

# THE BIOLOGICAL-ACTIVITY TESTING AND MODELING LABORATORY

A PLACE WHERE BIOLOGY, CHEMISTRY, AND MATHEMATICS CONVERGE

## General Information:

The Biological-Activity Testing and Modeling Laboratory is housed in the York Veterinary Teaching Hospital at The University of Tennessee Agricultural Institute.

The laboratory is coordinated by Prof. T. W. Schultz and includes technicians, graduate students, and collaborating scientists.

The mission of the laboratory includes the: 1) development, validation, and use of rapid and inexpensive assays for the evaluation of environmental toxicity; 2) development, validation, and use of structure-activity models for predicting toxic potencies, and 3) advancement of the basic understanding of toxicology.

The laboratory has academic ties to the Department of Comparative Medicine, College of Veterinary Medicine and Department of Ecology and Evolutionary Biology, College of Arts and Sciences.

The laboratory has research ties with Waste Management Research and Education Institute and the Center for Environmental Biotechnology. In addition, the laboratory has cooperative arrangements with several foreign research groups.

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## How to Contact Us:

If you have questions, please contact Prof. Schultz.

## ***Toxicity Test Systems***

The Biological-Activity Testing and Modeling Laboratory is the world leader in toxicological work with the freshwater ciliate *Tetrahymena pyriformis*. Current *Tetrahymena* toxicity assessment systems being used in the laboratory include the population growth inhibition assay {Schultz, T.W. 1997. TETRATOX: *Tetrahymena pyriformis* population growth impairment endpoint-A surrogate for fish lethality. Toxicol. Methods 7: 289-309.}, ciliate mortality assay {Schultz, T.W., Bryant, S.E. and Lin, D.T. 1994. Structure-toxicity relationships for *Tetrahymena*: Aliphatic aldehydes. Bull. Environ. Contam. Toxicol. 52: 279-285}, ciliate phototoxicity assay {Sink, G.D., Schultz, T.W. and Hunter, R.S. 1997. UVb-induced toxicity of PAHs: Effects of substituents and heteroatom substitution. Bull. Environ. Contam. Toxicol. 59: 1-8}.

Currently recombinant yeast (*Saccharomyces cerevisiae*) strains for assaying endocrine disruption are being used in the Biological-Activity Testing and Modeling Laboratory. These incorporate either the human estrogen receptor or the aryl hydrocarbon receptor. Both systems use a *lac-Z*-based reporter. In addition, *Lux*-based reporter strains are being developed in conjunction with the Center for Environmental Biotechnology.

Current prokaryotic toxicity assessment systems being used in the Biological-Activity Testing and Modeling Laboratory include the *Vibrio fischeri* (MICROTOX™) systems as well as other bacterial bioluminescent assays developed in conjunction with the Center for Environmental Biotechnology.

## ***TETRATOX Database***

The TETRATOX database is a collection of toxic potency data for more than 2,100 industrial organic compounds of which more than 1,500 have been published. The assay {Schultz, T.W. 1997. TETRATOX: *Tetrahymena pyriformis* population growth impairment endpoint-A surrogate for fish lethality. Toxicol. Methods 7: 289-309} is a short-term, static protocol using the common freshwater ciliate *Tetrahymena pyriformis* (strain GL-C). The 50% impairment growth concentration (IGC<sub>50</sub>) is the recorded endpoint.

Briefly, cultures are reared in 50 ml of a semi-defined medium in 250 ml Erlenmeyer flasks. In the TETRATOX assay, a range-finding assay followed by three replicate definitive tests is performed on each test material. Definitive test replicates consist of a minimum of five different concentrations of each test material with duplicate flasks of each concentration. Thus, a minimum of 30 data points comprises each analysis. Duplicate controls, which have no test material but were inoculated with *T. pyriformis*, and a blank are used to provide a measure of the acceptability of the test by indicating the suitability of the medium and test conditions, and a basis for interpreting data from other treatments. While several different time points were used over the course of more than 15 years, each was used with a medium and test condition regime to allow for 8 to 9 cell cycles in controls. Duplicate flasks are inoculated with an initial density of  $\approx 2,500$  cells/ml with log-growth-phase ciliates. Following  $\approx 40$  hours of incubation at  $27 \pm 1^\circ\text{C}$ , population density is measured spectrophotometrically and 50% effect levels determined.

