Aquatic toxicity and biodegradation of ionic liquids

A synthesis

ABSTRACT

Room-temperature ionic liquids (ILs) are non-volatile chemicals that are being developed as alternatives to many traditional industrial solvents. Although ILs are considered "environmentally friendly", their ecological effects have been understudied, despite their potential for release into freshwater ecosystems. Standard toxicological bioassays indicate that IL toxicity tends to be organism and chemical specific, though many ILs have toxicity comparable to traditional solvents. Pyridinium-based ILs have shown the greatest potential for biodegradation. We also discuss research challenges and provide recommendations for future studies. By promoting increased feedback and collaboration between scientists and engineers in academia, industry, and government, we can guide the development of these chemicals in a direction that both maximizes utility and minimizes environmental harm.

INTRODUCTION

Green chemistry is the use of a set of principles (Table 1) that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemicals (1). Two of the goals of green chemistry are to 1) reduce the toxicity of a chemical (i.e., its potential to have harmful effects on living organisms) and 2) increase its ability to degrade when no longer in use. An underlying assumption of such goals is that we understand if and why particular chemicals are hazardous, yet novel compounds are often designed with little or no environmental testing. Often, new chemicals prove to be as hazardous, though in different ways, as their predecessors (e.g., MTBE, CFCs), demonstrating the need to incorporate hazard assessment *prior to* widespread use. Rigorous environmental testing during the design phase might improve our ability to avoid such unintended consequences. Room-temperature ionic liquids (ILs) are an emerging class of chemicals being developed to reduce the use of volatile organic compounds (2). Ionic liquids include a wide variety of chemical structures, and therefore pose an unknown risk to the environment (3, 4). Many ILs are water soluble, and may enter and impact aquatic ecosystems (2).

1. Prevent waste instead of cleaning it up	7. Maximize atom economy
2. Design safer chemicals and products	8. Use safer solvents and reaction conditions
3. Design less hazardous chemical syntheses	9. Increase energy efficiency
4. Use renewable feedstocks	**10. Design chemicals to degrade after use**
5. Use catalysts, not stoichiometric reagents	11. Analyze in real time to prevent pollution
6. Avoid chemical derivatives	12. Minimize the potential for accidents

Table 1. The Twelve Principles of Green Chemistry (adapted from (1)). Principles addressed in this paper are bolded

A broad, interdisciplinary effort is being made to understand the chemical and environmental factors controlling IL toxicity and degradation and to incorporate those factors into IL design, applications, and disposal (4-6). A unique opportunity exists with ILs to develop chemicals that are as environmentally benign as possible but retain their functional properties (4, 5). Toxicity research has encompassed a variety of aquatic taxa, generally geared towards producing realistic predictions of potential environmental effects, while most degradation studies have focused on biodegradation pathways. Research on the environmental fate of ILs is limited, and has typically focused on interactions between ILs, soils, and sediments. We focus our review on IL toxicity and biodegradation because these are two important aspects of green chemistry, yet data are limited compared to the vast literature on IL generation and application (7). Here, we synthesize the current state-of-knowledge regarding IL aquatic toxicity and biodegradation.

