

Letter to our Readers

September 2005

Dear Colleague,

Our changing understanding of dose-response relationships for toxic chemicals has the potential to alter risk assessment methodologies more than any other advancement since the inception of the field. Non-linear dose-response curves, modes of action and margin of exposure concerns, and debates over hormesis are all contributing to the intense discussion now taking place.

Contributors to this issue include Dr. Lorenz Rhomberg, Gradient Principal and quantitative risk assessment expert, and Dr. Julie Goodman and Ms. Ari Schoen, both Gradient toxicologists. Joining them as our guest author is Dr. Edward Calabrese, Environmental Health Sciences professor at the University of Massachusetts in Amherst. Dr. Calabrese expresses his view of the role of hormesis in understanding the toxicity of chemicals.

We hope this issue of *Trends* will provide you with a new perspective on the excitement surrounding current research in the area of dose-response assessment.

Yours truly,



Neil Shifrin, Ph.D.  
 President and Founder

# Frontiers in Dose-Response Modeling

By Lorenz R. Rhomberg, Ph.D.

*Advances in carcinogenic dose-response modeling are focusing on the molecular level.*

Decision-making about emissions restrictions, environmental cleanup, and product safety hinges on how – and how well – scientists project low-dose risks in humans from high-dose testing of animals. Much of the history of quantitative risk assessment has revolved around debates on alternative approaches to extrapolating

risks from high experimental doses to low environmental ones, and from test animals to humans.

The first carcinogenic chemicals to be well studied (*i.e.*, components of coal tar) were found to have their effect through direct interaction with cellular DNA, producing gene mutations that disrupt the control of cell division. Because such mutations can

happen at even very low doses, this mode of carcinogenic action was traditionally thought to be without a dose threshold; that is, any exposure would result in some increased risk. Consequently, early dose-response analysis for carcinogens was based on a linear extrapolation from the high-dose animal tumor responses (*e.g.*, one-hundredth the dose gives one-hundredth the risk, and even small doses confer some excess risk).

As the investigation of chemical carcinogenesis has progressed, however, it has become clear that some agents that cause tumors in animals at high doses (*e.g.*, chloroform, carbon tetrachloride) do so by mechanisms other than direct DNA interaction. Instead, these chemicals may prompt excess division of normal cells through hormonal influences, toxicity to the cells (inducing cell division to replace

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# Frontiers in Dose-Response Modeling

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damaged cells), or other physiological disruptions. Importantly, the primary toxic effects of such compounds may have dose thresholds – that is, levels below which there is no risk. As such, doses that are low enough to prevent such toxicity will avoid the carcinogenic effect entirely. If low-dose risks for such chemicals are assessed with a low-dose linear dose-response approach, unnecessarily low limits on acceptable exposures may result.

Modern dose-response analysis tries to incorporate knowledge of the underlying mode of action of animal carcinogens

*The future of dose-response analysis is to look beyond simply fitting curves to tumor counts . . .*

and apply it to low-dose and cross-species extrapolations. Increasingly, regulatory guidance (such as the newly revised U.S. EPA guidelines)

entertains the case-by-case possibility of such analyses (see related article). The debate nowadays typically focuses on whether there is sufficient understanding of a particular chemical's underlying biological actions to support alternative extrapolation methods, thereby relaxing the conservative default calculations that became established in earlier regulatory approaches.

