. Orthologous AS3MT proteins are found in vertebrates ranging from primitive chordates to humans; expression of AS3MT in these species is the critical determinant of the capacity to metabolize arsenicals. Because epidemiological studies suggest capacity to methylate arsenic to be a risk factor for adverse health effects during chronic inorganic arsenic exposure, we are examining the role of *AS3MT* polymorphisms as determinants of capacity to methylate inorganic arsenic. The second project has focused on development and refinement of analytical techniques for quantitation of arsenicals with a particular goal of developing methods to identify the oxidation state of arsenic in various metabolites. This endeavor has demonstrated that the metabolism of arsenic in humans and other species yields the predicted array of metabolites, including those containing trivalent arsenic. It has also shown that volatile trimethylarsine is produced by AS3MT from inorganic arsenic. Continued refinement of these analytical techniques critically support the development of physiologically based pharmacokinetic models by providing detailed dosimetric data on the formation and fate of arsenicals at the cellular and organismic level. These improved techniques can also be used in epidemiological studies to provide a better dose metric.

**Impact and Outcomes***:*

- This work has shown the feasibility of identifying and quantifying of a wide range of arsenic-containing metabolites in biological samples, including tissue and urine.
- Data on the occurrence of arsenicals in tissues improve the internal dosimetry and contribute to better understanding of dose-response relationships.
- Identification of new metabolites of inorganic arsenic also contributes to the design and conduct of studies of the modes of action of arsenicals which may underline its actions as a toxicant and carcinogen.

**Poster LTG1-13**
**Discovering the Mode(s) of Action of Conazole Toxicity Using the Tools of Toxicogenomics and Toxicology for Harmonization, Interspecies Extrapolation, and Computational Toxicology**

**Presenter:** Stephen Nesnow (NHEERL)
**Contributors:** James Allen, Hugh Barton, Larry Claxton, David Dix, Don Delker, Susan Hester, Leon King, Michael Narotsky, Ann Richard, John Rockett, Jeffrey Ross, Sheau-Fung Thai, and Doug Wolf (NHEERL); Drew Ekman, (NERL); Stanley Barone (NCEA);Vicki Dellarco and Karl Baetcke (Office of Pesticide Programs); and Jacques Retief, Affymetrix Corporation

**Science Questions:**

Conazoles are azole antifungal agents used as pesticides and drugs. Approximately forty conazole fungicides are used in commerce. Conazoles are ergosterol biosynthesis inhibiting fungicides (EBIFs) and are used as fungicides on fruits, grains, ornamental flowers, and other crops. As pharmaceuticals, they are used to treat local and systemic infections. Conazole exposure has been associated with hepatotoxicity, thyroid and liver cancer, and developmental and reproductive toxicity. This project is designed to ask the following questions:

- Is there a common mode of action for the observed toxicities of conazoles?
- Is P450/XME modulation a common critical event in the toxicities of conazoles?
- Can the critical events in each conazole-induced toxicity be identified and combined with "omics" data for use in interspecies extrapolation?
- Can mode of action data be combined with patterns of gene expression and chemical structure to predict toxicities of new conazoles?

**The Research:**

This highly integrated research is designed to apply state-of-the-art molecular tools supported by traditional toxicological methods to study the mode(s) of action of conazoles that have toxicologically adverse outcomes. A basic tenet of this research is to select activity-inactivity pairs of conazoles for comparison of their effects (i.e., carcinogens and non-carcinogens; reproductive toxins and non-reproductive toxins). Tissues are collected from rats and mice who were administered selected conazoles and these tissues subjected to a battery of biochemical, hormonal, molecular, and histological evaluations. Global changes in gene expression (genomics) are compared across species, conazole, dose, and time to reveal those alterations associated with toxicity. Those genes and cellular and molecular ontological pathways identified as candidates are further explored at the protein, enzyme, and biochemical intermediate levels. These molecular beacons or roadmaps of modes of action are further studied using proteomics and metabolomics. Interspecies comparisons (rat, mouse, and human) are sought using both *in vitro* and *in vivo* approaches. This project is also a component of ORD's Computational Program, where toxicogenomic approaches are used to identify shared

structural features, “omics” patterns, and activity profiles of conazoles and related chemicals to serve as potential predictors of toxicological responses based on common modes of action.

