15 Emerging Contaminants and Their Potential Effects on Amphibians and Reptiles

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Serious threats to the health and sustainability of global amphibian and reptile populations have been well documented over the last few decades (Stuart et al. 2004). As many authors of this book have already indicated, habitat destruction and encroachment, increased ultraviolet B radiation, fungal diseases (e.g., chytridiomycosis), parasites, climate change, introduction of exotic species, and pollution have been cited as factors in these declines (Burrowes et al. 2004; Lips et al. 2006). Effects from pollutant exposure on wild populations are often difficult to discern due to their sublethal nature and interaction with other stressors that confound clear understanding to the causes behind population declines. The overall number of laboratory ecotoxicological studies carried out on amphibians and reptiles are small relative to other aquatic organisms, such as rainbow trout (Oncorhynchus mykiss), Daphnia magna, fathead minnow (Pimephales promelas; Chapter 1, this volume). A large number of these existing studies on amphibians have focused on metals or organohalogen pesticides such as DDT (4,4’-(2,2,2-trichloroethane-1,1-diyl) bis(chlorobenzene)) or polychlorinated biphenyls (PCBs), which have been banned for many years (Sparling et al. 2000). Other studies have included some second-generation pesticides like the organophosphate, carbamate, and pyrethroid insecticides (Cowman and Mazanti 2000; Chapters 6 and 7, this volume).

Over the last 5 to 6 years, many compound classes have been identified as “emerging contaminants.” Emerging contaminants may generally be defined as natural or synthetic chemicals or microorganisms that fall outside the normal list of typical pollutant classes and are released to the environment with the potential for toxic effects on humans or biota. Environmental science
has expanded in scope beyond the banned organochlorine insecticides, polychlorinated biphenyls, and pesticides in widespread use to include additives in industrial and consumer products and pharmaceuticals used by humans and in concentrated animal feeding operations. A classic example of an emerging contaminant is perfluorinated surfactants (PFOS) such as Scotchguard®, and their metabolites, which, after many decades of use, have become global contaminants with potential for adverse effects (Renner 2004). This discovery contributed to significant expansion in the area of environmental science focused on the fate and transport of perfluorinated chemicals.

As the capacity and technological capability of analytical chemistry have improved, development of methods to measure organic chemicals of varying properties in environmental matrices has become more rapid. Capillary gas chromatography with high-resolution mass spectrometry or tandem mass spectrometry has allowed for unambiguous measurement of complex mixtures of organic pollutants and their degradation products in samples as varied as air and precipitation to fish and whale blubber (Aono et al. 1997; Xie et al. 2007b; Yao et al. 2007; Young et al. 2007). More recently, high-performance liquid chromatography–mass spectrometry (HPLC-MS) equipment has become standard in most environmental science laboratories. The development of the electrospray interface between the HPLC and MS components combined with triple-quadrupole MS has increased both sensitivity and selectivity of analytical methods for more polar and higher-molecular-weight compound classes. Recent reviews of scientific publications related to developments in environmental mass spectrometry have documented the development in this research area (Richardson 2001, 2004; Richardson and Ternes 2005).

In the United States, several ongoing and newly formed efforts are aimed at identifying pollutants that may be causing serious toxic effects in humans or wildlife populations. In 1976, the Toxic Substances Control Act (TOSCA) Interagency Testing Committee was formed to identify chemicals with the potential for toxicity where minimal toxicological or environmental fate data are available. Reports from this committee are made annually to the administrator of the US Environmental Protection Agency (http://www.epa.gov/oppt/itc/), and chemicals are added to the Priority Testing List and testing information is requested from the manufacturer. The 1996 Food Quality Protection Act required that contaminants such as pesticides be screened for effects on the endocrine systems of humans and wildlife. From this USEPA has developed an Endocrine Disruptor Screening Program where substances are prioritized for uniform testing and results are made public (http://www.epa.gov/endo/). Environment Canada manages the Toxic Substances Research Initiative, which includes support for research on endocrine-disrupting substances (EDS) (http://www.hc-sc.gc.ca/sr-sr/finance/tsri-irst/index_e.html).

The purpose of this chapter is to provide information on several “new” or “emerging” chemicals that may pose risks to amphibians and reptiles (Tables 15.1 and 15.2). We do not intend this list to be exhaustive; that would require more space than this book allows. Rather, we selected a few chemicals that, based on production volume, usage, known toxicity, and other factors, may pose greater risks than others. Certainly, many readers would be able to add other chemicals to our list that pose equal or even greater risks. Many of these emerging contaminants are released along with wastewater treatment effluents; therefore, an evaluation of several compound classes associated with residential or industrial wastewater streams is included. The use of fungicides in US agriculture has increased over the last few decades and a number of new chemistries have been introduced to the environment, while little is known of their risk to amphibian or reptile populations. Outcomes from this effort are expected to identify research needs with respect to toxicological and developmental studies of amphibian species. The area of emerging contaminants and their study is a rapidly developing field, and undoubtedly several new papers will have been published by the time this chapter gets to press. Nevertheless, this is our perspective at the time of writing (July 2009).
TABLE 15.1
List of Specific Chemical Contaminants, Abbreviations, and Basic Physical Chemical Properties