AQUATIC ECOTOXICOLOGY

Ecotoxicology is the study of the toxic effects of natural or artificial substances on living organisms, and the interactions of these substances with the environment (8).
Because it can be illegal and unethical to release potentially hazardous chemicals into the environment, toxic effects are commonly studied in a laboratory using standard toxicity bioassays, which determine the relative toxicity of a substance by comparing its effects on test organisms with those of a control (9).
Acute bioassays expose an organism to a range of chemical concentrations for a short amount of time and determine an LC50 value for the chemical, the concentration that produces 50 percent mortality (i.e., Lethal Concentration) in a test population over a specific time period (10). If an effect other than mortality is investigated (e.g., growth or reproduction), the term EC50 is used to denote the Effect Concentration that is estimated to cause a 50 percent decrease or increase in the effect.
Standard metrics, such as LC50's, are comparable across organisms and chemicals (Tables 2, 3) and may be used to determine which chemicals are the most toxic to a particular organism, or which organism is most sensitive to a particular chemical.
The effects of ILs on aquatic organisms have been examined for different trophic levels (Figure 1), including primary producers (11-15) (e.g., algae and plants), primary consumers (16, 17) (e.g., invertebrates), and secondary consumers (18) (e.g., fish). Ionic liquid toxicity varies widely across organisms and trophic positions, as demonstrated by the IL 1-butyl-3-methylimidazolium bromide (bmimBr) (Table 2). Such tests provide important baseline information about the toxicity of ILs, and can inform future studies and modeling efforts. With *Daphnia magna*, a primary consumer, standard bioassays have shown several ILs to be as toxic, if not more so, than many currently used volatile solvents (Table 3). However, standard bioassays typically do not mimic the conditions in which an organism would be exposed in the environment,

Trophic Group	Organism (Reference #)	EC50 or LC50 [mg/L]
Decomposer (microbe)	*Vibrio fischeri* (15, 44, 45)	257.5 - 2248.4
1° producer (algae)	*Chlamydomonas reinhardtii* (11)	2153.0
1° producer (algae)	*Pseudokirchneriella subcapitata* (14)	38.5 - 45.0
1° producer (algae)	*Scenedesmus quadricauda* (11)	2.4
1° producer (algae)	*Scenedesmus vacuolatus* (15)	24.5
1° producer (vascular plant)	*Lemna minor* (12, 15)	8.6 -115.3
1° consumer (crustacean)	*Daphnia magna* (14, 16, 36)	3.2 - 14.8
1° consumer (mollusk)	*Dreissena polymorpha* (46)	1290.6
1° consumer (mollusk)	*Physa acuta* (17)	229.0
2° consumer (fish)	*Danio rerio* (18)	>100
2° consumer (fish)	*Pimephales promelas* (47)	302.0

Table 2. Summary of acute toxicity of [bmim]halide ([1-butyl-3-methylimidazolium] Br^- or Cl^-) to various aquatic test organisms

Chemical Name (Reference #)	LC50 (mg/L)
omimBr (16)	**0.013**
Chlorine (48)	0.12-0.15
ompyrBr (29)	**0.72**
hmpyrBr (29)	**1.00**
hmimBr (16)	**1.5**
Ammonia (49)	2.90-6.93
tetrabutylphosphoniumBr (29)	**2.99**
bmimBr (16)	8.03
tetrabutylammoniumBr (29)	**9.41**
Phenol (50)	10-17
bmimBF_4 (16)	**10.68**
bmpyrBr (29)	**13.30**
bmimCl (14, 16)	**6.5-14.80**
bmimPF_6 (14, 16)	**19.91-24**
Trichloromethane (51)	29
Tetrachloromethane (51)	35
Toluene (52)	86 – 313
Benzene (53)	356-620
Methanol (54)	3289
Acetonitrile (55)	3600
Sodium hexafluorophosphate (16)	9345
Acetone (56)	23500

Table 3. Acute toxicity (48-h LC50) to Daphnia magna of ILs (in bold) compared to other commonly used industrial compounds