The 50% inhibitory growth concentration in mg/l (IGC<sub>50</sub>) and the 95% fiducial interval are determined for each test compound. The IGC<sub>50</sub> is calculated by probit analysis using the percent control-normalized absorbance as the dependent variable and the toxicant concentration in mg/l as the independent variable. Both the slope and intercept of the probit regression equation is recorded as well as the Chi-squared value. The latter is an indicator of the fit of the data to the probit model. Normally the Chi-squared value is greater than 0.9.

In the majority (> 85%) of the more than 2,100 tests completed, it is possible to generate a statistically valid concentration-response curve. However, some chemicals (e.g., neutral organics with 1-octanol/water partition coefficients greater than 5.0) are not toxic at saturation; others do not attain the measured 50% effect endpoint at saturation, and still other chemicals (e.g., highly bioreactive toxicants) have a very narrow concentration-response range that precludes proper statistical analyses.

## ***Structure-Toxicity Modeling***

Structure-toxicity modeling work in the Biological-Activity Testing and Modeling Laboratory focuses on acute and aquatic toxicity and involves endpoints representing different trophic levels. Current model development involves bacterial, protozoa, algae, daphnid, and fish endpoints. The modeling of relationships between acute and chronic endpoints is also being examined.

The premise of structure-toxicity modeling is that changes in the structure of a chemical may influence the type and potency of its toxic action. This principle is a continuation of the concept that all chemical-toxicological effects are the result of an interaction between the chemical and one or more components of the living system. These interactions may be reversible or covalent in character. Components of living systems that are capable of reversible or covalent binding with chemicals are referred to as molecular sites of action.

The objectives of structure-toxicity modeling are two-fold. First, determine as accurately as possible the limits of variation in the structure of a chemical that are consistent with the production of a specific biological effect (i.e., can a chemical elicit a specific biological endpoint). Second, define the ways in which alterations in structure and thereby the overall properties of a compound influence potency. If enough data related to a specific biological effect becomes available, a hypothesis can be developed regarding the molecular basis of interaction between the toxicant and its active site. This is indeed the case for molecules that are specific covalent reacting. However, a non-covalent narcosis or anesthetic response, especially elicited by neutral organic molecules, represents a nonspecific effect, in which no moiety or molecular substructure requirement is implicated and toxic potency is totally dependent on the hydrophobicity of the entire molecule.

The development of a structure-toxicity model requires three components. A data set is required that provides toxicity for a group of chemicals. This group is defined typically by some selection criteria. Also required of this group of chemicals are property data (i.e., descriptors). These two data arrays then must be related usually via a statistical analysis method. Subsequent to initial model development, additional data are used as a means to define the scope and limitations of the preliminary model and to develop an improved and more robust predictive model. Collectively, these latter steps are referred to often as model validation.

Structure-toxicity models provide a rational, rapid, and inexpensive means of predicting toxic effects. There are two types of structure-toxicity models. The first type is qualitative. A qualitative relationship is a general rule-type of model that provides either yes/no, or at best,  $A > B > C$  information. It can be developed with lower quality non-continuous-type data. The second type is quantitative. It can only be developed using continuous valued data and provides a mathematical model that describes toxic potency based on descriptors of the chemicals.

Chemical descriptor(s) embody empirical, quantum chemical, or non-empirical parameters. Empirical descriptors maybe measured or estimated and include physicochemical properties. Physicochemical properties include hydrophobic, electronic, and steric descriptors. Non-empirical descriptors are typically structural properties based

on topological or graph theory as such they are 2-D indices. Quantum chemical descriptors are based on an optimized 3-D structure of molecules.

Properties of compounds are related to their molecular structure. While chemicals are normally thought of in a two-dimensional structure, toxicity is a manifestation of the three-dimensional structure of a molecule. Properties also are typically manifestations of three-dimensional structure. Thus, property-based models are generally preferred. Chemical descriptors may be based on atom, substituent, or whole molecule. While atom-type descriptors and substituent constants have been used in modeling toxicity, the more global whole-molecule descriptors are preferred.

Methods used in the development of structure-toxicity modeling are of two sorts; correlative and pattern recognition. The most common correlative method is regression analysis. Regression analyses are quantitative in character. Regression analysis is a simple approach that leads to a result that is easy to understand and often provides a mechanistic basis for the modeled toxicity. For this reason, most quantitative models in toxicology are derived using regression analysis. In contrast, pattern recognition techniques are qualitative in character. They are a complex approach, the results of which are often difficult to interpret.