**Impact and Outcomes:**

- A collaborative effort involving researchers from ORD, OPP and industry and with a Cooperative Research and Development Agreement with The US Triazole Task Force has been established.
- The emerging research directly addresses the needs to derive a commonly accepted set of principles defining how mode of action information can be used in chemical risk assessments, particularly as it relates to interspecies and high to low dose extrapolation issues.
- This project addresses both qualitative and quantitative methods that could be eventually applied to risk assessment activities.
- The results from this project can impact the regulation of several conazoles, as well as other pesticides that act by similar MOAs.
- In harmonization, a common paradigm for dose-response assessments for all toxicity endpoints will simplify the human health risk assessment of these agents and make them biologically consistent.
- In mode of action, the elucidation of the mode of action for each toxicity and the interspecies approaches will improve, and make scientifically defensible, the existing human health risk assessments and provide models for future risk assessments.
- The emerging approach combines genomic and proteomic technologies in combination with traditional toxicological approaches and should make it possible to assess common modes of action for the improved prediction of human health risk of chemicals.

**Poster LTG1-14**
**Mode of Action Studies of Conazoles in Rats: Determination of Pathways in Carcinogenesis**

**Presenter:** Douglas Wolf (NHEERL)
**Contributors:** James Allen, Larry Claxton, Don Delker, Susan Hester, Leon King, Stephen Nesnow, Jeffrey Ross, and Sheau-Fung Thai (NHEERL); and Vicki Dellarco and Karl Baetcke (Office of Pesticide Programs)

**Science Question:**

Conazoles are used as fungicides in both agricultural and pharmaceutical settings. The US EPA Office of Pesticide Programs has approximately 17 registered conazole fungicides with more being submitted. Many conazoles are reported to be hepatotoxic and hepatocarcinogenic in mice, some are reported to induce thyroid tumors in rats, and a few induce both tumor types. The relevance for human health risk assessment of the lesions reported in rodents after treatment needs to be determined. The present work within NHEERL on these compounds is designed to identify if a common mode of action is driving the responses in rats and mice and if a concordant mechanism is operative in human tissues in order to determine the relevance of the responses in rodents for human health risk assessment. Specifically, the science question being addressed is:

- Is the rat thyroid tumorigenic activity of conazoles mediated through alteration of thyroid hormone-related metabolic pathways and subsequent TSH stimulation of the thyroid gland?

The answer will help define the nature of the low dose tumor response for exposures to carcinogenic conazoles. The current risk assessment recommendations suggest a non-linear or margin of exposure approach if a hormonal pathway for rat thyroid cancer is operative. The influence of conazole-induced changes in hepatic function will also be delineated.

**The Research:**

This research program is new and began in January 2004. The aim of these studies is to completely describe the thyroid pathways disrupted in the rat. To define the thyroid endocrine disruption and tumor development, both carcinogenic and non-carcinogenic conazoles are being tested. In addition, comparisons are being made between target and non-target tissues as well as between mouse and rat thyroid. Comparing the results for different chemicals and tissues should help to specifically distinguish a carcinogenic from non-carcinogenic pathway. Ultimately, these data will be used in developing comparative models for extrapolation and prediction across species, tissues, and between chemicals. Three conazoles with different potentials for cancer induction are being evaluated in rats. Triadimefon induces thyroid tumors in rats and liver tumors in mice; propiconazole induces liver tumors only in mice, and myclobutanil is negative for a tumor response. The response endpoints that include histology, cell proliferation,

apoptosis, induction of cytochrome P450s and other metabolizing enzymes, thyroid hormone analysis, and gene and protein expression. The hypothalamic-pituitary-thyroid (HPT) system in the rat is very sensitive to xenobiotics that impair normal thyroid gland and thyroid hormone function. Tissue responses in thyroid and liver coupled with gene expression analysis will more completely describe the chemical's effects. The data from these studies will be used in developing comparative models for extrapolation and prediction across species, tissues, and between chemicals. Using direct comparison of responses between animals, studies, and tissues, commonalities of toxicity pathways will be determined. It should then be possible, through direct comparison of tissue responses and altered gene expression, to determine common cellular pathways that are perturbed in rats and mice and as well as human liver cells. Utilizing this cross study and cross response approach, common toxicity pathways for this series of conazoles, if they exist, will be determined. The data collected to date indicate that all three conazoles induce metabolizing enzymes in the liver including the UGT that metabolizes T4. All three conazoles decrease circulating T4 with triadimefon also decreasing circulating T3. None of the conazoles result in increased TSH secretion. Only triadimefon causes transient histopathology after 30 days of treatment at the high dose. These studies show that altered metabolism in the liver is a key event in the development of thyroid hormone disruption associated with exposure to triadimefon. The liver enzyme changes, liver and thyroid histology, and thyroid hormone alterations are not consistent with a TSH mediated thyroid tumor development in the rat.