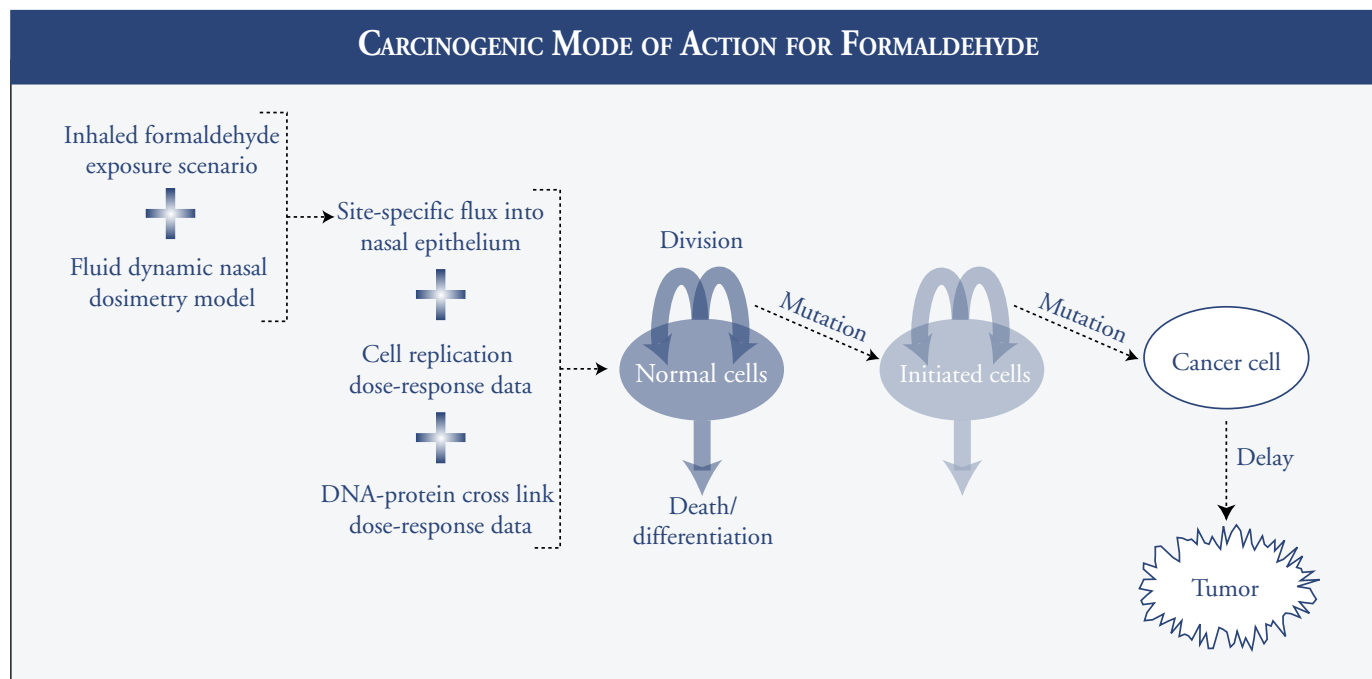
Some mechanisms of carcinogenic action are unique to particular species, and animal results may not apply to humans. For some chemicals, human exposures are insufficient to exceed

thresholds for the tissue toxicity necessary to induce carcinogenic action. A more complex situation occurs when the body's uptake and processing of the chemical (pharmacokinetics) or the critical physiological impacts on target tissues (pharmacodynamics) differs quantitatively between experimental rodents and humans or between high doses and low ones. Mathematical models are being developed that describe the complex interactions of multiple underlying processes that together determine the ultimate probability of developing a tumor.

As an example of what is possible, scientists at the CIIT Centers for Health Research have conducted a dose-response analysis of inhaled formaldehyde's ability to cause nasal epithelial tumors as seen in rats at life-long high air concentrations (Conolly, *et al.*, 2003, 2004). Computerized models of airflow in rat and human nasal cavities are used to estimate nasal uptake and indicate notable rat-human differences. Molecular aspects of formaldehyde toxicity were shown to occur disproportionately at high dose levels. The impact of these nonlinear patterns on the risk of nasal tumors was described by a model that shows human risks from ambient formaldehyde concentrations to be very small, far below what would be calculated by traditional methods.

The "omics" revolution (see related article) promises to even further expand our detailed understanding of the intricate and interlinked molecular and epigenetic processes that underlie toxicity. Ever-more sophisticated models are being developed. The future of dose-response analysis is to look beyond simply

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Modified from: Chemical Industry Institute of Toxicology. 1999. "Formaldehyde: Hazard Characterization and Dose-Response Assessment for Carcinogenicity by the Route of Inhalation." September 28.

# Bioinformatics

By Julie E. Goodman, Ph.D.

*Complex molecular changes in the biological response to toxic exposures can be measured and utilized through this emerging methodology.*

Biological systems respond to environmental chemicals,

*In the future, bioinformatics could theoretically contribute to individual “designer” risk assessments...*

events, and stresses by altering the quantity and/or chemical form of a large number of molecules, including their own DNA, RNA, and proteins. Owing to practical constraints,

traditional laboratory experiments, by design, examine only a limited number of biological responses. Recent technological advances, however, have made it possible to measure thousands of responses simultaneously. Bioinformatics is a tool that has been developed to find patterns among these thousands of responses and bring this information to bear on solving statistically complex biological problems.