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chemical Name</th>
<th>Abbreviation</th>
<th>CAS Registry Number</th>
<th>Chemical Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>Melting Point (°C)</th>
<th>Aqueous Solubility (mg/L)</th>
<th>Vapor Pressure (Pa)</th>
<th>Log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brominated flame retardants</td>
<td></td>
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<tr>
<td>Decabromodiphenylether</td>
<td>2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxyl)benzene</td>
<td>Deca-BDE or BDE 209</td>
<td>1163-19-5</td>
<td>C_{12}Br_{10}O</td>
<td>959.17</td>
<td>304–307</td>
<td>&lt;0.001 at 25 °C</td>
<td>4.63 × 10^{-6}</td>
<td>6.3–12.6</td>
</tr>
<tr>
<td>Hexabromocyclododecane</td>
<td>1,2,5,6,9,10-Hexabromocyclododecane</td>
<td>HBCD</td>
<td>3194-55-6</td>
<td>C_{12}H_{18}Br_{6}</td>
<td>641.7</td>
<td>180–185</td>
<td>3.4 at 25 °C</td>
<td>6.27 × 10^{-3}</td>
<td>5.625 at 21 °C</td>
</tr>
<tr>
<td>Pentabromodiphenylether</td>
<td>1,2,4-Tribromo-5-(2,4-dibromophenoxyl)benzene</td>
<td>BDE-47</td>
<td>32534-81-9</td>
<td>C_{12}H_{12}Br_{5}</td>
<td>564.69</td>
<td>–7 to –3</td>
<td>0.0109</td>
<td>1.45 × 10^{-3}</td>
<td>6.46–6.97</td>
</tr>
<tr>
<td>Tetrabromobisphenol-4</td>
<td>2,2′,6,6′-Tetrambrmo-4,4′-isopropylidenephenol</td>
<td>TBBPA</td>
<td>79-94-7</td>
<td>C_{14}H_{20}Br_{5}O_{2}</td>
<td>543.88</td>
<td>181</td>
<td>1.26 at 25 °C at pH 7</td>
<td>1.19 × 10^{-7}</td>
<td>8.024 at 20 °C</td>
</tr>
<tr>
<td>Perfluorinated Compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>N-Methyl perfluorooctane sulfonamidoethanol</td>
<td>N-MeFOSE</td>
<td></td>
<td>24448-09-7</td>
<td>C_{12}H_{22}F_{13}NO_{2}S</td>
<td>557.23</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N-Ethyl perfluorooctane sulfonamide</td>
<td>N-Ethyl-1, 1, 2, 3, 4, 5, 5, 5, 6, 6, 7, 7, 8, 8, 8-Heptadecafluoro-octanesulfonamide</td>
<td></td>
<td>4151-50-2</td>
<td>C_{12}F_{14}SO_{2}NHCH_{2}CH_{3}</td>
<td>527.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorooctanesulfonic acid</td>
<td>1-Octanesulfonic acid</td>
<td>PFOS</td>
<td>1763-23-1</td>
<td>C_{12}F_{12}SO_{3}^{-}</td>
<td>500.13</td>
<td>&gt;400</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Perfluorooctanoic acid</td>
<td>Pentadecafluoroocctanoic acid</td>
<td>PFOA</td>
<td>335-67-1</td>
<td>C_{12}F_{12}COO^{-}</td>
<td>414.07</td>
<td>45–50</td>
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<td></td>
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<tr>
<td>Perfluorooctane sulfonamide</td>
<td>N-Ethyl-1, 1, 2, 3, 4, 5, 5, 5, 6, 6, 7, 8, 8-Heptadecafluoro-octanesulfonamide</td>
<td></td>
<td>754-91-6</td>
<td>C_{12}F_{12}SO_{2}NH_{2}</td>
<td>499.14</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Anionic Surfactants</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>4-Nonylphenol</td>
<td>NP</td>
<td>104-40-5</td>
<td>C_{15}H_{20}O</td>
<td>220.35</td>
<td>43–45</td>
<td></td>
<td>6.2 × 10^{-6} at pH 7</td>
<td>3.8–4.77</td>
</tr>
<tr>
<td>Octylphenol</td>
<td>4-(1, 1, 3, 3-Tetramethylbutyl)phenol</td>
<td>OP</td>
<td>140-66-9</td>
<td>C_{14}H_{22}O</td>
<td>206.33</td>
<td>79–82</td>
<td></td>
<td>0.21 at 20 °C</td>
<td></td>
</tr>
<tr>
<td>Antibacterial Compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triclosan</td>
<td>5-Chloro-2-(2, 4-dichlorophenoxyl)phenol</td>
<td></td>
<td>3380-34-5</td>
<td>C_{12}H_{22}Cl_{2}O_{2}</td>
<td>289.54</td>
<td>180</td>
<td>1.97–4.6</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chemical Name</th>
<th>Abbreviation</th>
<th>CAS Registry Number</th>
<th>Chemical Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>Melting Point (°C)</th>
<th>Aqueous Solubility (mg/L)</th>
<th>Vapor Pressure (Pa)</th>
<th>Log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl triclosan</td>
<td>5-Chloro-2-(2,4-dichlorophenoxy)anisole</td>
<td></td>
<td>4640-01-1</td>
<td>C₁₃H₁₀Cl₂O₂</td>
<td>303.57</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Triclocarban</td>
<td>3-(4-Chlorophenyl)-1-(3,4-dichlorophenyl)urea</td>
<td>OC</td>
<td>101-20-2</td>
<td>C₁₃H₁₂Cl₂N₂O</td>
<td>315.59</td>
<td>255.3</td>
<td>0.11 at 20 °C at pH 6.1–6.3</td>
<td>&lt;1 hPa at 50 °C</td>
<td>4.2 at 22.6 °C</td>
</tr>
<tr>
<td>Benzophenone-1</td>
<td>2,4-Dihydroxybenzophenone</td>
<td></td>
<td>131-56-6</td>
<td>C₁₃H₁₀O₂</td>
<td>214.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzophenone-2</td>
<td>2', 4', 4'-Tetrahydroxy-benzophenone</td>
<td></td>
<td>131-55-5</td>
<td>C₁₃H₁₀O₂</td>
<td>246.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Benzophenone-3</td>
<td>2-Hydroxy-4-methoxy-benzophenone</td>
<td>BP-3</td>
<td>131-57-7</td>
<td>C₁₃H₁₀O₂</td>
<td>228.24</td>
<td>62–65</td>
<td>210 at 25 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylhexylmethoxyccinnamate</td>
<td>(3-(4-Methoxyphenyl)-2-propanoic acid 2-ethylhexyl ester</td>
<td>EHMC</td>
<td>5466-77-3</td>
<td>C₁₃H₁₂O₂</td>
<td>290.4</td>
<td>-25</td>
<td>150 at 25 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Methylbenzylidene camphor</td>
<td>(3-(4'-Methyl-benzylidene)boman-2-one)</td>
<td>4-MBC</td>
<td>36861-47-9</td>
<td>C₁₉H₂₀O</td>
<td>254.37</td>
<td>66–69</td>
<td>5.1 at 25 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octocrylene</td>
<td>2-Cyano-3,3-diphenyl-2-propanoic acid 2-ethylhexylester</td>
<td>OC</td>
<td>6197-30-4</td>
<td>C₁₉H₂₇NO₂</td>
<td>361.48</td>
<td>14</td>
<td>0.2 at 25 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fungicides</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Trifloxystrobin</td>
<td>Methyl (E)-methoxyimino-[(E)-a-[(α,β,α,β- trifluoro-m-tolyl)ethylideneamino]oxyl-o-tolyl]acetate</td>
<td></td>
<td>141517-21-7</td>
<td>C₁₉H₁₅F₆N₂O₄</td>
<td>408.4</td>
<td>72.9</td>
<td>0.61 at 25 °C</td>
<td>4.5 x 10⁻⁴ Pa at 25 °C</td>
<td></td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>Methyl (E)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-xyloxy]phenyl]-3-methoxyacrylate</td>
<td></td>
<td>131860-33-8</td>
<td>C₁₇H₁₇N₃O₅</td>
<td>403.38</td>
<td>116</td>
<td>6.0</td>
<td>1.1 x 10⁻¹⁰ Pa at 25 °C</td>
<td></td>
</tr>
<tr>
<td>Fluoxatstrobacin</td>
<td>(E)-2-[6-(2-Chlorophenoxy)-5-fluoropyrimidin-4-xyloxy]phenyl</td>
<td>5.6-dihydro-1,4,2-dioxazin-3-ylmethanone O-methyloxime</td>
<td>361377-29-9</td>
<td>C₁₇H₁₈ClF₄N₂O₂</td>
<td>458.8</td>
<td>103–105</td>
<td>2.3 at pH 7</td>
<td>5.63 x 10⁻¹⁰ at 20 °C</td>
<td>2.86</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>1-[4-[4-[(2S, 4R)-2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-3-dioxolan-4-yl]methoxy]phenyl)piperazin-1-yl]ethanone</td>
<td></td>
<td>65277-42-1</td>
<td>C₂₀H₂₈Cl₂N₂O₄</td>
<td>531.43</td>
<td>148–152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetraconazole</td>
<td>(RS)-2-(2, 4-Dichlorophenyl)-3-(1H-1, 2, 4-triaza-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether</td>
<td></td>
<td>112281-77-3</td>
<td>C₁₃H₁₁Cl₂F₄N₂O</td>
<td>372.16</td>
<td>-29.2</td>
<td>159</td>
<td>1.8 x 10⁻⁴</td>
<td>3.56 at 23 °C</td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Structure</td>
<td>Molecular Formula</td>
<td>Log K OW</td>
<td>Solubility (g/L)</td>
<td>Mp (°C)</td>
<td>Vp (mm Hg)</td>
<td>Ps (Pa)</td>
<td>pH 7</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Prothioconazole&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(RS)-2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;15&lt;/sub&gt;Cl&lt;sub&gt;3&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;OS</td>
<td>178928-70-6</td>
<td>344.26</td>
<td>139.1-144.5</td>
<td>5.0 at pH 4 at 20 °C</td>
<td>&lt;4 × 10⁻² at 20 °C</td>
<td>3.82 at pH 7</td>
<td></td>
</tr>
<tr>
<td>Cyazofamid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4-Chloro-2-cyano-&lt;i&gt;N&lt;/i&gt;,&lt;i&gt;N&lt;/i&gt;-dimethyl-5-&lt;i&gt;p&lt;/i&gt;-tolylimidazole-1-sulfonamide</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;ClN&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>120116-88-3</td>
<td>324.9</td>
<td>152.7</td>
<td>0.107 at pH 7 at 20 °C</td>
<td>&lt;1.33 × 10⁻³ at 20 °C</td>
<td>35 °C</td>
<td></td>
</tr>
<tr>
<td>Dimethomorph&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(EZ)-4-[3-(4-Chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]-morpholine</td>
<td>C&lt;sub&gt;23&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;ClNO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>110488-70-5</td>
<td>375.9</td>
<td>125-149</td>
<td>18 at pH 7 at 20 °C</td>
<td>10⁻⁸ to 9.7 at 25 °C</td>
<td>2.63-2.73 at 20 °C</td>
<td></td>
</tr>
<tr>
<td>Boscalida&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2-Chloro-&lt;i&gt;N&lt;/i&gt;-(4'-chlorobiphenyl-2-yl)nicotinamide</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;ClN&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>188425-85-6</td>
<td>343.21</td>
<td>143.4-143.6</td>
<td>6 at 20 °C</td>
<td>7 × 10⁻² Pa at 20 °C</td>
<td>2.96 at pH 7</td>
<td></td>
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<tr>
<td>Famoxadone&lt;sup&gt;e&lt;/sup&gt;</td>
<td>(RS)-3-Anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;</td>
<td>131807-57-3</td>
<td>374.39</td>
<td>140.3-141.8</td>
<td>111 at pH 7 at 20 °C</td>
<td>6.4 × 10⁻⁷ Pa at 20 °C</td>
<td>4.65 at pH 7</td>
<td></td>
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</table>