nor do they take into account the effects of chemicals on interactions among organisms and with their environment (19). The fate of ILs in aquatic environments depends on interactions with both abiotic and biotic components, as well as characteristics of the ILs and environments themselves. Lab studies have shown that ILs have the capacity to sorb to soils and sediments (20-22), which could potentially reduce their risk to aquatic biota, though a recent study found a limited capacity for the retention of imidazolium-based ILs on natural aquatic sediments (23). The community of organisms present in an aquatic environment will also determine what effects ILs may have. Interactions among organisms are often overlooked in ecotoxicology, and research is needed to examine the effects of ILs on predator-prey interactions (but see (24)), competition, and other interspecific interactions (25). Several ILs have been shown to have low octanol-water partitioning coefficients and low lipophilicity, indicating that they are likely to remain in the aqueous phase and not bioaccumulate in organisms (26, 27). The toxicity of ILs can also be mediated by environmental conditions, such as dissolved organic matter (12), nutrients (11), and salinity (13). Predicting the effects of ILs in natural environments is challenging due to the many possible IL structural configurations (which coincidentally make them so attractive to industry), and the complex effects of ILs on entire food webs. One approach to addressing these challenges is to use structure-based modeling of novel IL configurations coupled with food web models of aquatic ecosystems. Quantitative structure-property relationship (QSPR) modeling can be used to determine which structural factors affect the toxicity of an IL (28-30), and can give chemists an estimate of toxicity while avoiding the costs associated with the production and testing of a new IL. Food web models can be used to predict the direct and indirect effects of pollutants in complex food webs (31, 32). We can apply similar techniques to understand the potential effects of ILs on multiple organisms and the interactions among them, although empirical data regarding IL effects on species interactions is lacking. Single-species toxicity bioassays coupled with more complex multi-species bioassays and predictive models can provide a better understanding of the environmental hazards of ILs.

BIODEGRADATION

Biodegradation is the microbial catabolism of a chemical compound into metabolically useful products (33). Understanding the biodegradation pathway(s) of any chemical is important to determine its potential environmental effects. With any organic compound, including ILs, the end products of biodegradation are ultimately carbon dioxide, biomass, and water. However, incomplete biodegradation may produce intermediary molecules of differing toxicities compared to the initial parent compound. Theoretical biodegradation pathways proposed for the bmim cation suggest the production of several potential metabolites of the molecule (5). However, relatively few empirical studies of IL biodegradation exist.

Because ILs are new compounds, they cannot be readily studied using traditional biodegradation methods that include radioactively labeled elements. Thus, most published IL biodegradation studies have been performed according to the Organisation (sic) for Economic Cooperation and Development (OECD) standard tests. These tests generally rate a compound as "readily biodegradable" if at least 60 percent mineralization occurs within a 10-day period falling within 28 days of incubation with the microbial inoculate, usually consisting of wastewater treatment plant activated sludge (34).

Most biodegradability tests to date have indicated that the bmim cation is very recalcitrant, resisting microbial biodegradation for over 60 days (14, 35-38). Longer alkyl chain-substituted imidazoliums (e.g., hmimBr and omimBr) can be partially mineralized, but do not achieve the OECD pass levels for biodegradability using the DOC Die-Away test (35). ^{1}H-NMR results suggest that the partial catabolism measured is due to degradation of the alkyl chain substitution and not of the imidazolium ring itself (35). Substitution with side-chain ester groups can enhance biodegradation, but still does not achieve OECD pass levels with the imidazolium cation (37). The IL bmim octylsulfate can achieve adequate OECD pass levels for biodegradability, likely because of the addition of a labile anion rather than through imidazolium ring catabolism (38). One drawback of the OECD method is that no direct chemical analyses or further toxicity measures are required to assess