Although bioinformatics includes numerous applications, those that could have the largest impact on risk sciences are “omics,” which are studies of complete sets of cellular molecules. Genomics is the study of the entire set of DNA molecules in a cell, transcriptomics is the study of the complete set of RNA molecules in a cell, proteomics is the study of protein synthesis and cell signaling, and metabonomics is the study of systemic biochemical profiles of low molecular weight compounds (see figure). To use proteomics as an example, a set of thousands of proteins can be measured in urine, blood, or even cell culture. A scientist could then distinguish the effects of a treatment, exposure, or disease by quantitating protein profiles in the different groups, creating what has been called a fingerprint or a biomarker. Different groups can be distinguished by determining which aspects of profiles in the same group are similar to each other but different from profiles in other groups. After

this occurs, unknown samples can be categorized based on the profile to which they are most similar. For example, once the profiles of lung cancer patients *vs.* those of healthy individuals have been compared, likelihood of lung cancer can be predicted in a person based on whether his or her profile is more similar to the patients’ or the healthy controls’ profiles. Furthermore, the current profile of a healthy individual could indicate whether that individual is likely to develop lung cancer in the future.

Beyond this, if a scientist categorizes certain molecules in specific biological pathways, bioinformatics can reveal which pathways (*vs.* individual molecules) are different between groups. In this way, “omics” can begin to address biological mechanisms and potentially identify a mode of action for certain toxins. If certain profiles are associated with specific adverse effects, chemicals can be screened for toxicity based on the profile they produce. This could eventually be applied to dose-response models that incorporate biochemical mechanisms of toxins. All of these tools can be applied in risk assessments.

Risk assessments are generally performed for one disease outcome at a time, but bioinformatics could dramatically change this approach to risk assessment. Profiles could be used in place of disease as the risk assessment endpoint, as the profile could be a more specific and earlier marker. Bioinformatics may also allow the division of the population into more and less susceptible subgroups based on specific “omic” profiles, and separate assessments could be performed for each group, reducing uncertainty in calculations. In the future, bioinformatics could theoretically contribute to individual “designer” risk assessments; a person’s “omic” profile could be entered into a model, and his or her personal risk assessment could be generated. A person could then choose to alter his or her behavior or lifestyle based on this profile to lower his or her individual disease risk.

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## “OMIC” TECHNOLOGIES

“Omic”	Biological System	Endpoint	Experimental System
Genomics	DNA	Single nucleotide polymorphisms	Microarrays
Transcriptomics	RNA	DNA transcription	Microarrays
Proteomics	Protein	Protein expression	Mass spectrometry
Metabonomics	Metabolites	Expression of low molecular weight molecules	NMR, HPLC, GC/MS

# Mode of Action in Cancer Risk Assessment

By Ari Schoen, M.S.

*Growing understanding of the mode of action of carcinogens is leading to more enlightened risk assessments for some chemicals.*

The relationship between animal data and human health risk assessment has been rocky, at best. Although risk assessors often rely heavily on information from animal studies, biological differences among species and the use of high experimental doses often lead to uncertainties that are not easily addressed by traditional risk assessment methodologies. In an attempt to keep pace with scientific advances, the U.S. EPA recently released its revised "Guidelines for Carcinogen Risk Assessment." These guidelines provide a basic framework for overcoming some of these uncertainties, and have reinforced a paradigm shift in which

*The flexibility offered in the new cancer guidelines to assess the human relevance of animal MOAs on a case-by-case basis is an important step forward in cancer risk assessment.*

positive cancer bioassays are no longer presumed to have low-dose human relevance. Rather, these results serve as a starting point for evaluating the human relevance of observed animal tumor(s) and selection of an appropriate dose-response model.

At the heart of this human relevance assessment is a comparative evaluation of the carcinogenic mode of action (MOA) in animals *vs.* humans. The EPA describes the MOA as "a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." Carcinogenic MOAs can be broadly divided into two types: those that involve direct DNA damage (and likely have a linear dose-response) and those that act through more indirect interactions, such as enhancement of cell proliferation. Chemicals that have a non-DNA reactive MOA likely have a nonlinear or threshold dose-response and may require a margin of exposure (MOE) analysis to best describe cancer risk.