**Impact and Outcomes:**

- Conazole mode of action studies of rat thyroid tumorigenesis are part of a collaborative EPA (NHEERL, NERL, NCEA, and OPP) research program supported by industry partners, and with a Materials Cooperative Research and Development Agreement with the US Triazole Task Force
- These studies show that altered metabolism in the liver is a key event in the development of thyroid hormone disruption but not consistent with TSH mediated thyroid tumor development in the rat.
- These results have the potential to affect the conazole risk assessments performed by OPP as a class because the default assumptions used in the assessments are not supported by scientific data.
- Identifying the initiating key event driving the toxicity and subsequent carcinogenicity from pesticide exposure will allow the development of more targeted and cost effective studies as well as risk assessments based on the most relevant adverse health effects.

**Poster LTG1-15**

## Toxicogenomic and Traditional Approaches Applied to Identifying the Mode(s) of Action of Conazole-Induced Mouse Liver Cancer

**Presenter:** James Allen (NHEERL)
**Contributors:** Larry Claxton, Don Delker, Susan Hester, Leon King, Stephen Nesnow, Jeffrey Ross, Sheau-Fung Thai, and Doug Wolf (NHEERL); Vicki Dellarco and Karl Baetcke (Office of Pesticide Programs)

**Science Question**:

Conazoles are azole antifungal agents used as pesticides and drugs. A number of these fungicides induce liver tumors in mice fed these conazoles, while rats do not develop liver tumors. Mouse liver tumors are the most common tumors found in surveys of long-term cancer bioassays of environmental agents in rodents. There are many modes of action described for the induction of mouse liver tumors, however only a few are applicable to humans. It has been hypothesized that, because conazoles are predicted to be or have been shown to be non-genotoxic, they may produce mouse liver tumors by the induction of P450 isoforms with associated liver hypertrophy, cell proliferation, inhibition of apoptosis, and selected clonal expansion. This proposed carcinogenesis pathway is thought to be similar to that for phenobarbital which is tumorigenic in mice but not considered to be a human carcinogen. Therefore, the key science questions are:

- What are the mode(s) of action by which some conazoles induce liver toxicity and hepatic tumors in mice?
- Are these modes of action similar to that of phenobarbital?
- Are these modes of action concordant with processes that could occur in humans and are they predictive of human health risks?

**The Research**:

This research program is new and began in January 2004. These studies are specifically aimed at delineating the critical pathways involved in conazole-induced mouse liver toxicity and cancer. Using traditional toxicological approaches we are examining the histological changes, enzymatic activities, biochemical intermediates, and receptor levels in the livers from mice fed tumorigenic (triadimefon, propiconazole), non-tumorigenic (myclobutanil) conazoles, and phenobarbital. Using genomic and proteomic analyses of these tissues, we are examining changes in gene expression patterns and their associated proteins, to identify changes associated with toxicological responses. To explore the contribution of *in vivo* genotoxicity, studies with transgenic mice are being undertaken. Approaches taken to determine the relevant mode(s) of action are based on 1. The identification of candidate aberrant gene expression pathways through analyses by microarray technology. 2. Testing the proffered hypothesis that liver cancer arises from P450 induction and resulting oxidative stress and enhanced mitogenesis. The results

observed in mouse tissues are compared to those found in rat tissues. Human relevance is assessed by "parallelogram" studies comprising mouse (*in vivo* and *in vitro*) and human (*in vitro*) hepatocyte gene expression data to distinguish between species-specific and non-specific pathway responses to conazoles. Analyses of the genomics data from mice treated *in vivo* with each of the three conazoles reveals a number of over-expressed ontological pathways. Some pathways were found in tissues for all three conazoles tested and some were unique to a specific conazole. The changes in the over-expression of specific P450 isoforms, as well as changes in P450 enzymatic activities, and histological effects were similar for all three conazoles. These data suggest that these P450 genes are not associated with the tumorigenic process. Data mining activities are identifying key pathways that might be associated with tumorigenic activities.

**Impact and Outcomes**:

- Conazole mode of action studies of mouse liver tumorigenesis are being studied in a collaborative EPA (NHEERL, NERL, NCEA, and OPP) research program supported by industry partners, and with a Materials Cooperative Research and Development Agreement with the US Triazole Task Force.
- These studies form an essential component of current Agency efforts based on the Proposed Guidelines for Carcinogen Risk Assessment to determine how mode of action information can be used in chemical risk assessment, particularly as it relates to quantitation and human extrapolation.
- Research will provide a model approach for combining genomic technologies with traditional toxicological methods to assess cross-species/tissues commonality in modes of action for chemical induction of tumors and other toxicities, and should better inform the process for assessing and predicting human health risk.
- The specific risk assessments of the group of conazoles in commerce will be scientifically strengthened both in hazard identification and dose response assessments
- The combination of genomic, proteomic and traditional toxicology approaches should enhance our ability to identify and classify new environmental agents according to mode of action
- Computational toxicological approaches will be developed to prioritize the testing of new environmental agents through better prediction of their toxicological activities.

