The Knoxville Workshops on Reactivity Toxicity: Relationships with Aquatic Hazard

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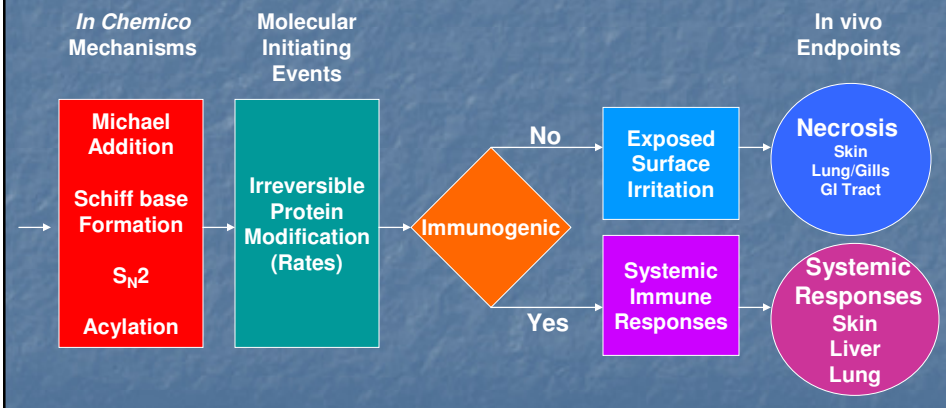
Workshop Goals

- Identify gaps in QSAR capabilities for modeling regulatory endpoints
- Develop a framework for modeling reactive toxicity
- Encourage the development of new, high quality databases for QSAR applications

Reactive Toxicity

- Involves the irreversible and often non-specific interaction of a xenobiotic chemical with endogenous molecules that include proteins, nucleic acids, and lipids
- Identified as the major gap in our ability to model regulatory endpoints

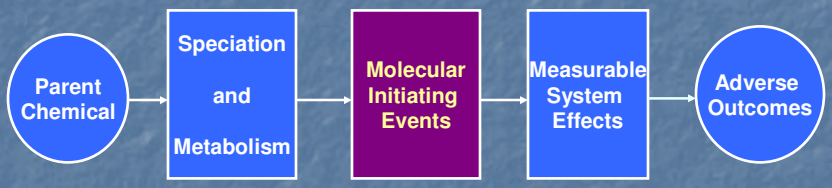
Primary Pathways for Reactive Toxicity from Soft Electrophiles



Why Reactive Toxicants in Aquatic Toxicity?

- "Nonspecific Narcosis" the QSARs of the 1980s
- Currently 100s of QSARs for such physical toxicity
- All fail to accurately modeling reactive chemicals
- Since FATS, little progress has been made in classifying or modeling reactive toxicants

Knoxville Workshop's Framework for Transparent QSAR Models



Rather than developing statistical models of complex endpoints, molecular initiating events are modeled as well-defined QSAR endpoints and are used to estimate the probabilities for important biological effects

Key Issues of the 1st & 2nd Knoxville Workshops

- Rules for chemical reactivity
- *In Chemico* Assays for reactive data
- Define the domains of reactivity
- Linking reactivity to risk assessment endpoints
- Development of an open source chemical evaluation platform

Rules for Chemical Reactivity

- The general rules of organic chemical reactions are a good starting point for identifying reactivity toxicity
- Mechanism-based Roberts's Rules of Chemical Reactivity

  (Aptula et al., 2005; Aptula and Roberts, in press)

In Chemico Assays

- Quantitative, rapid, inexpensive based on a series of model nucleophiles
- Verify mechanism-based rules of reactivity
- Define the application domain of a reactive mechanism
- Formulate a reactive profile (acrolein)
- Thiol assay (Schultz et al., 2005)
- Amine assay (under development)

Modeling Reactive Aquatic Toxicity

- Establish Plausible Molecular Initiating Events (Roberts's Rules)
- Design Database for Abiotic Binding Affinity/Rates (Thiol Binding EC50)
- Explore Correlations and Pathways to Downstream Effects (Regression Equations with TETRATOX Data)

In Chemico Thiol Reacivity Assay

- Abiotic spectrophotometric assay
- Measures % free thiol with GSH as model nucleophile
- Endpoint 50% effect concentration (mM)
- Calculated by probit analysis of concentrations-response data

