Developing Molecular Design Guidelines for Reduced Toxicity

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Green Chemistry Principle #4

Chemical products should be designed to preserve efficacy of function while reducing toxicity and other environmental hazards.

Status of the field of safer chemical design

Founding the field
P. Anastas
J. Warner
S. DeVito
N. Anastas
R. Boethling
P. Wender
R. Garrett
E. Ariens

Industry practice
Dow
DuPont
Rohm & Haas
Sherwin Williams
Clarke
Procter & Gamble
.and others

Field development

Articles in RSC Journal of Green Chemistry (1999-present)

- Designing Safer Chemicals
- Toxicity
- Sustain./Education
- Other
- Alternative Processes
- Catalysis
- Solvents

(Winners of Presidential Green Chemistry Awards)
Roche Biochemical Pathways Wallchart
Pharmaceuticals vs Industrial Chemicals

- **Pharmaceuticals**
  - Designed to be biologically active
  - Performance criteria include a consideration of side effects
  - Produced in relatively small volumes
  - Well defined use scenarios

- **Industrial chemicals**
  - No intentional biological activity
  - Performance is generally separate from toxicity
  - Can be produced in multi-billion pound quantities
  - Extremely diverse use scenarios
The physiological “gates” of chemical exposure

1. Chemical is not bioavailable
2. Chemical rate of distribution in blood is reduced
3. Promote chemical detoxification and elimination
4. Prevent toxicodynamic interaction responsible for toxic effect.

Mechanistic layers of reducing toxicological hazard.

Physiochemical Properties Relevant to Toxicity

- Gastrointestinal absorption
- HERG K⁺ channel blockage
- Skin, Caco-2 and MDCK cell permeability
- Human serum albumin binding

- Vapor Pressure
- Melting & boiling point
- Solid-solid interactions

- Partition coefficients of water/gas, octanol/gas, hexadecane/gas, octanol/water
- Water solubility

- Dipole moment
- Polarizability
- HOMO/LUMO energies
- Polar surface area

- Molecular weight
- Globularity
- Surface Area
- Molecular Volume
Property-based guidelines for bioavailability

Absorption

- Organs:
  - GI tract
  - Skin
  - Lungs
  - Eyes

Substances:
- Acidic: pKa < 7
  - Unionized at pH > pKa
  - Un-ionized at pH > pKa
- Basic: pKa > 7
  - Lipid soluble
  - Absorbed across intestine membrane into blood

Chemical processes:
- Lipid soluble absorption
- Unionized at pH > pKa
- Acidic or basic substances

Organic structures:
- Upper Respiratory Tract:
  - Nasal Cavity
  - Pharynx
  - Larynx
- Lower Respiratory Tract:
  - Trachea
  - Primary bronchi

Anatomical details:
- Skin:
  - Skin surface
  - Sweat pore
  - Capillaries
  - Nerve ending
  - Adipose tissue
  - Venule
  - Venule

Green Chemistry:
- Center for Green Chemistry & Green Engineering at Yale
Physicochemical properties that affect bioavailability

- **Absorption organs**
  - Water solubility
  - Molecular size<br>  - Vapor pressure < 0.001 mmHg

- **GI tract**
  - Molecular size < 500 Da
  - Log $P_{ow}$ 0 - 5
  - Non-ionization at GI pH

- **Eyes**
  - Particle size < 5 μm
  - Molecular size < 400 Da
  - Vapor pressure < 0.001 mmHg

- **Skin**
  - Molecular size < 400 Da
  - Log $P_{ow}$ 0 - 6
  - Presence of solvents
  - Ionization (polar, ionized)

Lipinski rules for drug likeness (oral bioavailability)

Lipinski, 1997
~90% of drugs on the market have the following properties in common:

**Lipinski’s Rules for Druglikeness**
1. Not more than 5 hydrogen bond donors
2. Not more than 10 hydrogen bond acceptors
3. Molecular weight under 160-480 D
4. Octanol-water coefficient (logP) < 5
5. 20-70 atoms
6. Molecular refractivity from 40-130 m³/mol.
7. At least one N or O
8. Less than 6 rings

Reducing Distribution & Storage

- **Volume of Distribution** the theoretical volume of fluid into which the total xenobiotic administered would have to be diluted to produce a certain observed concentration in the plasma.