**Poster LTG1-16**
**A Toxicogenomic Approach to Determining Modes of Action for the Reproductive Toxicity of Conazoles**

**Presenter:** David Dix (NHEERL)
**Contributors:** Hugh Barton, Michael Narotsky, John Rockett, Douglas Wolf (NHEERL); Stanley Barone (NCEA); Drew Ekman, (NERL); Vicki Dellarco, Karl Baetcke (Office of Pesticide Programs); Jacques Retief (Affymetrix Corporation).

**Science Questions:**

This research addresses the following questions:

- Can gene expression profiling identify toxic modes of action either common to conazoles or specific to individual members of the conazole class of fungicides?
- Do the conazole fungicides share common modes of action across chemicals, tissues, or toxicities which are relevant to individual and grouped risk assessments?

**The Research:**

Conazole fungicides are the subject of an integrated toxicological, genomic and pharmacokinetic research effort utilizing rodent *in vivo* and *in vitro* exposures. Three agricultural and one pharmaceutical conazole, each containing the common 1,2,4-triazole moiety, were selected for this study. Results from the initial short-term study of adult exposures in rats and mice indicated that while histopathology and modulation of cytochrome P450 (CYP) enzyme activities in the liver and testis did not differentiate between the conazoles, gene expression profiles identified multiple candidate toxicity pathways in the liver, testis and brain. Some of these pathways were common to all three conazoles, while others were distinct to a subset of the compounds and potentially predictive of specific toxicities. Subsequent studies with rats exposed from gestation through adulthood identified a broad range of effects on pregnancy outcomes and reproductive development. Increased pre- and postnatal mortality attributed to effects on parturition, and increased anogenital distance in both male and female pups, suggested possible aromatase inhibition by several of the conazoles. Exposure to high concentrations of these same conazoles adversely impacted female reproductive development, also indicating inhibition of estrogen synthesis or function. Male reproductive development and function was also altered by conazoles, resulting in delayed puberty, increased liver, thyroid, testis and accessory sex gland weights, increased serum testosterone, decreased thyroxine levels, and impaired male fertility. *In vitro* studies have confirmed the conazoles ability to inhibit steroidogenesis (*i.e.,* CYP17) in the testis. The next step in this toxicogenomic study will be to use gene expression profiling in these affected tissues to identify toxicity pathways relevant to specific conazole exposures and correlated to adverse effects. These genomic results will define toxic modes of action either common to or unique for

the various conazole fungicides, consistent with the observed adverse reproductive and developmental effects.

**Impact and Outcomes:**

- This research effort is ongoing in ORD to better define and understand the reproductive effects of conazole fungicides.
- If common modes of action are defined from this toxicogenomic approach, this information will help the Office of Pesticides Programs (OPP) to determine whether there is sufficient scientific basis to group the triazole-bearing conazole pesticides by a common mechanism of reproductive toxicity.
- Determination of mechanisms common between reproductive and cancer outcomes will inform progress on harmonizing cancer and non-cancer risk assessments.
- The methods and practices developed in this toxicogenomic study will be useful to EPA's development of science policy and procedures for applying this genomic data to risk assessments.

**Poster LTG1-17**
**Overview of Use of Mechanistic Data in Risk Assessment**

**Presenter:** Hugh A. Barton (NHEERL)
**Contributors:** Jeff Gift and Resha Putzrath (NCEA); R. Woodrow Setzer (NHEERL); and Chris Saint (NCER)

**Science Question**:

- How does information describing biological processes leading to toxicity inform evaluation of risks from chemicals?

**The Research**:

Risk assessment approaches have been evolving since the 1970's to make increasingly sophisticated use of scientific information. Improvements in dose-response analysis arise from a shift from a simple exposure-response perspective to a multistep process describing exposure, pharmacokinetic (toxicokinetic) processes producing internal tissue dosimetry of the active form(s) of the chemical, and pharmacodynamic (toxicodynamic) processes describing the toxicity pathway leading to the observable toxic response. Exposure and pharmacokinetic modeling are described in greater detail in other thematics areas (e.g., aggregate/cumulative risk assessment, source-to-effect modeling for children as a susceptible population). Mode of action analyses are described in this theme for a number of endpoints and toxic chemicals.