<sup>a</sup> EPA (2008).
<sup>b</sup> Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/opppthpv/quicksearch.display?pChem=100412).
<sup>c</sup> Vapor pressure values from Tittlemier et al. (2001) and other values from Peltola and Ylä-Mononen (2000).
<sup>d</sup> Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/opppthpv/quicksearch.display?pChem=100413).
<sup>e</sup> OECD (2002).
<sup>f</sup> Melting point from Boit (1975) and vapor pressure from Kaiser et al. (2005).
<sup>g</sup> EPA (2005a).
<sup>h</sup> Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/opppthpv/quicksearch.display?pChem=100234).
<sup>i</sup> Estimated values of melting point, vapor pressure, and Log K OW from Halden and Paul (2005).
<sup>j</sup> Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/opppthpv/quicksearch.display?pChem=101315).
<sup>k</sup> Solubility and Log K OW of UV filters from Díaz-Cruz et al. (2003).
<sup>l</sup> USEPA (1999).
<sup>m</sup> USEPA (1997).
<sup>n</sup> USEPA (2005b).
<sup>o</sup> USEPA (2005c).
<sup>p</sup> USEPA (2007).
<sup>q</sup> USEPA (2004).
<sup>r</sup> USEPA (1998).
<sup>s</sup> USEPA (2003a).
<sup>t</sup> USEPA (2003b).
**TABLE 15.2**
Results of Toxicological Studies of Amphibian Species with Selected Emerging Contaminants Arranged by Contaminant Class

<table>
<thead>
<tr>
<th>Species</th>
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<tbody>
<tr>
<td><em>Pseudacris regilla</em></td>
<td>Tetrabromobisphenol-A</td>
<td>Thyroid hormone-mediated activity on tadpoles</td>
<td>Aqueous at 5.4 and 54.4 µg/L over 96 hours</td>
<td>May act as agonist of TH action and potentiate TH-mediated gene expression leading to accelerated anuran metamorphosis</td>
<td>Veldhoen et al. 2006a</td>
</tr>
<tr>
<td><em>Rana rugosa</em></td>
<td>Tetrabromobisphenol-A</td>
<td>Thyroid hormone agonist activity on tadpoles</td>
<td>Aqueous at 540, 54, and 5.4 µg/L over 9 days</td>
<td>Agonist activity to T3 at lowest treatment</td>
<td>Kitamura et al. 2005</td>
</tr>
<tr>
<td><em>Xenopus laevis</em></td>
<td>BDE-47</td>
<td>Effect on time to complete metamorphosis</td>
<td>Single intraperitoneal injection of 1 or 100 µg/tadpole</td>
<td>Significant effect at 100 µg/tadpole</td>
<td>Balch et al. 2006</td>
</tr>
<tr>
<td></td>
<td>BDE-99</td>
<td>Effect on time to complete metamorphosis</td>
<td>Single intraperitoneal injection of 1 or 100 µg/tadpole</td>
<td>No observable effect</td>
<td>Balch et al. 2006</td>
</tr>
<tr>
<td></td>
<td>BDE-206</td>
<td>Thyroid hormone agonist activity on tadpole tail tips</td>
<td>Culture media at 0.88 to 880 µg/L for 6 days in presence of T3 or 880 µg/L alone</td>
<td>Significant reduction in tail tip regression in all treatments with T3; no effect with BDE-206 alone</td>
<td>Schriks et al. 2006</td>
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<tr>
<td></td>
<td>DE-71 commercial formulation</td>
<td>Effect on time to complete metamorphosis</td>
<td>Single intraperitoneal injection of 0.6, 6, or 60 µg/tadpole</td>
<td>Significant effect at 60 µg/tadpole</td>
<td>Balch et al. 2006</td>
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<td></td>
<td>DE-71 commercial formulation</td>
<td>Effect on time to complete metamorphosis</td>
<td>Dietary at 1, 1000, or 5000 µg/g for 14 days</td>
<td>Significantly inhibited metamorphosis in all 3 treatments</td>
<td>Balch et al. 2006</td>
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<tr>
<td></td>
<td>HBCD</td>
<td>Thyroid hormone agonist activity on tadpole tail tips</td>
<td>Culture media at 0.64 to 640 µg/L for 6 days in presence of T3 or 640 µg/L alone</td>
<td>Significant reduction in tail tip regression in all treatments with T3; no effect with HBCD alone</td>
<td>Schriks et al. 2006</td>
</tr>
<tr>
<td><em>Xenopus tropicalis</em></td>
<td>BDE-47</td>
<td>Effect on time to complete metamorphosis for tadpole</td>
<td>Dietary at 100, 1000, or 10000 µg/g for 14 days</td>
<td>Significant mortality at 10 000 µg/g; reduced hind limb length and body length at 1000 µg/g</td>
<td>Carlsson et al. 2007</td>
</tr>
<tr>
<td></td>
<td>BDE-99</td>
<td>Effect on time to complete metamorphosis</td>
<td>Dietary at 100, 1000, or 10000 µg/g for 14 days</td>
<td>Significant mortality at 10 000 µg/g; reduced hind limb length and developmental stage at 1000 µg/g</td>
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<tr>
<td><em>Rana pipiens</em></td>
<td>PFOS</td>
<td></td>
<td>Effects on survival and development from early embryogenesis through complete metamorphosis</td>
<td>0.03 to 10 mg/L through metamorphosis</td>
<td>Survival was significantly decreased at 10 mg/L (90%), but survival was not affected at the lower treatment levels’ an increased time to initial metamorphosis was observed in the 3.0 mg/L treatment</td>
</tr>
<tr>
<td><em>Xenopus laevis</em></td>
<td>PFOS</td>
<td></td>
<td></td>
<td>LC50 96-hour FETAX</td>
<td>LC50 = 15.6 mg/L, and NOEC and LOEC were calculated as 4.82 and 7.97 mg/L, respectively</td>
</tr>
<tr>
<td></td>
<td>Anionic Surfactants</td>
<td></td>
<td></td>
<td>Exposure resulted in a significantly increased proportion of females at nominal concentrations of 100 and 25 mg/L, but not at 50, 12.5, or 6.25 mg/L.</td>
<td>Blandin et al. 1996</td>
</tr>
<tr>
<td>NP</td>
<td>Developmental effects on tadpoles</td>
<td>Aqueous flow through at 6.25 to 100 mg/L</td>
<td></td>
<td></td>
<td>Bevan et al. 2003</td>
</tr>
<tr>
<td>NP</td>
<td>Developmental effects on embryos compared with natural estrogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>Examine interaction between OP sublethal exposure and UV-B radiation on mRNA expression in the brain and effects on metamorphosis, specifically growth rate and hind limb emergence</td>
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<td>Combined 1 μM OP and UV-B treatment were heavier than other treatments, and displayed significant acceleration of hind limb emergence</td>
<td>Crump et al. 2002</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
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<tr>
<td><em>Rana catesbeiana</em></td>
<td>Triclosan</td>
<td>Changes in metamorphosis process</td>
<td>Aqueous at 0.3 to 30 μg/L over 4 days</td>
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<tr>
<td><em>Rana pipiens</em></td>
<td>Triclosan</td>
<td>Activity level, startle response, survivorship, and growth on tadpoles</td>
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<td>Activity level reduced in all treatments; startle response and survivorship were lower at 230 μg/L treatment; no interaction with acetaminophen exposure</td>
<td>Fraker et al. 2004</td>
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<tr>
<td><em>Rana temporaria</em></td>
<td>Azoxystrobin</td>
<td>Acute toxicity to tadpoles</td>
<td>Aqueous at 0.5, 0.13, and 0.03 mg/L</td>
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<td>Johansson et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Azoxystrobin</td>
<td>Chronic toxicity to tadpoles</td>
<td>Aqueous at 10 and 1 μg/L</td>
<td>No negative effects observed</td>
<td>Johansson et al. 2006</td>
</tr>
</tbody>
</table>