- $V_D = \text{total amount of drug in body}$
  - concentration of toxicant in blood

- Controlled by water solubility

- Special membranes, e.g. blood brain barrier

- Binding to blood proteins

- Storage in liver, kidneys, bones & lipids
Reducing bioactivation

CYT P-450 metabolism

\[ \Delta G_{\text{desolv}} = -0.025 \text{ SASA} \]
\[ \Delta G_{\text{part}} = -RT \ln P_{o/w} \]

\[ \Delta G_{\text{bind}} = 2.3 \cdot RT \ln(K_m) \]
\[ \pi-\pi \text{ stacking, H-bond donors, molecular planarity (area/depth}^3) \]

Turnover:
\[ k_{\text{cat}} = \frac{V_{\text{max}}}{[E_t]} \]
\[ = \frac{RT}{N \exp(\Delta S^*/R)} \exp(-\Delta H^*/RT) \]
\[ \Delta H \text{ of hydrogen-abstraction, ionization potential and/or } E_{\text{HOMO}} \]
Reducing bioactivation

• Factors that have been shown to affect bioactivation:
  – Frontier orbital energies
  – Steric hindrance of reactive functional groups
  – Molecular shape
  – Redox potential
Minimizing interaction with biomolecules

• Biomolecules of interest:
  – Proteins
  – DNA/RNA
  – Enzymes (suicide inhibition)
  – Receptors
  – ...

Mutagenicity and carcinogenicity of halogenated olefins were associated with the two-center bond energies of the corresponding epoxides. Thus when this energy falls between -14.1 eV and -12.9 eV, the epoxides formed are highly toxic.

The following epoxides fall outside of that range, and were tested in-vitro to be non-oncolgenic.

\[
\begin{align*}
\text{H}_2\text{C} & \text{Cl} & \text{F} & \text{Cl} & \text{F} & \text{Cl} & \text{F} & \text{H} & \text{Cl} & \text{Cl} \\
-14.38 \text{ eV} & 15.44 \text{ eV} & 15.47 \text{ eV} & 14.83 \text{ eV} & 14.73 \text{ eV}
\end{align*}
\]

Jones, R. B.; Mackrodt, W. C.; Biochem. Pharmac. 32, 2359-2362.
Ab initio molecular modeling of epoxides

What method should be used for calculation?

TS $\Delta G^\ddagger$ for Sn2 epoxide opening

LUMO orbital
Property differences

**dE-MP2**
- Positive: [Graph]
- Negative: [Graph]

**Charge C (unsub)**
- Positive: [Graph]
- Negative: [Graph]

**Olefins**
- Mutagenicity: [Graph]
- Dipole (D): [Graph]
- Positive: [Graph]
- Negative: [Graph]

**Epoxide**
- Mutagenicity: [Graph]
- Dipole (D): [Graph]
- Positive: [Graph]
- Negative: [Graph]
Crossroads of computational chemistry and toxicology
Is it feasible to develop one combined set of design guidelines for particular groups of species?
Strategy for developing combined design guidelines

Mechanistic & Statistical analysis of in-vivo toxicity data and physical/molecular properties

- not QSAR
- not based on structural alerts
- not “predictive”, but a design guideline
Design guidelines for reduced acute aquatic toxicity

Fathead minnow
LC$_{50}$, 96-h assay

Japanese medaka
LC$_{50}$, 96-h assay

*Daphnia magna*
EC$_{50}$, 48-h assay

*P. subcapitata*
EC$_{50}$, 72-h

U.S. E.P.A.
671 chemicals

Japan Ministry of Environment
285 chemicals

363 chemicals

300 chemicals

4 categories based on EPA thresholds of concern level

- LC$_{50}$/EC$_{50}$: 0 – 1 mg/kg
- LC$_{50}$/EC$_{50}$: 1 – 100 mg/kg
- LC$_{50}$/EC$_{50}$: 100 – 500 mg/kg
- LC$_{50}$/EC$_{50}$: > 500 mg/kg
Acute Aquatic Ecotoxicity

Boxplots help explore differences in distributions of properties by toxicity group.