ORD has a range of research activities addressing applications of mode of action data in risk assessment in terms of developing general approaches and specific case-study examples. This research includes development of guidance for general methods such as statistical analyses of dose-response relationships or application of mode of action data in risk assessments for cancer and other endpoints. Case studies represent an important research tool for exploring the implementation of advanced methods in risk assessments. The modes of action described in this theme are examples where ORD researchers are generating data and then, along with others in EPA, participating in the application of those data for risk assessments for relevant chemicals. The application of biologically informed risk assessment methods for neurological and cancer endpoints arising from perchlorate illustrates how the expertise of ORD scientists can be applied to evaluating a chemical that is not a focus of an EPA laboratory research effort.

A particularly challenging area is mathematical modeling of pharmacokinetic and pharmacodynamic processes to facilitate biologically based dose-response analyses. Earlier research funded by ORD and others focused on clonal growth modeling for cancer and explored approaches to modeling developmental malformations. Due to the importance of cell growth, death, and differentiation during development, more recent extramural efforts are addressing applications of clonal growth modeling for developmental toxicities. Human variability in pharmacokinetics and pharmacodynamics

is another area of substantial importance that has been subject of intramural and extramural research. This information could be incorporated into a biological modeling approach, but it can also be used to inform risk assessments using empirical dose-response methods. Addressing human variability and other factors (e.g., interspecies extrapolation) is a component of recently initiated activities addressing data-based uncertainty factors and the development of methods for probabilistic analyses in non-cancer risk assessment.

**Impact and Outcomes:**

- The roles of tissue dosimetry and mode of action in risk assessment, particularly dose-response assessment, are increasingly prominent in EPA guidance documents and risk assessments for chemicals, reflecting the level of importance of research activities in ORD.
- Both the draft cancer guidelines and supplemental guidance for early-life exposures are organized around the concept of mode of action.
- Implementation of benchmark dose analysis is an important tool for analyzing dose-response relationships, whether based upon exposures or internal doses predicted by pharmacokinetic modeling.
- Risk analyses for specific chemicals including atrazine, chloroform, perchlorate, and butoxyethanol have been modified substantially through the use of mode of action data.

**Poster LTG1-18**
**Dose-Response Modeling**

**Presenters:** Jeff Gift (NCEA) and R. Woodrow Setzer (NHEERL)
**Contributors:** Kenneth G. Brown (Kbinc); Gary Foureman and John Fox (NCEA); Yiliang Zhu (University of South Florida)

**Science Question**:

- What can be done to support risk assessors in their efforts to model and characterize dose-response data for the purposes of Agency risk assessments?
- What are the various study designs, chemical modes of action, and dose-response shapes a risk assessor is likely to encounter?
- What guidance and training are needed to ensure that the results are interpreted and used in a consistent and scientifically defendable manner?

**The Research:**

To answer this question the various study designs, chemical modes of action and dose-response shapes an assessor may encounter need to be considered. Once the dose-response has been modeled, the assessor needs additional support in the way of guidance and training to ensure that the results are interpreted and used in a consistent and scientifically defensible manner. Ongoing dose-response modeling research and support activity within the Agency can be divided into five areas: (1) accounting for the various study designs risk assessors will encounter, (2) accounting for a chemical's mode of action (MOA) and the various dose-response shapes an assessor will encounter, (3) determining what can be credibly inferred from model results, (4) developing user-friendly and consistent interfaces for dose-response models, and (5) providing guidance and training on the application of models and the linkage of dose-response modeling to risk assessment. The Agency has developed models for its benchmark dose software (BMDS) that account for standard chronic bioassay study designs involving dichotomous (e.g., tumor), continuous (e.g., organ weight) and nested (e.g., effect in pups following parental exposures) dose-response data. ORD laboratories and centers are working together to develop new models that will be able to assess studies which measure responses (e.g., neurotoxicology batteries) over time following acute exposures. Other models are planned which will allow for the analysis of dose-response for effects measured over time during subchronic or chronic exposure studies, and for more flexible models for continuous and quantal data. . The Agency has also developed models to evaluate chemicals with distinct MOAs and dose-response shapes. Models included in BMDS were chosen for their ability to analyze a broad spectrum of saturable (e.g., Hill model), nonlinear (e.g., log-logistic model), linear (e.g., Multistage/Polynomial) and nested effects. Future plans include the development of EPA BMDS models that will allow for a consistent Agency approach to the assessment of chemicals that induce early mortality and early tumor responses.