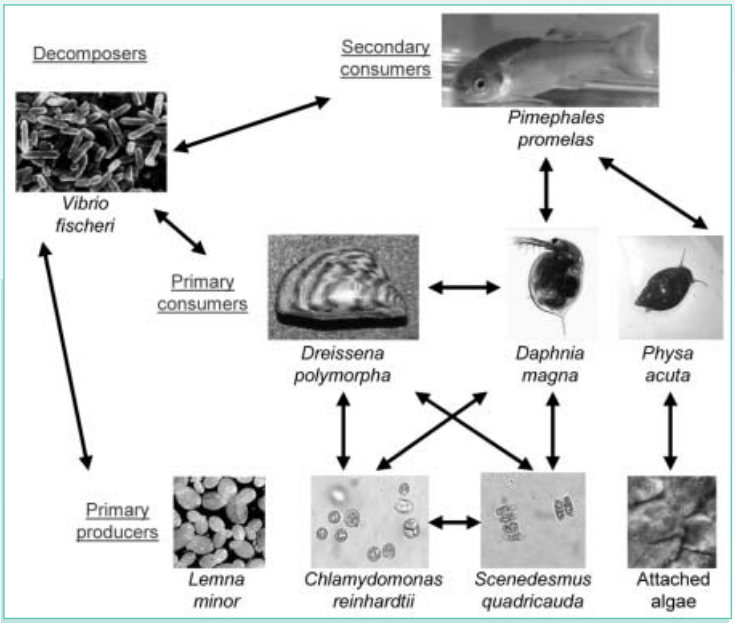

Figure 1. A simple aquatic food web with example members whose IL toxicity has been measured at the University of Notre Dame. Arrows indicate interactions between the different trophic levels

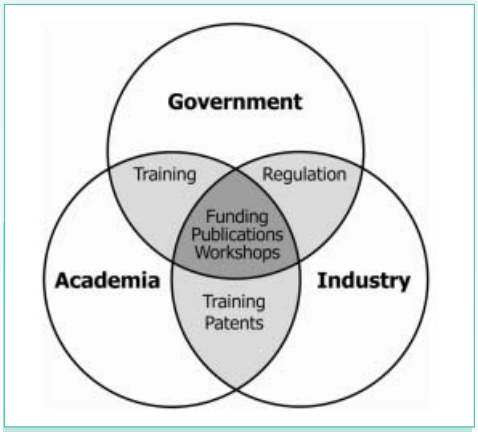

Figure 2. Desirable interactions between government, academia, and industry. Each overlapping area, especially the center, would enhance the development and use of green chemicals

"readily biodegradability", so a chemical can be considered biodegradable even if it is not catabolized into non-toxic products.

In contrast to imidazolium-based ILs, pyridinium-based ILs can be fully catabolized by activated sludge microorganisms. However, only 1-octyl-3-methylpyridinium bromide (ompyrBr) (35) and 1-$C_{11}H_{23}$-oxymethyl-3-hydroxypyridinium salts (39) can be definitively classified as readily biodegradable by OECD standards. Full mineralization of bmpyrBr and hmpyrBr can be achieved with longer incubation periods, as verified with ^{1}H-NMR and RP-HPLC-MS detection of biodegradation products (7, 35). While most IL synthesis and toxicity studies have focused on imidazolium-based cations, full biodegradability of pyridinium ILs makes them attractive as green chemicals because they can be completely converted to carbon dioxide and biomass. Although increasing the length of a substituted alkyl chain on an IL increases it biodegradability, it also increases its toxicity (4, 7, 39). Future studies should expand the range of compounds to better understand the link between IL chemical structure and biodegradability, thereby allowing IL synthesis that balances archetypal green chemical engineering properties, biodegradability and low toxicity, with functionality. Molecular techniques (e.g., denaturing gradient gel electrophoresis) can be used to characterize microbial communities involved in IL degradation. Particular microbial consortia appear critical for IL breakdown, although these consortia change as biodegradation progresses (35). This information has implications for "priming" wastewater treatment plants that are most likely to receive ILs in waste streams. Specific information on the actively biodegrading microbial community will also be useful for creating on-site industrial waste-treatment bioreactors.

Overall, biodegradation studies suggest that ILs can be synthesized to be biodegradable (7, 39). Although microorganisms exist that can break down ILs, these microbial consortia are likely to be absent or in much lower densities in natural environments as compared with wastewater treatment plants. Furthermore, multi-organism communities may be responsible for IL biodegradation, making isolation of a specific microorganism for use in future bioreactor or "priming" experiments more challenging (7). Because some ILs do not readily biodegrade, even a diverse wastewater microbial community may not degrade all ILs considered for industrial applications in the future. The goal should be to synthesize chemically green solvents, according to engineering standards, that also have low toxicity and high biodegradability.