<table>
<thead>
<tr>
<th></th>
<th>Fathead minnow</th>
<th>Jap. medaka</th>
<th>Daphnia magna</th>
</tr>
</thead>
<tbody>
<tr>
<td>logP&lt;sub&gt;(o/w)&lt;/sub&gt;</td>
<td>3.66, 2.23, 1.38, 0.22</td>
<td>3.87, 2.52, 1.70</td>
<td>3.56, 2.22, 0.86, -0.07</td>
</tr>
<tr>
<td>LUMO (eV)</td>
<td>-0.301, 0.186, 0.402, 2.59</td>
<td>-0.069, 0.176, 0.544</td>
<td>-0.065, 0.184, 0.181, 0.20</td>
</tr>
<tr>
<td>ΔE (eV)</td>
<td>9.10, 9.33, 10.0, 12.4</td>
<td>8.99, 9.40, 9.11</td>
<td>8.88, 9.51, 10.5, 11.8</td>
</tr>
</tbody>
</table>

Median value:

Level of concern for acute aquatic toxicity
ΔE (eV) vs logP (o/w)

(a) Mean acute toxicity value
Fathead minnow, Jap. medaka, D. Magna

(b) Acute toxicity value
Pseudokirchneriella subcapitata

End Disr | logP(o/w) | dE (eV)
----------|-----------|------
BPA       | 1.10      | 3.48
phthalate | -4.47     | 4.45
atrazine  | 1.18      | 5.62
PBDE      | 6.33      | 4.36
Identifying key properties: statistically and mechanistically

- \( \log P_{(o/w)} \)
- Frontier orbital energies

Nucleophiles in the body:
Protein residues (e.g. cysteine)
DNA bases
Thiols (e.g. glutathione)

Electrophiles in the body:
phosphates
sugars

Therefore **low** LUMO energies and **high** HOMO energies promote chemical reactivity with biological macromolecules.
### Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Fathead (96-h)</th>
<th>minnow (96-h)</th>
<th>J. killifish (96-h)</th>
<th>Daphnia (48-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% “desirable” compounds captured in logP(o/w) &lt; 2 and dE &gt; 9</td>
<td>88%</td>
<td>75%</td>
<td>92%</td>
<td></td>
</tr>
<tr>
<td>% “undesirable” compounds captured in logP(o/w) &lt; 2 and dE &gt; 9</td>
<td>38%</td>
<td>26%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>Mean LC&lt;sub&gt;50&lt;/sub&gt; or EC&lt;sub&gt;50&lt;/sub&gt; of compounds with logP(o/w) &lt; 2 and dE &gt; 9 (mg/L)</td>
<td>2265</td>
<td>3151</td>
<td>105.2</td>
<td></td>
</tr>
<tr>
<td>Mean LC&lt;sub&gt;50&lt;/sub&gt; or EC&lt;sub&gt;50&lt;/sub&gt; of all compounds in data set (mg/L)</td>
<td>969</td>
<td>1172</td>
<td>39.7</td>
<td></td>
</tr>
<tr>
<td>Median LC&lt;sub&gt;50&lt;/sub&gt; or EC&lt;sub&gt;50&lt;/sub&gt; of compounds with logP(o/w) &lt; 2 and dE &gt; 9 (mg/L)</td>
<td>135</td>
<td>47.2</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Median LC&lt;sub&gt;50&lt;/sub&gt; or EC&lt;sub&gt;50&lt;/sub&gt; of all compounds in data set (mg/L)</td>
<td>21.55</td>
<td>14.8</td>
<td>4.1</td>
<td></td>
</tr>
</tbody>
</table>

Chronic Aquatic Toxicity Design Guidelines

<table>
<thead>
<tr>
<th>Cat</th>
<th>EPA Concem level</th>
<th>Lower limit (NOEC mg/L)</th>
<th>Upper limit (NOEC mg/L)</th>
<th>Number of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High</td>
<td>0.1</td>
<td>10</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>0.1</td>
<td>10</td>
<td>333</td>
</tr>
<tr>
<td>3</td>
<td>Low</td>
<td>0.1</td>
<td>10</td>
<td>92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>LB value</th>
<th>U value</th>
<th>NOEC (mg/L)</th>
<th>Median NOEC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2N-kHa</td>
<td>7803-57-8</td>
<td>4170-96-3</td>
<td>0.073 mg/L</td>
<td>0.02 mg/L</td>
</tr>
<tr>
<td>117-81-7</td>
<td>10 mg/L</td>
<td>3319-31-1</td>
<td>56 mg/L</td>
<td></td>
</tr>
<tr>
<td>0548-65-3</td>
<td></td>
<td></td>
<td>30 mg/L</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EPA level of concern</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Mean NOEC (mg/L)</th>
<th>Median NOEC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All data</td>
<td>74</td>
<td>206</td>
<td>46</td>
<td>9.55</td>
<td>0.48</td>
</tr>
<tr>
<td>logP(o/w) &lt; 2 &amp;</td>
<td>6</td>
<td>56</td>
<td>29</td>
<td>26.67</td>
<td>4.2</td>
</tr>
<tr>
<td>dl(AM1) &gt; 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>logP(o/w) &gt; 2 &amp;</td>
<td>34</td>
<td>31</td>
<td>2</td>
<td>1.38</td>
<td>0.084</td>
</tr>
<tr>
<td>dl(AM1) &lt; 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Chemical structures]
70-80% of the compounds that have low acute and chronic aquatic toxicity concern to the four species have a defined range of values for octanol-water partition coefficient, $\log P_{o/w}$, and $\Delta E$ (LUMO-HOMO energy).