15.1 EMERGING COMPOUND CLASSES AND RELEVANCE TO HERPETOFAUNA

15.1.1 BROMINATED FLAME RETARDANTS

There are 3 major types of brominated flame retardants (BFRs): 1) Tetrabromobisphenol-A (TBBPA) is added during the production of epoxy and polycarbonate resins used in circuit boards and other products. It becomes part of the polymer backbone, making it less available for loss to the environment. However, this chemical is also used as an additive in acrylonitrile-butadiene-styrene plastics for products like television casings (BSEF 2007). 2) Polybrominated diphenyl ethers (PBDEs) are added to different polymers, but they are not chemically bound to the polymer backbone and thus are easily released to the environment. 3) Hexabromocyclododecane (HBCD) is added to polystyrene insulation foams used in building construction and is used in the back coating of textiles like upholstered furniture.

At present, the risk from TBBPA is deemed low due to its incorporation into the polymer backbone, and this product remains in heavy use. This chemical has primarily been detected in sewage sludge and in sediment samples collected near industrial sources (Law et al. 2003). A recent report by Xie et al. (2007a) described decreasing atmospheric concentrations of TBBPA with increasing latitude from the North Sea to the Arctic, suggesting that the potential for long-range transport is
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limited. TBBPA is similar in structure to thyroxine (T4) and can alter thyroid hormone-responsive genes in the laboratory amphibian model *Xenopus laevis* (Jagnytsch et al. 2006). Developmental effects have also been observed in *Rana rugosa* at $10^{-8}$ to $10^{-6}$ M (Kitamura et al. 2005), and in the Pacific tree frog, *Pseudacris regilla*, at $10^{-8}$ to $10^{-7}$ M (Veldhoen et al. 2006a).

PBDEs are a class of chemical compounds in which up to 10 bromine atoms are attached to a diphenyl ether molecule. There are 209 different possible compounds, called congeners, depending on the number and position of the bromine atoms. PBDEs have properties similar to those of polychlorinated biphenyls (PCBs), are persistent in the environment, and undergo long-range atmospheric transport, as demonstrated through their bioaccumulation in the arctic marine food web (Muir et al. 2006). PBDEs function in multiple ways to inhibit thyroid activity and act as endocrine disruptors. Two industrial PBDE mixtures, called penta-BDE and octa-BDE, were banned in the European Union in 2004 due to concern over their potential for toxicity and biomagnification. Analysis of archived guillemot (*Uria aalge*) eggs from the Baltic Sea revealed increasing PBDE concentrations from early 1970 to approximately 1990, followed by a steep decline through 2001 (Sellström et al. 2003). A similar study of tawny owl (*Strix aluco*) eggs from Northern Europe observed an average decline of 6% per year in total PBDEs between 2001 and 2004, reflecting a decline in usage in the region (Bustnes et al. 2007). The primary manufacturers of these products in the United States have voluntarily phased out their production as of 2004.

PBDEs residues have been measured in turtles and other reptiles (e.g., de Solla et al. 2007; Wu et al. 2008; see Chapter 10, this volume). However, studies have not gone beyond residue determination into exploring the possible effects of these chemicals in reptiles.

The deca-BDE formulation, primarily used in plastics for electronics equipment, is still widely used in the United States and Europe. The higher brominated congeners appear to be less prone to bioaccumulation (Ciparis and Hale 2005). Amphibians and reptiles are important components of many food webs and may become exposed by eating contaminated prey or become a source of BDEs for organisms at higher trophic levels. The decabrominated congener BDE-209 has been detected in Peregrine falcon (*Falco peregrinus*) eggs in Sweden (Lindberg et al. 2004). Uptake by fish such as rainbow trout (*Onchorhynchus mykiss*) and common carp (*Cyprinus carpio*) appears to be minimal, but biotransformation of BDE-209 to lower brominated PBDE congeners was observed in the liver of these species (Stapleton et al. 2006). PBDEs were also detected in the livers of frog samples (*Rana temporaria*) collected along a transect of the Scandinavian Peninsula (ter-Schure et al. 2002). The tetrabrominated congener BDE-47 was the most frequently detected PBDE, but PBDE concentrations were 10 to 100 times lower than the most abundant PCB congeners. Recent research by Balch et al. (2006) with *Xenopus laevis* and by Carlsson et al. (2007) with *Xenopus tropicalis* has demonstrated the potential for developmental effects from exposure to BDE-47.

HBCD is a widely used BFR with a reported global production of 600 000 metric tonnes per year in 2000 (Alaee et al. 2003). The HBCD commercial mixture contains 3 diastereomers, α-, β-, and γ-HBCD, which may be preferentially assimilated. From work by Zegers et al. (2005) it appears that the β- and γ-HBCD are susceptible to enzyme-mediated biotransformation while α-HBCD is resistant. Each of the 3 diastereomers also have 2 enantiomers (Janák et al. 2005), making the environmental fate of these chemicals in biological systems all the more complex. Alpha-α-HBCD caused thyroid disruption in *Xenopus laevis* tadpoles in laboratory studies (Schriks et al. 2006), but further investigations are required under environmentally relevant concentrations.