FUTURE CHALLENGES AND RECOMMENDATIONS

Challenges

We see four important challenges facing the IL research and development community. First, the mode-of-action of IL toxicity is poorly understood. While ILs have been shown to affect different systems (40-42), and disruption of cellular membranes has been proposed (5) and shown (43) in some systems, more research is needed to demonstrate the mechanisms of IL toxicity. Second, standard tests (as described above) may be inadequate to fully predict the (eco)toxicity and biodegradability of given ILs in the environment. Third, information about IL toxicity and biodegradability is not always easily available to chemists developing ILs. Lastly, society now expects that chemists, engineers, toxicologists, and biologists will work together to design effective *and* environmentally benign chemicals.

Recommendations

We offer two global recommendations for IL development and application.

First, more informative and realistic experimental systems are needed to assess the toxicity of ILs. Such experimental systems should include a broader range of conditions that more closely mimic natural environments.

Furthermore, experiments should be designed to provide the information needed to parameterize predictive models, which extend beyond simple LC50 values.

Second, future development of effective 'green' ILs requires interaction among chemical engineers, toxicologists, and ecologists. In particular, mechanisms are needed to enhance information exchange among these disciplines, as well as

among researchers in industry, academia, and government (Figure 2). Activities such as round-table discussions, workshops, limited disclosure of patented and/or trademarked information, and interdisciplinary journals encouraging short, more accessible communications would facilitate such exchanges.
Recent examples include the BATIL (Biodegradability And Toxicity of Ionic Liquids; 2007) and COIL (Congress On Ionic Liquids; 2005, 2007, 2009) meetings. By increasing interdisciplinary collaboration and exchange, ILs can be developed that are as effective and environmentally benign as possible.

CONCLUSIONS

Important tradeoffs often exist in the development of new technologies. The research highlighted here demonstrates that for ILs, such tradeoffs are apparent between toxicity and biodegradability, air and water pollution, and perhaps even functionality and the "greenness" of a chemical. While many ILs are toxic to aquatic organisms, they remain an improvement over traditional solvents due to their non-volatility.
Current IL research illustrates the need to be proactive in assessing the ecological hazards posed by new chemicals. The benefits of addressing potential hazards at the development stage rather than after impacts have occurred will far outweigh the costs of assessment over the long term.