Recent evaluations by the EPA and other scientific panels have formally recognized that certain chemicals that are animal carcinogens are not human carcinogens, based on divergent MOAs. For example, in rodents, a class of chemicals called peroxisome proliferators (PP) bind to a receptor called PPAR- $\alpha$ , which causes abnormal increases in cell proliferation and eventual liver tumors. Although humans have the PPAR- $\alpha$  receptor, they have substantially lower quantities compared to rodents, making humans resistant to PP-induced liver tumors. Because

PP-induced liver tumors have an MOA that is not relevant to humans, a quantitative carcinogenic evaluation is not appropriate.

In some cases, an MOA may be biologically plausible in humans, but improbable because of the high doses required to cause animal tumors. Several chemicals operate *via* an MOA described as "cytotoxicity followed by regeneration." This threshold phenomenon occurs when high experimental doses produce tissue death, and, in response, cells start to proliferate abnormally and tumors develop. Based on this MOA, the EPA departed from the traditional linear dose-response model and used an MOE approach to determine that chloroform is unlikely to pose a carcinogenic hazard to humans. This decision was based on liver tumors developing in rodents only at cytotoxic doses that were sufficiently above any reasonable human exposure.

The flexibility offered in the new cancer guidelines to assess the human relevance of animal MOAs on a case-by-case basis is an important step forward in cancer risk assessment. By discouraging the use of overly conservative default assumptions that tended to overestimate risk, and advocating the use of the best available scientific information, the EPA has enabled the development of the most scientifically robust health-based risk assessments.

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## References:

U.S. EPA. 2005. Guidelines for carcinogen risk assessment (Final). Risk Assessment Forum. Washington, D.C. March. EPA/630/P-03/001B.

## BY THE WAY...

**Contrary to current popular opinion, a recent study has found that intermittent sun exposure may actually increase survival from melanoma. Mechanistic hypotheses include sun exposure stimulating the vitamin D pathway and/or increasing DNA repair capacity.**

Source: Berwick, M., B.K. Armstrong, L. Ben-Porat, J. Fine, A. Kricker, C. Eberle, and R. Barnhill. 2005. Sun exposure and mortality from melanoma. *J. Nat. Cancer Inst.* 97 (3): 195-199.

## What's New at Gradient

### Upcoming Presentations

**Ann Arbor, MI. September 15-16, 2005. Teresa S. Bowers** will moderate a roundtable discussion at the "Calculation to Communication" symposium at University of Michigan's new Center for Risk Science and Communication.

**Philadelphia, PA. September 29, 2005. Eric L. Butler.** "Better Litigating Through Chemistry: A Case Study in the Use of Experts to Assist in the Allocation of Responsibility for the PCB Contamination at the Paoli Rail Yard," presentation to the Philadelphia Bar Association.

**Minneapolis, MN. October 3-6, 2005. Christopher M. Long.** "Measurement, Fate, and Exposure Potential of Ultrafine Particles in Indoor Air: Lessons Learned for Nanotechnology," poster session to be presented at the 2nd International Symposium on Nanotechnology and Occupational Health.

**Amherst, MA. October 19, 2005. David E. Langseth.** "Estimating the Timing of a Chlorinated Solvent Release: A Case Study," presentation at the Soils, Sediments, and Water conference.

**Baltimore, MD. November 13-17, 2005.** SETAC North America 26<sup>th</sup> Annual Meeting poster sessions:

- **Teresa S. Bowers, Jennifer K. Saxe, and Margaret C. Pollock.** "The Influence of Forage Area Geometry on Remedial Action Levels in Ecological Risk Assessment."
- **Jennifer K. Saxe, Manu Sharma, and Timothy J. Ward.** "Critical Analysis of the Proposed European Framework for Ecological Assessment of Pharmaceuticals."

- **Timothy J. Ward, Manu Sharma, Grace I. Kim, and Jennifer K. Saxe.** "Predicting Metal Bioavailability and Toxicity in Stream Bank Samples."

- **Timothy J. Ward** and William E. Robinson. "Artificial Selection for Cadmium Resistance in a Laboratory Population of *Daphnia magna*: Long-term Fitness Implications."

**Boston, MA. November 28-December 2, 2005. Barbara D. Beck, Melissa A. Hoffer, and Lorenz R. Rhomberg.** "Regulation of Nanotechnology in the Environment and Workplace: Comparative Approaches," presentation at the 2005 Materials Research Society Fall Meeting.