The relative risk of BFRs to herpetofauna is difficult to assess. Clearly the potential for thyroid function-disrupting or developmental effects on amphibians in the environment exists for all 3 types of BFRs. However, exposure to toxicologically significant levels of BFRs may be limited to amphibian populations downstream or downwind from urban or industrial areas. Despite the reduction in
usage of the penta-BDE formulation, a huge reservoir of BFRs exists in consumer products still in use, and the potential for exposure by amphibian populations will continue into the future.

15.1.2 PERFLUORINATED CHEMICALS

Perfluorinated chemicals (PFCs) are industrial products utilized in a variety of consumer and agricultural products. They are used as refrigerants, agrochemicals, chemical catalysts/reagents, surfactants, and in fire-fighting foams. The strength of the carbon-fluoride bond, and the presence of multiple C-F bonds in PFCs contribute to their resistance to biotic and abiotic degradation (Key et al. 1997). The study of PFCs in the environment is an active area of research.

Perfluorooctane sulfonate fluoride is the primary building block used in polymers to treat fabrics and other textiles to repel water and stains. Some common perfluorooctanesulfonyl fluoride-derived products are N-methyl perfluorooctane sulfonamidoethanol and N-ethyl perfluorooctane sulfonamidoethanol. Another PFC, N-ethyl perfluorooctane sulfonamide, is an insecticide used in ant bait products. These neutral, semivolatile fluorinated chemicals have the potential for long-range atmospheric transport and have been detected in arctic air (Shoeib et al. 2006). Perfluoroalkyl sulfonamidoethanols and fluorotelomer alcohols are precursors to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the most commonly detected perfluorinated environmental contaminants.

Evidence for global environmental contamination by PFOS and PFOA was initially published in 2001 (Giesy and Kannan 2001; Hansen et al. 2001; Kannan et al. 2001). Subsequent investigations and improvements in analytical methods have led to the identification of additional PFCs in the environment (Powley et al. 2008). Results from a survey of PFCs in the arctic food chain (zooplankton to whale) suggest that PFOS biomagnifies in the food chain; however, biotransformation of other PFCs to PFOS is a complicating factor in determining biomagnification factors (Tomy et al. 2004). PFOS and PFOA are chemically persistent, highly water soluble, and can even be used as tracers for ocean circulation patterns (Yamashita et al. 2008).

Published results from standard laboratory toxicity testing experiments indicate that PFOS is toxic to many aquatic organisms, but effects are generally seen at concentrations well above measured environmental concentrations (Ankley et al. 2005). For example, PFOS affected the survival of the aquatic midge (Chironomus tentans) at an EC50 of 92 μg/L in a chronic life cycle test, but no toxic effects were seen for PFOA (MacDonald et al. 2004). In another study of PFOS toxicity, 2 green algae species, a floating macrophyte, and 2 invertebrates were tested, and an overall acute toxicity concentration of 100 mg/L was determined from the most sensitive species (Boudreau et al. 2003).

In an experiment that exposed northern leopard frog (Rana pipiens) larvae to 0.03 to 10 mg/L of PFOS (Ankley et al. 2004), survival was affected at the highest concentrations and metamorphosis was delayed at lower concentrations. During these same experiments, Ankley also found that tadpoles bioaccumulated PFOS via the water. Beach et al. (2006) reported a lowest observable effects level (LOEL) for PFOS of 7.97 mg/L and an LC50 of 15.6 mg/L using a 96-hour embryo teratogenesis assay with Xenopus laevis.

PFOS has been measured in tissues of turtles, so we know that they are being exposed. In a study on the Great Lakes, Kannan et al. (2005) determined that PFOS was the most common perfluorinated compound in the food chain. Zebra mussels accumulated PFOS concentrations that were approximately 1000 times greater than in the water, and concentrations in predators were 5 to 10 times greater than in their prey. The livers of 2 male snapping turtles had PFOS concentrations of 105 and 169 ng/g, while 2 female turtles had only 1 and 8.8 ng/g. For comparison, adult green frog livers contained 50 to 285 ng/g PFOS. Keller et al. (2005) found that among 12 PFCs, PFOS had the highest concentration in plasma of loggerhead (Caretta caretta) and Kemp’s ridley (Lepidochelys kempii) sea turtles; mean concentrations were 3.2 to 11.0 ng/mL in loggerheads and
3.57 to 39.4 ng/mL in Kemp’s. However, there are no studies that we know of that have examined the effects of perfluorinated chemicals in any reptile.

As an endocrine disruptor, PFOS did not appear to have a strong effect on the thyroid hormone system of rats when introduced orally (Chang et al. 2008). However, results from a recent study with mice by Johansson et al. (2008) suggested that PFOS and PFOA are potential developmental neurotoxins. In aquatic organisms, endocrine-disrupting effects of PFCs have been observed by measuring estrogenic activities in cultured tilapia hepatocytes (Liu et al. 2007). Estrogenic activity was also expressed in minnows exposed to PFOA concentrations of 3 to 30 mg/L over 14 to 28 days (Wei et al. 2007).

Although information on PFC exposure by amphibian populations is not available at present, the stability of PFCs in water and their potential for long atmospheric range transport suggest that amphibian populations in all regions of the world have a potential for exposure to PFCs. Aquatic reptiles such as turtles and crocodilians may also be at risk. An aquatic benchmark to protect aquatic organisms of 1.2 µg/L PFOS has been proposed by Beach et al. (2006). Recent measurements of PFOS in river samples collected upstream and downstream from a wastewater treatment plant effluent in Germany were 0.8 to 3.5 ng/L and 0.7 to 15 ng/L, respectively (Becker et al. 2008). In a study conducted in rivers in China, PFOS concentrations ranged from 0.90 to 99 ng/L and <0.01 to 14 ng/L in samples from the Pearl River and Yangtze River, respectively (So et al. 2007). Therefore, despite widespread contamination, measured concentrations of the more bioaccumulative perfluorinated chemical, PFOS, does not appear to pose a significant threat to amphibians or aquatic reptiles.

### 15.1.3 ANIONIC SURFACTANTS

Anionic surfactants are widely used in consumer products to improve the effectiveness of detergents, pesticides, and other products. While many different types of surfactants are used, one of the most studied groups of surfactants is the 4-alkylphenol polyethoxylates. The technical product is a complex mixture of 4 to 20 carbon chain ethoxylates with differing levels of branching (Giger et al. 1981). The ethoxylate chains are easily biodegraded under anaerobic conditions, leaving behind the more persistent metabolites, mono- and diethoxylates, nonylphenol, and octylphenol (Ahel et al. 1994). Alarmingly high concentrations of these surfactant metabolites were detected in sewage treatment sludge in the early 1980s (Giger et al. 1984).

Octylphenol and nonylphenol have been observed in sediments and surface waters in many regions of the world (Rice et al. 2003; Li et al. 2004; Vitali et al. 2004; Nagy et al. 2005; Chen et al. 2006; Li et al. 2007b; Fiedler et al. 2007) and have recently been found in the air and surface waters of remote regions like the North Sea (Xie et al. 2007b). Their hydrophobic nature favors partitioning into sediments, generally creating a zone of high concentrations around sources, that is, wastewater treatment plant discharges.