Therefore, the most useful structure-toxicity models are derived using regression analysis and continuously valued data for both toxic potency and 3-D, whole molecule-based properties. Such regression models are based on the concept that changes in a property (e.g., partition coefficient) will be reflected in changes in free energy if such correlations are made on a double logarithmic scale. These models are extra-thermodynamic relationships.

Over the past fifteen years, schemes for structure-toxicity modeling have changed. The simple congeneric series approach was replaced by a chemical class-based approach. The latter approach was replaced by the mechanism of toxic action approach. More recently a descriptor-limited, multiple regression approach or the response-surface has been explored.

Regardless of the approach, the limiting factor in the development of toxicological models has been and still remains the availability of high quality experimental toxicity data. For this reason the overriding goal of the Biological-Activity Testing and Modeling Laboratory has been the production of high quality toxicity data.

## ***Current Projects***

On going projects in the Biological-Activity Testing and Modeling Laboratory include:

- Continued development of the TETRATOX database
- Standardization of the *T. pyriformis* population growth assay
- Development and validation of structure-toxicity models
- Development and validation of knowledge-based expert systems to predict toxic endpoints
- Cellular and molecular aspects of bioreactive mechanisms of toxic action
- Estimation of no observable effects concentrations from other endpoints
- Bioactivities of polycyclic aromatic hydrocarbons and metabolites
- Development and validation of the *Lux*-based reported systems for selected toxicological endpoints

## ***Recent Publications***

Peer-reviewed publications authored by members of the Biological-Activity Testing and Modeling Laboratory number over 200. These include manuscripts on: 1) development, standardization, and validation of methods for toxic hazard assessment; 2) structure-activity relationships for toxic endpoints and industrial organic chemicals, and 3) elucidating mechanism of toxic action.

- Kaiser, K.L.E., Dearden, J.C., Klein, W. and Schultz, T.W. 1999. A note of caution to users of ECOSAR. *Water Quality Research Journal Canada* 34: 179-182.
- Swann, J.M., Kennedy, J.R. and Schultz, T.W. 1999. Evaluation of two *in vitro* ciliated epithelial systems, dog trachea and frog palate, for potential as screens for sensory irritation. *In Vitro and Molecular Toxicology* 12: 17-32.
- Schultz, T.W. and DeWeese, A.D. 1999. Structure-toxicity relationships for selected lactones to *Tetrahymena pyriformis*. *Bulletin of Environmental Contamination and Toxicology* 62: 463-468.
- Akers, K.S., Sinks, G.D. and Schultz, T.W. 1999. Structure-toxicity relationships for selected halogenated aliphatic chemicals. *Environmental Toxicology and Pharmacology* 7: 33-39.
- Schultz, T.W. and Cronin, M.T.D. 1999. Response-surface analyses for toxicity to *Tetrahymena pyriformis*: Reactive carbonyl-containing aliphatic chemicals. *Journal of Chemical Information and Computer Sciences* 39: 304-309.
- Muccini, M., Layton, A.C., Sayler, G.S. and Schultz, T.W. 1999. Aquatic toxicities of halogenated benzoic acids to *Tetrahymena pyriformis*. *Bulletin of Environmental Contamination and Toxicology* 62: 616-622.
- Bearden, A.P., Sinks, G.D. and Schultz, T.W. 1999. Acclimation to sublethal exposure to a model nonpolar narcotic: Population growth kinetics and membrane lipid alterations in *Tetrahymena pyriformis*. *Aquatic Toxicology* 46: 11-21.
- Layton, A.C., Gregory, B.W., Schultz, T.W. and Sayler, G.S. 1999. Validation of genetically engineered bioluminescent surfactant resistant bacteria as toxicity assessment tools. *Ecotoxicology and Environmental Safety* 43: 222-228.
- Bearden, A.P., Sinks, G.D., Vaes, W.H.J., Ramos, E.U., Hermens, J.L.M. and Schultz, T.W. 1999. Bioavailability, biodegradation, and acclimation of *Tetrahymena pyriformis* to 1-octanol. *Ecotoxicology and Environmental Safety* 44: 86-91.
- Schultz, T.W. 1999. Structure-toxicity relationships for benzenes evaluated with *Tetrahymena pyriformis*. *Chemical Research in Toxicology* 12:1262-1267.
- Seward, J.R. and Schultz, T.W. 1999. QSAR analyses of the toxicity of aliphatic carboxylic acids and salts to *Tetrahymena pyriformis*. *SAR QSAR in Environmental Research* 10: 557-567.
- Sanseverino J., Menn, F.-M., Gregory, B., Sampsel, E., Shi, Z., Ghosh, M., Schultz, T.W. and Sayler, G.S. 1999. Bioavailability, estrogenicity, and toxicity of surfactant-PCB mixtures after biodegradation and photolysis. In: *Bioremediation of Nitroaromatic and Haloaromatic Compounds*. (B.C. Alleman and A. Leeson, eds.) pp. 155-160.
- Bearden, A.P., Sinks, G.D. and Schultz, T.W. 1999. Population growth kinetics of *Tetrahymena pyriformis* exposed to selected electrophiles. In: Henshel, D.S., Black, M.C. and Harrass, M.C. (eds.) *Environmental Toxicology and Risk Assessment: Standardization of Biomarkers for Endocrine Disruption and Environmental Assessment: Eighth Volume*,