With respect to inferences that can be made from modeling results... There are still outstanding theoretical statistical issues related to the calculation and use of BMD. Research sponsored by an NCER has explored confidence interval calculation and sensitivity of the calculated interval to mis-specifying the model. Ongoing research in ORD is directed towards understanding the consequences of and adjusting for the fact that we do not know the true dose-response functions when we estimate BMDs using dose-response modeling. This results in uncertainty that increases as we require inferences for responses that are far removed from the data.

The Agency has also developed categorical regression (CatReg) software to evaluate dose-responses for chemicals that elicit effects that can be categorically graded by severity. Several research projects have been initiated in categorical regression. To obviate the inherent difficulties with categorization schemes applied across various species, sensory responses of humans on exposure to formaldehyde and Sarin that have been precisely categorized with respect to severity grade were analyzed with CatReg. To evaluate the capability of CatReg in estimating the actual duration-concentration relationship, specific endpoint data (mortality) in a single species (rats) were empirically analyzed and compared, for hydrogen sulfide, with options currently available on CatReg. Results from these studies will allow the Agency to better define and refine the application of categorical regression to dose-response.

Ultimately, Agency risk assessors need guidance and training in the use of models, and the interpretation of their results. To this end, a web-based, online training program was developed by ORD. The online training course for BMDS reviews the benchmark dose methodology, consistent with the most recent draft of our BMD technical guidance document (EPA, 2000), and the application of BMDS to the various types of data sets that risk assessors may encounter. ORD plans to continue to improve the online training program to keep up with the latest Agency needs and risk assessment methods.

**Impact and Outcomes***:*

- Recently developed benchmark dose methods are integral to the development of the cancer slope factors, reference doses (RfDs) and reference concentrations (RfCs) used along with other scientific information to set these standards.
- Benchmark dose methods are currently used or considered in all Agency IRIS and most other program office risk assessments.
- Over the past few years, the Agency has made BMDS easier to use by improving its user interface and modeling capabilities to keep up with the state-of-the-science in this important and growing risk assessment field.
- The customer base for BMDS has expanded to over 2,000 registered users in areas of industry, academia and government from over 80 countries.
- The Agency plans to maintain and improve dose-response modeling software such as BMDS and CatReg so that they continue to provide a valuable resource to Agency Program Office, regional, State and international risk assessors.

**Poster LTG1-19**
**Mode of Action as the Biological Basis for Cancer Risk Assessment**

**Presenter:** Resha Putzrath (NCEA)
**Contributors:** Hugh A. Barton and R. Woodrow Setzer (NHEERL); Risk Assessment Forum Technical Panel for Cancer Guidelines and Technical Panel for Early-Life Guidance (see listing on poster)

**Science Question**:

- How do modes of action of chemicals inform evaluation of cancer risks for children and adults?

**The Research:**

Procedures that are harmonized biologically based, and dependent on data rather than defaults offer the potential advantages of a less-fragmented, more integrated, and more biologically consistent approach to risk assessment. Approaches to addressing these issues are formulated within the NAS framework for the assessment of toxic effects that arise from the biological responses to tissue doses of chemicals following exposure. EPA's mode of action framework links exposure and responses through key pharmacokinetic (or toxicokinetics) and pharmacodynamic (or toxicodynamic) processes. Although this approach is applicable to a wide range of toxicities, the research described here focuses on carcinogenesis.

The current Agency draft cancer risk assessment guidelines are designed to capture the significance of evaluating all the data, including data on mode of action and tissue dosimetry of the active form of the chemical. These guidelines outline a framework for evaluating the weight of evidence for a mode of action and its applicability to humans, as well as describing some implications for cancer risk assessment of different modes of action. Dose-response analysis initially determines a point of departure from which subsequent extrapolation methods are determined by the mode of action information, or lack thereof.

Based upon recommendations of the EPA's Science Advisory Board in their review of the draft cancer risk assessment guidelines, research was also undertaken to determine if specific approaches were needed for evaluating cancer risks for children. A review of published literature indicated that very limited data were available for humans with the strongest data for radiation and the potent estrogen, diethylstilbesterol, indicating that exposures during pregnancy or early in life were potentially of greater concern than adult exposures. Data on the age-dependence of chemical carcinogenesis in animals are available, with the majority for chemicals causing cancer through DNA adduct-induced mutagenesis; limited data for a number of other modes of action were identified. A Bayesian dose-response analysis was used to calculate ratios comparing cancer responses following early-life exposure to those observed following adult exposures. Ratios

greater than one suggest children are more susceptible. Studies with repeated exposures used routes that are relevant to humans, e.g., inhalation, drinking water. Injection studies using chemicals with a mutagenic mode of action are not typically used for risk assessment, but they provide valuable information because the exposure dose is rigorously controlled to a degree that is not feasible in the repeated dose studies. The analysis demonstrated that young animals were generally more susceptible than adults to induction of cancer via a mutagenic mode of action. The response was clearest for liver tumors, but was also observed for other tumor sites.