Research carried out since the 1990s established that 4-nonylphenol in particular acted as an endocrine disruptor in aquatic organisms (Jobling and Sumpter 1993; White et al. 1994; Jobling et al. 1996; Baldwin et al. 1997; Gimeno et al. 1997; Ashfield et al. 1998; Bistodeau et al. 2006) and in amphibians (Blandin et al. 1996; Bevan et al. 2003). Endocrine disruption by nonylphenol in embryonic diamondback terrapins (Malaclemys terrapin) and snapping turtles was confirmed when application of the chemical caused sex reversal in embryos incubated at male-inducing temperatures (Place et al. 2001). Also, octylphenol may disrupt hypothalamic development in young snapping turtles (Trudeau et al. 2002) and amphibian larvae (Crump et al. 2002). Extensive research shows that 4-nonylphenol and related compounds are estrogenic in various fish species (Ackermann et al. 2002; Arsenault et al. 2004). This chemical is often used to evaluate new bioassay methods and biomarkers to detect estrogenic activity (Allner et al. 1999; Belt et al. 2003). Herpetofaunal exposure to octyl- and nonylphenols and mono- and diethoxylates will be greatest in urban and suburban streams and wetlands receiving effluents from wastewater treatment systems.
The National Recommended Ambient Water Quality Criteria for nonylphenol are not to exceed a 1-hour average concentration of 28 μg/L or a 4-day average concentration of 6.6 μg/L more than once over 3 years (King 2006). A recent review of previous measurements of alkylphenol ethoxylates and related metabolites in surface waters of the United States and subsequent exposure analysis concluded that 97% of samples fell below the National Recommended Ambient Water Quality Criteria for nonylphenol. Therefore, chemicals related to 4-alkylphenol polyethoxylate surfactants are a concern with respect to endocrine-disrupting effects on aquatic organisms and herpetofauna, but exposure levels of these chemicals in aquatic environments may fall well below those that cause endocrine-disrupting effects. Further work to determine the sensitivity of various species to this class of chemicals is needed.

15.1.4 Antibacterial Products

Triclosan and triclocarban are bactericides used in numerous consumer products like cosmetics, toothpaste, hand soaps, shampoos, and plastics. Triclocarban has been included in the high-production-volume chemical challenge by the EPA (http://iaspub.epa.gov/oppthpv/quicksearch.display?pChem=101315). Due to the nature of the products in which they are used, residues of these chemicals are generally rinsed into wastewaters very quickly after use. Therefore, wastewater effluents and solids from wastewater processing have been identified as the most important sources of these 2 chemicals. Both chemicals are relatively lipophillic, with estimated log Kow values of 4.9 for trichlocarban and 4.8 for triclosan (Halden and Paull 2005), and both chemicals are persistent in soils under anaerobic conditions. However, triclosan has a shorter half-life in aerobic soils than triclocarban (18 vs. 108 days, respectively; Ying et al. 2007).

Triclosan is susceptible to photodegradation in aqueous solutions under certain conditions (Lindström et al. 2002), but in general is relatively stable in surface waters. Examination of triclosan fate in modern wastewater treatment plants indicates it is very effectively removed from influent water (87 to 95%), with methyl triclosan concentrations higher in effluent waters than in the influent (Bester 2005). Both biological degradation and sedimentation were important removal processes during waste treatment, thereby concentrating triclosan in resulting biosolids material (Ying and Kookana 2007). In an aquatic toxicity assessment of triclosan using chronic and acute toxicity measurements across 14 species of fish, invertebrates (no amphibians), and algae (Capdevielle et al. 2008), algae were more sensitive than the other species with a no observable effects concentration (NOEC) of 0.69 μg/L. The resulting risk assessment concluded that risks to aquatic species were low even near wastewater discharge points. Similar conclusions of minimal risks to aquatic organisms were made in a separate ecological risk assessment of triclosan (Reiss et al. 2002). Reiss et al. (2009) published a risk assessment of the chemical in terrestrial systems and concluded that risk to birds and mammals was low, but they did not include amphibians or reptiles in either study. Results from a study of triclosan toxicity on Bufo americanus tadpoles were inconclusive in that the intermediate exposure level (2.3 μg/L) had highest mortality rates, but the highest survivorship was observed at the highest exposure level (230 μg/L; Smith and Burgett 2005). A similar study with Rana pipiens tadpoles (Fraker and Smith 2004) with the same exposure levels as Smith and Burgett (2005) found lower survivorship and startle response at the highest exposure level.

Triclocarban, triclosan, and methyl triclosan residues bioaccumulated in algae and snails immersed in water near the outfall of a wastewater treatment plant (Coogan and La Point 2008). Recent reports on triclocarban indicate that while it is not an endocrine disrupter, it enhances the activity of estradiol (E2)-dependent or testosterone-dependent activation of estrogen- and androgen-responsive gene expression (Ahn et al. 2008), suggesting a new mode of action for endocrine-disrupting chemicals. Much less information has been published on the toxicity of triclocarbon relative to that of triclosan. This new information on the potential for triclocarban to enhance the activity
of other pollutants suggests that this chemical requires further examination with respect to herpeto-
fauna toxicity.

### 15.1.5 UV Filters

Another group of chemicals associated with personal care products are ultraviolet light-filtering compounds used in sunscreens and cosmetics. Four of these compounds, benzophenone-3 (BP-3), 4-methylbenzylidene camphor (4-MBC), ethylhexyl methoxyphenylamine (EHMC), and octocrylene (OC), have been found in surface waters, wastewaters, and fish tissue in Swiss lakes (Balmer et al. 2005; Buser et al. 2006), and 23 others were also listed in a recent publication by Diaz-Cruz et al. (2008). Examination of the fate of selected UV filters in wastewater plants suggests typical processing is only partially effective at removing residues from effluents (Li et al. 2007a). Some UV filters are lipophilic in nature with log $K_{ow}$ values in the same range as other persistent organic pollutants (Table 15.1). UV filters such as BP-3, 4-MBC, and OC have exhibited estrogenic characteristics in rats (Schlumpf et al. 2001), and both in vitro and in vivo estrogenic effects were seen in rainbow trout and fathead minnows, with vitellogenin induction occurring at 435 μg/L with benzophenone-1 and 4900 μg/L with benzophenone-2 (Kunz and Fent 2006). Benzophenone-2 also was bioaccumulated in fathead minnow and exhibited negative effects on reproduction at a concentration of 1.2 mg/L, and complete cessation of spawning activity was observed at 9.7 mg/L (Weisbrod et al. 2007). Relatively little is known of the fate of these chemicals in the environment and potential exposure by amphibians and reptiles, and no published studies of toxicity or effects on reproduction on herpetofauna are available at present.

### 15.1.6 Fungicides

A number of new fungicide chemistries have been developed over the last few years, and fungicide use has increased significantly in the United States, with the invasion of Asian soybean rust disease beginning in 2004.

#### 15.1.6.1 Strobilurins

Strobilurins include at least 9 products, including trifloxystrobin (Bayer), azoxystrobin (Syngenta), and fluoxatstrobin (Bayer) (www.alanwood.net/pesticides/class_fungicides.html), and are one of the classes of fungicides recommended for control of soybean rust disease. Their mode of action includes inhibition of the electron transport system. These chemicals are practically nontoxic to birds and mammals but are highly to very highly toxic to fish and other aquatic animals. Trifloxystrobin is a broad-spectrum fungicide, which, in different formulations, is registered for cucurbit vegetables, peanuts, pome fruits, grapes, turfgrass, and ornamentals. Application rates depend on formulation and crop. It has an LC50 of 0.014 mg/L for rainbow trout and 0.054 mg/L for bluegill (Lepomis macrochirus). However, it rapidly degrades in the environment with a half-life measured in hours or days to a more stable acid metabolite of unknown toxicity (USEPA 1999).