### Recent Articles

**Beck, B.D.** 2005. An evaluation of the EPA definition of risk assessment. *Belle Newsletter* 13(1):4-6.

**Shifrin, N.S.** 2005. Pollution management in the twentieth century. *J. Environ. Eng.* 131:676-691.

**Wait, A.D.** 2005. The impact of the Data Quality Act on environmental regulatory science. In Proceedings of the 2005 NGWA Ground Water and Environmental Law Conference, July 21, 2005. Baltimore, MD. 16-29.

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## Frontiers in Dose-Response Modeling

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fitting curves to tumor counts, and instead to incorporate into dose-response analysis the underlying network of molecular interactions in normal tissue and how it is perturbed by exposure to chemical and other stressors.

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### References:

Conolly R.B., J.S. Kimbell, D. Janszen, P.M. Schlosser, D. Kalisak, J. Preston, and F.J. Miller. 2003. Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. *Toxicological Sciences* 75(2):432-447.

Conolly R.B., J.S. Kimbell, D. Janszen, P.M. Schlosser, D. Kalisak, J. Preston, and F.J. Miller. 2004. Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. *Toxicological Sciences* 82(1):279-296.

# Guest Editorial: Hormesis

By Edward J. Calabrese, Ph.D.

*The seemingly unconventional concept of hormesis is gaining some momentum in the toxicological mainstream.*

Hormesis is a dose-response phenomenon characterized by a low-dose stimulation and a high-dose inhibition, appearing as either an inverted U- or J-shaped dose curve depending on the endpoint measured. Relegated to the trash bins of pharmacological and toxicological histories, hormesis has made a Sea Biscuit-like comeback in recent years to gain considerable attention.

*Relegated to the trash bins of pharmacological and toxicological histories, hormesis has made a Sea Biscuit-like comeback in recent years to gain considerable attention.*

The principal motive behind the re-exploration of hormesis has been an industry-encouraged hope to find a toxicologically valid way to challenge the several decades long decision by the U.S. EPA to use Linear Non-Threshold (LNT) modeling as the default in cancer risk assessment. Given limited experimental data in animal bioassays, it has been very difficult to differentiate the linear and threshold models, leading the EPA automatically to default to the LNT. Hormesis, with its J-shape, was thought to offer a better and more likely means to challenge LNT and to lead to a rejection, on toxicological grounds, of the LNT.

To the surprise of most, when scrutiny was given to below no observed adverse effect level (NOAEL) responses, the hormetic response was observed to reliably occur, regardless of biological model, the endpoint measure or chemical studied. Even more so, in valid head-to-head comparisons, the hormetic model notably and easily out-did its rival models. Continuing assessments with other biological systems have provided

substantial support and generalizability to these conclusions, leading to the incorporation of the concept of hormesis into major toxicological textbooks, featured symposia at major society meetings and even the creation of a new society (*i.e.*, International Hormesis Society – [www.hormesisociety.org](http://www.hormesisociety.org)).

This decade-long rise in recognition raises many questions and poses many challenges to the toxicological and risk assessment communities. The most obvious is how could a major discipline like toxicology, which now has over 10,000 practitioners worldwide, have continued to miss and misunderstand the most fundamental nature of their discipline – the nature of the dose-response. How could regulatory agencies, guided by numerous National Academy of Science committees, likewise have missed and essentially totally ignored this phenomenon?

These are important questions for the toxicological and risk assessment communities to address and not to simply pass off as professional evolution. Other critical questions must consider how the fields of toxicology and risk assessment will address and incorporate the hormetic dose-response model into their practices.

Despite all the advances made by the concept of hormesis over the past decade, the field of toxicology remains a high-dose/few-doses discipline that is disconnected from human reality. It is time for multiple independent groups (*e.g.*, the Society of Toxicology, the Society of Environmental Toxicology and Chemistry, the Society for Risk Analysis, as well as appropriate industry associations) to develop independent assessments of hormesis. These would aid in the development of a broader consensus on the topic and perspective on how to proceed.

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## In the next issue:

*Overview of Dietary Supplements*

*Documenting Benefits of Use*

*Limitations of Test Methods*

*Guest Editorial: Supplement Labeling Liability*

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