**Impact and Outcomes:**

- The U.S. EPA has developed new cancer risk assessment guidelines that incorporate explicit consideration of all biologically relevant information, including data on the mode of action by which the chemical causes cancer in animal species or humans.
- The draft guidelines are already having significant effects in EPA, e.g., use of a nonlinear analysis for cancer for chloroform.
- Draft supplemental guidance has also been developed for assessing cancer susceptibility from early-life exposures to chemicals with a mutagenic mode of action associated with increased susceptibility in young animals.
- This guidance serves as a model for development of guidance for other modes of action, e.g., hormonally-mediated cancer may be subject for a future analysis based, in part, on ongoing research by the National Institute of Environmental Health Sciences.

**Poster LTG1-20**
**ORD's Computational Toxicology Research Program**

**Presenter:** Robert J. Kavlock (NCCT)

**Science Questions:**

- Given the need to become more efficient and effective in the use of animals in screening and testing programs, what role can computational approaches play in characterizing environmental stressors?
- How can 'omics' approaches be used in the context of quantitative risk assessments?

**The Research**:

Computational Toxicology is defined in the "Framework for a Computational Toxicology Research Program" as the application of mathematical and computer models and molecular biological approaches to improve the Agency's prioritization of data requirements and risk assessments. Initiated by a Congressional reprogramming action in FY02, the computational toxicology program has undertaken several proof-of-concept studies for endocrine disruption, as it was felt that EPA already had a strong research presence in this area, and that there was a good understanding of the relevant toxicity pathways. Several projects were launched within the Computational Toxicology Program that will provide both short term and long term products to the Human Health Research Program. In FY03, ORD developed and had peer reviewed its Framework document that is intended to guide the use of the increasing level of resources being dedicated to the program. In FY04, a Computational Toxicology Implementation and Steering Committee (CTISC) was formed to begin to translate the Framework into action. The CTISC awarded 10 "Augmentation" projects funding to immediately expand 'omics' research across ORD, and later awarded 7 "New Start" projects that represented significant investments over a three year period. In FY05, ORD institutionalized the computational efforts by creating the National Center for Computational Toxicology. The Center is staffed with systems biologists, computational chemists and bioinformaticians, and will be a focal point for computational toxicology efforts across ORD. One of the main early challenges to the program will be to develop approaches for prioritizing chemicals of concern to the Agency for screening and testing purposes. Ideally this will lead to tailored testing schemes for environmental stressors and to the more effective and efficient use of animals in research. This, in turn, will lead to improved risk assessment methodologies and outcomes.

**Impact and Outcomes**:

- The Computational Toxicology program will continue developing tools and approaches for the prioritization of screening and testing needs in the areas related to human health research, e.g., agents with a Luteinizing Hormone MOA.

- Application of Computational Toxicology approaches to the screening and testing needs of EPA program offices (e.g., the Office of Prevention, Pesticides, and Toxic Substances and the Air Office) will also be evaluated.
- The Computational Toxicology program also expects to deliver the first alternative assay for animal testing of environmental toxicants in FY06. This will be accomplished with an in vitro cell line to study the potential of environmental stressors to stimulate the excessive production of steroids within living systems.
- The Computational Toxicology program will add a number of new toxicological databases to the Distributed Structure-Searchable Toxicity (DSSTox) system, expand the breadth of chemicals evaluated through computational models of nuclear receptor-ligand docking preferences, provide an expanded list of environmental stressors tested using in vitro approaches, and communicate the outputs of two conferences on the application of genomic technologies to eco-toxicological and human health risk assessment processes.
- The Computational Toxicology program will also be working to establish the STAR-funded Center for Environmental Bioinformatics in order to ensure that the data emanating from ORD research projects in 'omics' can be appropriately mined for information.

**No Poster Presented**
**Perchlorate Environmental Contamination: An Integrated Characterization of Human Health and Ecotoxicological Risk Based on Mode of Action**

**Presenters:** Annie M. Jarabek (NCEA) and Kevin M. Crofton (NHEERL)
**Contributing authors:** The ORD Perchlorate Risk Assessment Team (see list on poster)

**Scientific Question:**

- How can mode of action data be best utilized in a risk assessment to support regulatory readiness of an emergent contaminant?