Relationship of EC50 to Reaction Kinetics

Log (EC50) = 3.87 – 1.07 log (kGSH)

n = 26, s = 0.34, r^2 = 0.819, q^2 = 0.788

F= 109, relationship covers 4 log units

Relationship of Thiol Reactivity to Aquatic Toxicity

S_N2 (α-halo carbonyl compounds)

Log ($IGC50^{-1}$) = 1.13 (log $EC50^{-1}$) – 3.11

n = 20, s = 0.45, r^2 = 0.969, q^2 = 0.961
F= 568, relationship covers 9 log units

Relationship of Thiol Reactivity to Aquatic Toxicity

Michael Acceptors

Log ($IGC50^{-1}$) = 1.05 (log $EC50^{-1}$) + 1.53

n = 20, s = 0.39, r^2 = 0.975, q^2 = 0.973
F= 699, relationship covers 9 log units

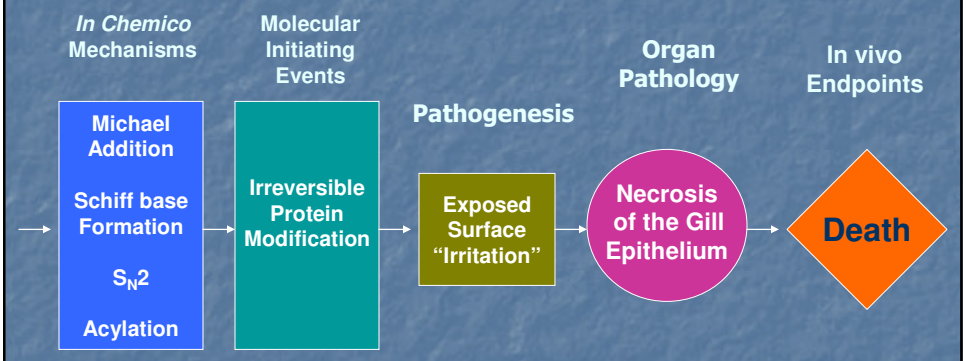
Relationship of Thiol Reactivity to Aquatic Toxicity

S_NAr electrophiles

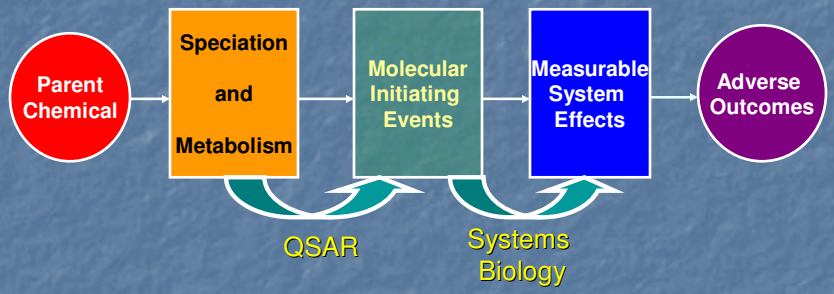
Log ($IGC50^{-1}$) = 0.79 (log $EC50^{-1}$) + 4.29

n = 13, s = 0.69, r^2 = 0.821, q^2 = 0.776
F= 51, relationship covers 6 log units

Major Pathway for Reactive Toxicity To Fish



Steps to the Development of QSAR for Reactive Toxicants



1. Establish Plausible Molecular Initiating Events
2. Design Database for Abiotic Binding Affinity/Rates
3. Explore Correlations/Pathways to Downstream Effects
4. Explore QSARs to Predict Initiating Event from Structure

Where are We?

- Roberts's Rules for Michael acceptors and S_NAr electrophiles
- Verified rules for Michael acceptors
- Shown a proof of concept that *in chemico* reactivity correlates with aquatic toxicity by reactive mechanism

Where We Need to Go

- Build *in chemico* reactivity data bases for other reactive mechanisms
- Develop *in chemico* assay for other nucleophiles
- Develop correlations between reactivity and other endpoints
- Predict reactivity from structure

QSARs for Reactivity from Structure

- Not a trivial task
- As a start we will provide measured thiol reactivity data for the Michael acceptor domain
- to include 70+ reactive and 30+ non-reactive compounds plus data for 30+ validation compounds
- ALL RESULTS MUST BE FREE & OPEN

KEY PUBLICATION

Schultz, T.W., Carlson. R.E., Cronin, M.T.D., Hermens, J.L.M., Johnson, R., O'Brien, P.J., Roberts, D.W., Siraki, A., Wallace, K.D. and Veith, G.D. 2006. A conceptual framework for predicting toxicity of reactive chemicals: Models for soft electrophilicity. SAR QSAR Environ Res (in press)