- ASTM STP 1364*. American Society for Testing and Materials, West Conshohocken, PA. pp. 319-328.
- Schultz, T.W., Sinks, G.D. and Seward, J.R. 2000. Estrogenicity of benzophenones evaluated with a recombinant yeast assay: Validation of a rules-based system of prediction. *Environmental Toxicology and Chemistry* 19: 301-304.
- Leblond, J.D., Applegate, B.M., Menn, F.-M., Schultz, T.W. and Sayler, G.S. 2000. Structure-toxicity assessment of metabolites of the aerobic bacterial transformation of substituted naphthalenes. *Environmental Toxicology and Chemistry*. 19: 1235-1246.
- Schultz T.W. and Seward, J.R. 2000. Health-effects related structure-toxicity relationships: a paradigm for the millennium. *The Science of the Total Environment* 249: 73-84.
- Cronin, M.T.D., Bower, G.S., Sinks, G.D. and Schultz, T.W. 2000. Structure-toxicity relationships for aliphatic compounds encompassing a variety of mechanisms of toxic action to *Vibrio fischeri*. *SAR QSAR in Environmental Research* 11: 301-312.
- Seward, J.R., Sinks, G.D. and Schultz, T.W. 2000. Population growth kinetics of *Tetrahymena pyriformis* exposed to select pyridines. *European Journal of Protistology* 36: 139-149.
- Dimitov, S.D., Mekenyan, O.G. and Schultz, T.W. 2000. Interspecies modeling of narcotic toxicity in aquatic animals. *Bulletin of Environmental Contamination and Toxicology* 65: 399-406.
- Niculescu, S.P., Kaiser, K.L.E. and Schultz, T.W. 2000. Modeling the toxicity of chemicals to *Tetrahymena pyriformis* using molecular fragment descriptors and probabilistic neural networks. *Archives of Environmental Contamination and Toxicology*. 39: 289-298.
- Layton, A.C., Gregory, B.W., Seward, J.R., Schultz, T.W. and Sayler, G.S. 2000. Mineralization of steroidal hormones in municipal sewage treatment systems in Tennessee USA. *Environmental Science and Technology*. 34: 3925-3931.
- Schultz, T.W., Sinks, G.D. and Cronin, M.T.D. 2000. Effects of substituent size and dimensionality on potency of phenolic xenoestrogens. *Environmental Toxicology and Chemistry* 19: 2637-2642.
- Schultz, T.W. and Seward, J.R. 2000. Dimyristoyl phosphatidylcholine /water partitioning-dependent modeling of narcotic toxicity to *Tetrahymena pyriformis*. *Quantitative Structure-Activity Relationships* 19: 339-344.
- Seward, J.R., Cronin, M.T.D. and Schultz, T.W. 2001. Structure-toxicity analyses of *Tetrahymena pyriformis* exposed to pyridine – An examination into extension of surface-response domains. *SAR QSAR in Environmental Research* 11: 489-512.
- Leblond, J.D., Schultz, T.W. and Sayler, G.S. 2001. Observations on the preferential biodegradation of selected components of polyaromatic hydrocarbon mixtures. *Chemosphere* 42: 333-343.
- DeWeese, A.D. and Schultz, T.W. 2001. Structure-activity relationships for aquatic toxicity to *Tetrahymena*: Halogen-substituted aliphatic esters. *Environmental Toxicology* 16: 54-60.
- Seward, J.R., Sinks, G.D. and Schultz, T.W. 2001. Reproducibility of toxicity across mode of toxic action the *Tetrahymena* population growth impairment assay. *Aquatic Toxicology* 53: 33-47.
- Sinks, G.D. and Schultz, T.W. 2001. Correlations of *Tetrahymena* and *Pimephales* toxicity: Evaluation of 100 additional compounds. *Environmental Toxicology and Chemistry* 20: 917-921.
- Cronin, M.T.D. and Schultz, T.W. 2001. Development of quantitative structure-activity relationships for the toxicity of aromatic compounds to *Tetrahymena pyriformis*:

- Comparative assessment of methodologies. *Chemical Research in Toxicology* 14:1284-1295.
- Cronin, M.T.D., Manga, N., Seward, J.R., Sinks, G.D. and Schultz, T.W. 2001. Parameterization of electrophilicity for the prediction of the toxicity of aromatic compounds. *Chemical Research in Toxicology* 14: 1498-1505.
- Cronin, M.T.D., Sinks, G.D. and Schultz, T.W. 2001. Modelling of toxicity to the ciliate *Tetrahymena pyriformis*: the Aliphatic Carbonyl domain. In: Rainbow, P.S., Hopkin, S.P. and Crane, M. (eds). *Forecasting the Environmental Fate and Effects of Chemicals*. John Wiley & Sons, Ltd. Chichester, UK pp. 113-124
- Schultz, T.W., Sinks, G.D. and Miller, L.A.. 2001. Population growth impairment of sulfur-containing compounds to *Tetrahymena pyriformis*. *Environmental Toxicology* 16: 543-549.
- Seward, J.R., Cronin, M. T.D. and Schultz, T. W. 2001. Effect of precision of molecular orbital descriptors on the modeling of toxicity of selected pyridines. SAR QSAR in Environmental. Research (in press).
- Seward, J.R., Hamblen, E.L. and Schultz, T.W. 2001. Regression comparison of *Tetrahymena pyriformis* and *Poecilia reticulata* toxicity. *Chemosphere* (in press).
- Schultz, T.W. 2002. Estrogenicity of biphenylols: Activity in the yeast gene activation assay. *Bulletin of Environmental Contamination and Toxicology* 68: 332-338.
- Ren, S. and Schultz, T.W. 2002. Identifying the mechanism of aquatic toxicity of selected compounds by hydrophobicity and electrophilic descriptors. *Toxicology Letters*. 129:151-160.
- Schultz, T.W. and Sinks, G.D. 2002. Effects of molecular structure on xenoestrogenic activity: Structural alerts for PAHs. *Environmental Toxicology and Chemistry* (in press).
- Patel, H., Schultz, T.W. and Cronin, M.T.D. 2002. Estimating DMPC partitioning. *THEOCHEM* (in press).
- Schultz, T.W., Sinks, G.D. and Cronin, M.T.D. 2002. Structure-activity relationships for gene activation estrogenicity: Evaluation of a diverse set of aromatic compounds. *Environmental Toxicology*. (in press).
- Bradbury, S.P., Russom, C.L., Ankley, G.T., Schultz, T.W. and Walker, J.D. 2002. QSARs for predicting ecological effects of organic chemicals. *Environmental Toxicology and Chemistry*. (in press).
- Layton, A.C., Sanserverino, J., Gregory, B.W., Ester, J.P., Sayler, G.S. and Schultz, T.W. 2002. *In vitro* estrogen receptor binding of PCBs: Measured activity and detection of hydroxylated metabolites in a recombinant yeast assay. *Toxicology and Applied Pharmacology* (in press).
- Schultz, T.W., Sinks, G.D. and Bearden-Lowit, A.P. 2002. Population growth kinetics and membrane lipid alterations in *Tetrahymena pyriformis* upon expose to pentachlorophenol. *Cell Biology and Toxicology* (in press).
- Aptula, A.O., Netzeva, T.I., Valkova, I.V., Cronin, M.T.D., Schultz, T.W., Kühne, R. and Schüürmann, G. 2002. Multivariate discrimination between modes of toxic action of phenols. *Quantitative Structure-Activity Relationships* (in press).
- Schaeffer, D.O. and Schultz, T.W. 2002. Biology and Diseases of Amphibians. In: Fox, J.G. (ed.) *Laboratory Animal Medicine*. 2<sup>nd</sup> Ed (in press).
- Walker J.D. and Schultz T.W. 2002. Structure activity relationships for predicting ecological effects of chemicals. In: Hoffman, D. and Barnett M. (eds.) *Ecotox Handbook* 2<sup>nd</sup> Ed. CRC Press (in press).