**The Research:**

Appreciation of widespread environmental contamination with perchlorate emerged in 1997 when the reporting limit of the analytical method for detecting its presence in drinking water was improved from 100 ppb to 4 ppb. Perchlorate is the stable anion that results when salts such as ammonium perchlorate readily dissolve in water. Ammonium perchlorate is the largest single component of solid rocket fuel and is used in the NASA space shuttle booster rockets, military solid fuel missiles, military ordnance, and fireworks. Contamination of soils, sediments, or ground and surface waters results after improper disposal or incomplete open burn / open detonation operations. Perchlorate has been placed on the contaminant candidate list (CCL) as a research priority and remediation is anticipated to be required of many facilities across the US. Currently no primary drinking water regulation or Superfund guidance level exist to direct treatment technologies. ORD took the lead with an integrated approach to developing both a human health and an ecotoxicological risk estimate to support regulatory readiness.

ORD coordinated an integrated assessment approach to risk characterization based on the mode of action for perchlorate toxicity. Each center and laboratory within ORD, the program and regional offices, NIEHS, and NIOSH were involved. This approach applied state-of-the-science research in key areas: human health risk, ecotoxicological risk, analytical methods, indirect exposure and transport/transformation, treatment technology and technology transfer / communication.

***Human Health Risk*:** A conceptual model based on the interference of thyroid hormone economy as the mode of action for perchlorate was used to identify data gaps and to harmonize approaches for cancer and non-cancer toxicity into one risk assessment. A testing strategy was developed that targeted the expected neurodevelopmental and neoplastic sequelae. Based on the key event of iodide uptake inhibition as the precursor to both sequelae, the model served as a framework to facilitate the integration of the diverse data available from human ecological epidemiology and clinical studies with focused laboratory animal data. New analysis methods were also developed to perform dose-response assessment of key endpoints.

***Ecotoxicological Risk:*** A conceptual site model (CSM) was developed that served as the basis of a screening-level assessment and as template for specific occurrence and biotransport studies at six different contaminated sites. Available data indicate a concern for toxicity to certain ecological receptors at exposure levels identical to that in the laboratory animal studies.

***Analytical Methods*:** ORD has led efforts to formalize an ion chromatography (IC) method for detection of perchlorate in water and its extension to various media (soil, sediments, plant and animal tissues). This work is critical to characterizing the indirect exposure potential of perchlorate via sources other than direct ingestion of drinking water. Refined sample preparation for the IC method and an additional LC/MS/MS method are under evaluation to extend analytical capabilities to lower detection levels.

***Indirect Exposure and Transport / Transformation:*** Concern that perchlorate in some fertilizers may pose an indirect exposure problem via uptake into plants that are consumed as crops or feedstock led to a collaborative study between ORD and The Fertilizer Institute. This work established that fertilizer was not a source of concern. Additional soil, sediment, and uptake studies do show concentration of perchlorate contamination in some plants and that, in addition to direct drinking water ingestion, the indirect route may pose an exposure concern.

***Treatment Technology:*** Once established, human health and ecotoxicological health risk estimates will set goals for clean up levels. Treatment technologies will need to tailor to meet these goals for end uses such as agricultural or drinking water. Region 9 and ORD are currently evaluating the efficacy of available treatment technology for both large and small scale operations.

***Technology Transfer & Communication*:** An Interagency Perchlorate Steering Committee (IPSC) was established in 1997 to serve as a governmental clearinghouse and has a subcommittee devoted to each key topic area. The IPSC hosted stakeholder meetings in effected areas and these helped to identify research priorities.

**Impact and Outcomes:**

- EPA applied state-of-the science integrated risk assessment approaches across all areas key to characterizing the potential risk from the widespread contamination.
- The harmonization of cancer and non-cancer approaches, as well as of human health with ecotoxicological risk estimates illustrates the utility of mode-of-action modeling.
- Risk assessment approaches that were used serve to address similar toxicity data from future studies required by testing guidelines.
- The first of two external expert peer and public reviews on the risk characterization was conducted in 1999. Additional studies and analyses were performed in response to recommendations by the peer panel and a second peer review was held in 2002. Both reviews were favorable and endorsed the Agency's use of mode of action.
- Because of broad interest among Federal agencies and other affected parties, the health assessment was then taken to the National Academy of Sciences (NAS) in

2003. The NAS report is expected in early 2005 and their comments will be used to finalize the Agency's assessment.