Compounds with $\log P_{o/w}$ values less 2 and $\Delta E$ greater than 9 eV are significantly more likely to have low acute aquatic toxicity compared to compounds that do not meet these criteria. These results are mechanistically rationalized.

Guidelines for Reducing Pesticide Toxicity to Birds

American Bird Conservancy Data (781 chemicals)

<table>
<thead>
<tr>
<th>Toxity Category</th>
<th>EPA’s Ecotoxicity Category for Acute Oral Toxicity</th>
<th>Lower limit (LD$_{50}$ mg/kg)</th>
<th>Upper limit (LD$_{50}$ mg/kg)</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very Highly and Highly Toxic</td>
<td>0</td>
<td>50</td>
<td>135</td>
</tr>
<tr>
<td>2</td>
<td>Moderately and Slightly Toxic</td>
<td>&gt;50</td>
<td>2000</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>Practically Non-Toxic</td>
<td>&gt;2000</td>
<td>1.489 x 10$^9$</td>
<td>564</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>781</td>
</tr>
</tbody>
</table>
How do we design safe insecticides to birds?

Ratio = \frac{\text{#toxic}}{\text{#non-toxic}}

Phosphorus

- # Phos > 0 (8.83, 96 chem.)
- # Phos = 0 (0.41, 134 chem.)

Aqueous Solubility

- logS > 7.3 (0.59, 109)

Globularity

- Glob > 0.84 (1.04, 70)
- Glob < 0.84 (0.23, 39)

Rotatable bonds

- Rotors < 5 (1.14, 67)
- Rotors > 5 (0, 3)
- Rotors > 10 (0, 5)
- Rotors <10 (0.28, 34)
Safer herbicides to birds

Ratio = \[
\frac{\text{toxic}}{\text{non-toxic}}
\]

Herbicides (0.059, 254 chem.)

Glob < 0.9; (0.047, 201 chem.)

Glob > 0.9; (0.111, 53 chem.)

Globularity

logPoct/gas < 13
(0.093, 95)

logPoct/gas > 13
(0.010, 106)

logPoct/gas > 13
(0, 2)

logPoct/gas < 13
(0.116, 51)

# heavy atoms

# heavy atoms < 21
(0, 15)

# heavy atoms < 21
(0.113, 80)

# heavy atoms < 15
(0.156, 40)

# heavy atoms > 15
(0, 11)
EPA’s Toxic Release Registry Chemicals vs all other chemicals

What does this mean for molecular designers?

Chemical products should be designed to preserve efficacy of function while reducing toxicity and other environmental hazards.

YES THIS CAN BE, AND IS BEING DONE
Molecular Design Resources

Garrett, R. and DeVito, S. ACS Symposium Series


Handbook of Green Chemistry (Wiley, ed. Anastas, P.)

Volume 9: Designing Safer Chemicals (Eds. Boethling, R.; Voutchkova, A.; Anastas, P.)
Special thanks to

P.I.s and Collaborators
Paul Anastas
Julie Zimmerman
John Emerson
Bob Boethling
Steve DeVito
Richard Judson

Students
Justin Steinfeld
Marina Santiago
Lori Ferris

Candida Foundation
Johnson Family Foundation
Extra slides
Is there a good correlation between different species?

- For Fathead minnow LC50 (mg/L) vs. log(Jap. medaka LC50) (mg/L), the correlation coefficient is $R^2 = 0.782$, with a p-value of 0.005.
- For Fathead minnow LC50 (mg/L) vs. log(Daphnia magna EC50) (mg/L), the correlation coefficient is $R^2 = 0.744$, with a p-value less than 0.001.