Similarly, azoxystrobin is registered for a variety of fungicide diseases on golf courses and turf farms. Application rates vary by crop. It is moderately to highly toxic in freshwater fish, with a 96-hour LC50 of 0.47 mg/L for rainbow trout and 1.1 mg/L for bluegill (USEPA 1997). The principal degradeate of azoxystrobin has an LC50 in rainbow trout of >150 mg/L and is considered to be practically nontoxic. Aqueous exposures to relatively high concentrations of azoxystrobin (0.5 mg/L) were found to be acutely toxic to *Rana temporaria* tadpoles and had negative effects on body length, as well as at lower concentrations of 0.13 and 0.03 mg/L (Johansson et al. 2006). Chronic exposure to concentrations of 10 and 1 μg/L did not cause any measurable effects on growth, survival, or metamorphosis (Johansson et al. 2006). The only report on its stability is that the chemical is “chemically stable for at least 14 days” (USEPA 1997).
Fluoxastrobin is registered for controlling early and late blight, leaf spots and rust, and *Rhizoctonia solani* in peanuts, tuberous and corm vegetables, leaf petiole vegetable turf, fruiting vegetables, and seed potatoes (USEPA 2005b). Up to 2.5 kg/ha can be applied per season. Fluoxastrobin degrades slowly with a half-life estimated at 1 month to over 1 year. The 96-hour LC50 for rainbow trout is 0.435 mg/L, and the no observed adverse effects concentration (NOAEC) for a 28-day exposure was 0.055 mg/L, and predicted exposure concentrations were as high as 0.033 mg/L (USEPA 2005b).

As with many of the other compounds in this chapter, concern for these chemicals occurs for several reasons: 1) there are few or no data on measured concentrations in the field under actual operations; 2) whereas acute data exist for 1 or 2 species of fish, no data are available for amphibians or reptiles; 3) even if acute data were available for herpetofauna, toxicity tends to increase with exposure duration, so the studies should last for the entire larval period, and chemicals may be more toxic in one stage of the life cycle than others, so embryo and larval tests need to be conducted; and 4) mortality is only the most severe of effects — sublethal effects expressed at lower concentrations may be debilitating to individuals and populations.

### Triazoles and Imidazole

These are fungicides that inhibit the CYP51-mediated enzyme 14α-demethylase, which is involved with sterol production and cell membrane formation (Hegelund et al. 2004). They are most effective in controlling fungi prior to spore formation and are often used as a preventative and as an early curative to fungal disease. The various types of triazoles and imidazoles are broadly used on many different crops and plants throughout the world (Fishel 2005).

Triazoles and imidazoles pose hazards to wildlife because their effects may not be limited to CYP51. Ketoconazole is a pharmaceutical that is considered a model for the functioning of triazoles and imidazoles (Ankley et al. 2007). Previous studies have shown that ketoconazole can decrease testosterone production in mammals by inhibiting other CYPs involved with steroid production (e.g., Feldman 1986). Ketoconazole also inhibits steroid production *in vivo* with fish gonadal preparations (Villeneuve et al. 2007).

Hegelund et al. (2004) investigated the effects of ketoconazole on CYP1A and CYP3A enzymes in rainbow trout and killifish (*Fundulus heteroclitus*). CYP3A enzymes are involved in liver and intestinal functions in vertebrates, especially with the metabolism of lipophilic substances that include many of the pharmaceuticals currently in use. CYP1A enzymes affect the metabolism of several organic pollutants, including polyaromatic hydrocarbons (PAHs; see Chapter 9, this volume). Thus, inhibition of either enzyme complex could have important secondary effects with an animal's ability to cope with contaminant exposure. The authors injected juvenile rainbow trout intraperitoneally with 12 to 100 mg ketoconazole/kg body mass and adult killifish with 25 mg/kg. Compared to controls, ketoconazole increased liver CYP1A protein levels and enzyme activity in rainbow trout at all dosages, but the responses to 12 and 25 mg/kg were greater than those to 50 and 100 mg/kg. Induction of CYP1A was also seen in intestines and kidney. Killifish did not show a response in CYP1A activity when injected with 25 mg/kg ketoconazole. CYP3A was inhibited at all dosages in rainbow trout and by 25 mg/kg in killifish, compared to their respective controls. In killifish induction of CYP3A was sex dependent, with greater protein induction occurring in females. Induction of CYP1A and CYP3A gene expression was at lower dosages of ketoconazole than those needed for induction in mammals or birds. The authors concluded that ketoconazole was more potent in inducing CYP1A than CYP3A enzymes in rainbow trout, but that the reverse relationship held for killifish. In either species, however, it was clear that the fungicide affected more than its targeted CYP51.

Tetraconazole, which was registered in 2005 as a fungicide on *Cercospora* leafspot and powdery mildew in sugar beets, is considered to be of concern to birds and mammals by the USEPA (2005c). Chronic risk levels of concern (LOCs) were exceeded for small birds and small mammals living in grasslands. Presumably, reptiles and terrestrial life stages of amphibians may be at similar risk, although no testing has been conducted with these vertebrate classes. Tetraconazole
is relatively persistent, with a half-life in soil, sediments, or water ranging from months to over a year. Its primary breakdown product, 1,2,4-triazole, also may be toxic at environmentally realistic concentrations. Terrestrial animals can be exposed to this fungicide through ingestion of vegetation or invertebrates; direct contact; inhalation of vapors, aerosols, or residues on dust; or ingestion of contaminated water. Secondary transfer can occur by ingesting vegetation that has taken up the chemical systemically. Aquatic organisms, including amphibian larvae, invertebrates, and fish, can be exposed through dermal absorption or uptake by gills. Tetraconazole is soluble up to 159 mg/L. In mallards (Anas platyrhynchos), tetraconazole significantly reduced egg laying, embryo survival, number of normal hatchlings, survival of chicks to 14 days, and chick body weight at 14 days. The NOAEC and LOAEC were 10 and 50 mg/kg diet, respectively. Reproductive effects in mallards and in rats may be due to endocrine disruption. Estimated doses to birds and mammals ingesting contaminated foods in natural settings ranged from 1 to 46 mg/kg. Greatest risk was to small (≤20 g) birds and mammals, which would also include many species of lizards and amphibians. Toxicity data on aquatic species are limited, but the 96-hour LC50 for bluegill was 3.85 mg/L and the 28-day LOAEC for growth in fathead minnows was 0.96 mg/L. These data, of course, are not very predictive for chronic effects in amphibian larvae. The greatest risk from tetraconazole to reptiles and amphibians is probably related to its reputed (but unproven) endocrine-disrupting effects, which would occur at exposure concentrations less than those necessary to kill animals outright.

Other recently registered conazoles are only slightly less problematic. Prothioconazole is used as a broadcast fungicide on barley, canola, chickpeas, oil seed crops, beans, lentils, and wheat. Its half-life in soils ranges from 553 to 1386 days. Under aerobic conditions it changes rapidly in water (half-life 15 to 20 days) into a comparably toxic degradate, and in anaerobic sediment its half-life is from 2 to 8 months (USEPA 2007). Limited data do not reveal any great, direct threat to aquatic animals, including amphibians. The substance is practically nontoxic to birds and mammals and, by extension, probably not a major threat to reptiles. However, prothioconazole and its degradate, prothioconazole-destho, are highly toxic to freshwater aquatic plants and invertebrates. The greatest risk to amphibians and aquatic or semiaquatic reptiles, therefore, may be through habitat degradation.