Biologically-based Determinants of Down Stream Effects

The Knoxville Framework

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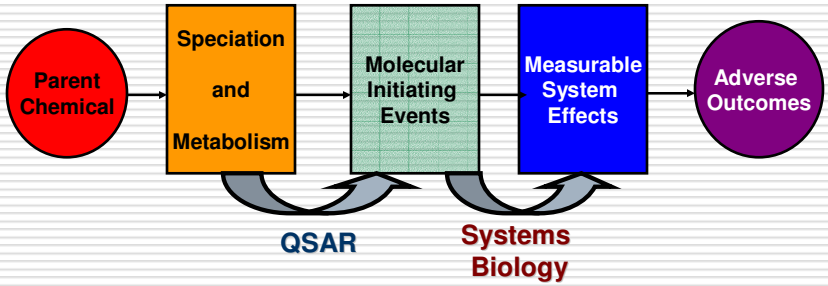
Steps to the Development of QSAR for Reactive Toxicants

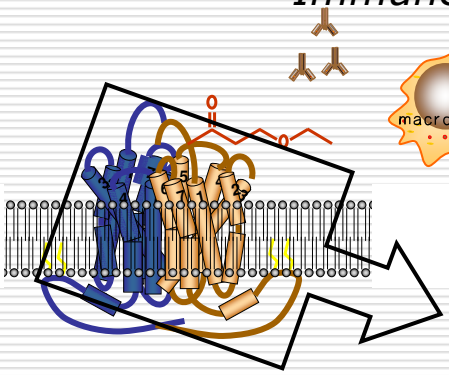


1. **Establish Plausible Molecular Initiating Events**
2. **Explore QSARs to Predict Initiating Event from Structure**
3. **Design Database for Abiotic Binding Affinity/Rates**
4. **Explore Correlations/Pathways to Downstream Effects**
   - **a) Reversible - receptor/non-receptor**
   - **b) Irreversible -**

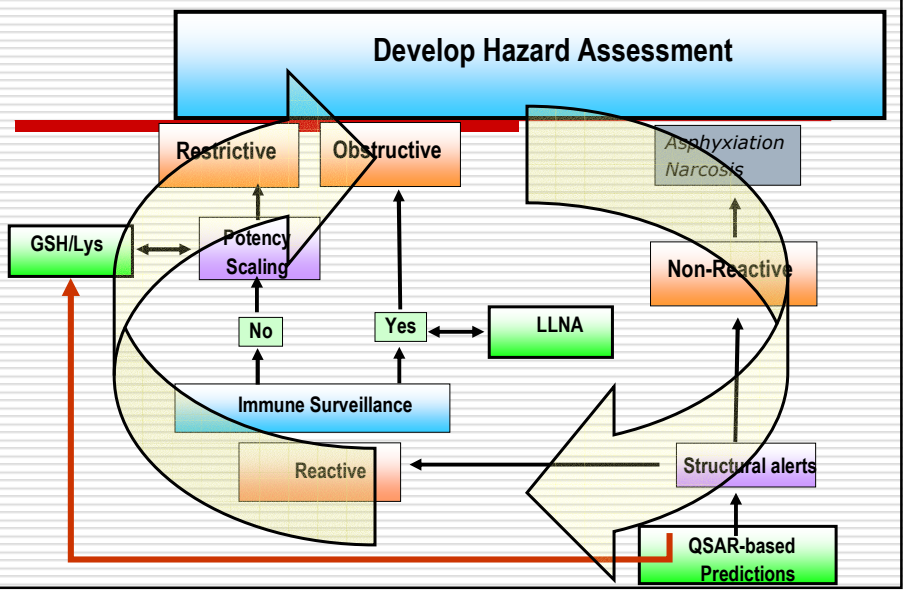
Biological Reactivity

- Assumptions of dosimetry
  - Species/route/duration
  - Chemical reactivity
- Irreversible protein modification
  - Oxidation
  - Adduct formation
- Stability of modified target

Irreversibly modified protein



The Biological Response



Conclusions

Molecular initiation is a function of:

- *Dosimetry*
- *Chemical reactivity*
  - *in silico/in chemico predictions*

Events downstream of molecular initiation are biologically-driven:

- *Identity and locale of biological target*
- *Stability of the modified biological target*
  - *Immune surveillance*
  - *Repair/replacement*

Individuality of the biological rules