Cyazofamid is classified as a cyanoimidazole fungicide for downy mildew on cucurbit vegetables and blight in tomatoes and potatoes. It has a short half-life of several days and is listed as practically nontoxic to birds and mammals (USEPA 2004). The water solubility of cyazofamid is not accurately known but is estimated as 0.107 mg/L. At 0.179 mg/L growth of larval fathead minnows was significantly reduced. This suggests that the fungicide may be problematic at ppb concentrations to larval amphibians. Significant reproductive effects, including reduction in nestling survival, thinned eggshells, reduced hatching success, and depressed body weights in adult females, were observed in northern bobwhite (Colinus virginianus) and Japanese quail (Coturnix coturnix). The EPA recommended that further endocrine testing with wildlife species should be done (USEPA 2004), but data from those studies, if performed, were not found.

Triazoles and imidazoles may present risk to both amphibians and reptiles, depending on the type of fungicide. There is potential risk due to endocrine disruption, habitat deterioration, and direct mortality at concentrations that are environmentally realistic. This risk is magnified by the environmental persistence shown by some of the chemicals included here. As with other contaminants in this chapter, there is a paucity of data and no field studies have been conducted. In fact, methods for detection of some triazoles and imidazoles in environmental matrices are not available. The use of these fungicides has expanded tremendously in the past few years, and their environmental consequences are yet to be determined.

15.1.6.3 Other Fungicides

Dimethomorph is a systemic morpholine fungicide registered for use on potatoes, tomatoes, grapes, and other vegetables and fruits (USEPA 1998). As the sole active ingredient in formulation, it has moderate toxicity to freshwater fish and should not pose extreme hazards to aquatic life stages of
amphibians. It is practically nontoxic to birds and mammals, in either acute or chronic presentations, and may not be of concern to terrestrial amphibians or reptiles when used appropriately. However, the Acrobat® formulation combines dimethomorph with the carbamate mancozeb. Neither pesticide alone seems to be highly toxic to fish. For example, the 48-hour LC50 for mancozeb is 2.2 mg/L in rainbow trout and 5.2 mg/L in catfish (*Ictalurus punctatus*; E. I. DuPont Nemours 1983). The combined toxicity, however, translates to a 96-hour LC50 of 0.03 mg/L dimethomorph and 0.26 mg/L mancozeb on freshwater fishes (USEPA 1998).

Boscalid is an extremely stable carboxamide fungicide registered for food crops, including “beans, berries, bulb vegetables, canola, carrots, fruiting vegetables, grapes, lettuce, peanuts, pistachios” and 10 other food groups (USEPA 2003a p 1). During the testing required for registration, half-lives under various conditions were not obtained. Instead, the pesticide fact sheet (USEPA 2003a p 13) states the following: “Boscalid is hydrolytically stable and is photolytically stable on soil and in water. The compound is not transformed to any significant extent in either aerobic or anaerobic aquatic systems, but is relatively rapidly transferred (dissipation half-lives of <2 weeks) from the water phase to the sediment phase of sorbing to the sediment.” “Based on the results obtained at 25 °C, the parent compound is not expected to hydrolyze in the environment, rendering hydrolysis an insignificant fate process for boscalid.” “… photodegradation is not expected to be a significant route of dissipation for boscalid in the environment.” “For assessment purposes, boscalid may be considered to be essentially stable to microbial degradation in anaerobic soils.” The degradation of boscalid in aerobic soils was slow, with half-lives ranging from 96 to 578 days. The majority of the compound’s apparent degradation is actually due to its transformation to bound residues rather than to actual degradation or complete mineralization of the compound. In other words, it appears that boscalid is inert to common degradation pathways, but that it binds to soil and sediment particles for indefinite duration. The binding may reduce the bioavailability of boscalid, but we cannot help but to question the possibility of long-term effects of such a stable compound and what it might mean for benthic organisms such as the larva of some species of amphibians. While boscalid is considered practically nontoxic to birds and mammals, the only aquatic toxicity data we could find was a 96-hour LC50 of 2.7 mg/L and a 97-day NOAEC of 0.12 mg/L in rainbow trout. “Boscalid is classified as ‘suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential’” (USEPA 2003a). Can we say the same about benthic organisms?

Famoxadone is another fungicide that is very highly toxic to aquatic organisms and poses some threat to amphibians (USEPA 2003b). It is used in formulation with the fungicide cymoxanil on peppers, tomatoes, potatoes, curcubits, lettuce, and grapes. Famoxadone is not persistent and has a half-life of a few days. The fungicide can bioconcentrate with bioconcentration factors in fish ranging from 971 to 3608. The acute, 96-hour LC50 to bluegill is 13 μg/L, with an NOAEC of 9.3 μg/L. In chronic exposures the NOAEC = 1.4 μg/L and the LOAEC = 4.1 μg/L. “Agency analysis indicates that famoxadone presents the greatest risks to fish and aquatic invertebrates through spray drift and runoff in the dissolved phase as compared to the other taxonomic groups evaluated in this assessment” (USEPA 2003b). It is fair to include amphibians in that risk as well. The USEPA also states that the risk quotients for herbivorous and insectivorous birds and herbivorous mammals exceeded the levels of concern in wildlife food items and that there could be chronic risks. Similar warnings may be added for reptiles, although there are no toxicity data on this group for famoxadone.

### 15.2 CONCLUSIONS

It is axiomatic that to be at risk from a chemical contaminant, wildlife must be exposed to it at sufficiently high concentrations to cause acute, often lethal, effects, or at lower concentrations for a longer time to produce chronic sublethal effects. A major handicap in predicting the threat from a contaminant is that most toxicological studies in amphibians have been conducted in the laboratory.
These highly controlled studies provide information on possible lethal and sublethal effects but often are deficient in estimating risk under more complex field situations. For the most part, we lack even laboratory studies in reptiles. For many of the chemicals described in this chapter we have neither field nor laboratory data for either amphibians or reptiles. Data on other wildlife such as fish, birds, or mammals can suggest possible risk, but cannot confirm it. In addition, new methods are often required to detect some contaminants in the environment or in tissues. These methods are often lacking or are in development so that ecotoxicologists are not always aware that the contaminants are even present.

The chemicals highlighted in this chapter were chosen because they show at least some evidence of effects, are manufactured in high to very high quantities, and demonstrate either widespread dispersal or broadscale use. Fungicides, for example, are used extensively in many types of agriculture and are often aerially sprayed, thus increasing the potential for dispersal. They can contact wildlife directly or enter waterways through runoff and pose problems for aquatic herpetofauna. Biocides and pharmaceuticals do not appear to present much risk as direct exposures to herpetofauna, but they enter waterways through municipal water treatment plants and may locally cause problems. Brominated flame retardants appear to be the “new PCBs.” They, like perfluorinated hydrocarbons, have become globally dispersed. While they do not appear to be acutely toxic at environmentally realistic concentrations, their possible sublethal effects on free-ranging populations of herpetofauna, including endocrine disruption, are not well known. Similar concerns can be raised about anionic surfactants whose endocrine-disrupting properties are better known. The bottom line is that we really do not know to what extent these chemicals pose risks to amphibian and reptile populations.

In this chapter we describe only a few of the thousands of emerging chemicals that potentially threaten wildlife. As we stated in the beginning, others may disagree with this list as being too limited or perhaps not including some candidates that they believe pose greater threats. The intent of this chapter was not to be exhaustive but to increase awareness.

REFERENCES


Emerging Contaminants and Their Potential Effects on Amphibians and Reptiles


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