REFERENCES AND NOTES

1. P. T. Anastas, J. C. Warner, "Green Chemistry: Theory and Practice", Oxford University Press, p. 135 (1998).
2. J. F. Brennecke, E. J. Maginn, AIChE J., 47, pp. 2384-2389 (2001).
3. T. Welton, Chem. Rev., 99, pp. 2071-2083 (1999).
4. B. Jastorff, K. Mölter et al., Green Chem., 7, pp. 362-372 (2005).
5. B. Jastorff, R. Störmann et al., Green Chem., 5, pp. 136-142 (2003).
6. J. Ranke, S. Stolte et al., Chem. Rev., 107, pp. 2183-2206 (2007).
7. K. M. Docherty, Ph. D. Dissertation, University of Notre Dame, USA (2007).
8. G. C. Butler (ed.), "Principles of Ecotoxicology", Wiley (1978).
9. EPA, Terms of Environment: Glossary, Abbreviations and Acronyms, http://www.epa.gov/OCEPAterms/bterms.html (2006)
10. G. M. Rand, P. G. Wells et al., "Introduction to Aquatic Toxicology", in Fundamentals of Aquatic Toxicology, G. M. Rand, Ed., Taylor & Francis, pp. 3-67 (1995).
11. K. J. Kulacki, G. A. Lamberti, Green Chem., DOI:10.1039/B709289J, In Press.
12. J. H. Larson, P. C. Frost et al., Environ. Toxicol. Chem., DOI:10.1897/06-540, In Press.
13. A. Latala, P. Stepnowski et al., Aquat. Toxicol., 73, pp. 91-98 (2005).
14. A. S. Wells, V. T. Coombe, Org. Process Res. Dev., 10, pp. 794-798 (2006).
15. M. Matzke, S. Stolte et al., Green Chem., (DOI:10.1039/b705795d) In Press.
16. R. J. Bernot, M. A. Brueseke et al., Environ. Toxicol. Chem., 24, pp. 87-92 (2005).
17. R. J. Bernot, E. E. Kennedy et al., Environ. Toxicol. Chem., 24, pp. 1759-1765 (2005).
18. C. Pretti, C. Chiappe et al., Green Chem., 8, pp. 238-240 (2006).
19. M. Crane, Hydrobiologia, 346, pp. 149-155 (1997).
20. P. Stepnowski, Aust. J. Chem., 58, pp. 170-173 (2005).
21. P. Stepnowski, W. Mrozik et al., Environ. Sci. Technol., 41, pp. 511-516 (2007).
22. D. J. Gorman-Lewis, J. B. Fein, Environ. Sci. Technol., 38, pp. 2491-2495 (2004).
23. J. J. Beaulieu, J. L. Tank et al., Chemosphere, DOI:10.1016/j.chemosphere.2007.07.046, In Press.
24. M. A. Evans-White, Ph. D. Dissertation, University of Notre Dame, USA (2005).
25. B. L. Preston, Environ. Manag., 29, pp. 311-323 (2002).
26. L. Ropel, L. S. Belvèze et al., Green Chem., 7, pp. 83-90 (2005).
27. P. Stepnowski, P. Storoniak, Environ. Sci. & Pollut. Res., 12, pp. 199-204 (2005).
28. S. Stolte, J. Arning et al., Green Chem., 9, pp. 760-767 (2007).
29. D. J. Couling, R. J. Bernot et al., Green Chem., 8, pp. 82-90 (2006).
30. J. Ranke, A. Muller et al., Ecotoxicol. Environ. Saf., 67, pp. 430-438 (2007).
31. D. Mackay, A. Fraser, Environ. Pollut., 110, pp. 375-391 (2000).
32. R. V. Thomann, J. P. Connolly, Environ. Sci. Technol., 18, pp. 65-71 (1984).
33. L. P. Wackett, C. D. Hershberger, "Biocatalysis and Biodegradation: Microbial Transformation of Organic Compounds", ASM Press (2001).
34. OECD, 301 A-F (1992).
35. K. M. Docherty, J. K. Dixon et al., Biodegradation, 18, pp. 481-493 (2007).
36. M. T. Garcia, N. Gathergood et al., Green Chem., 7, pp. 9-14 (2005).
37. N. Gathergood, M. T. Garcia et al., Green Chem., 6, pp. 166-175 (2004).
38. N. Gathergood, P. J. Scammells et al., Green Chem., 8, pp. 156-160 (2006).
39. M. Stasiewicz, E. Mulkiewicz et al., Ecotoxicol. Environ. Saf., DOI:10.1016/j.ecoenv.2007.08.011, In Press.
40. F. Stock, J. Hoffmann et al., Green Chem., 6, pp. 286-290 (2004).
41. S. Stolte, J. Arning, et al., Green Chem., 8, pp. 621-629 (2006).
42. P. Stepnowski, A. C. Skladanowski et al., Hum. Exp. Toxicol., 23, pp. 513-517 (2004).
43. K. O. Evans, Colloid Surface A, 274, pp. 11-17 (2006).
44. J. Ranke, K. Mölter et al., Ecotoxicol. Environ. Saf., 58, pp. 396-404 (2004).
45. K. M. Docherty, J. Kulpa, C.F., Green Chem., 7, pp. 185-189 (2005).
46. D. M. Costello, (Personal communication).
47. M. A. Brueseke, (Personal communication).
48. M. Kaniewska-Prus, Pol. Arch. Hydrobiol., 29, pp. 607-624 (1982).
49. D. I. Mount, T. J. Norberg, Environ. Toxicol. Chem., 3, pp. 425-434 (1984).
50. U. M. Cowgill, D. P. Milazzo, Arch. Environ. Contam. Toxicol., 20, pp. 211-217 (1991).
51. G. A. LeBlanc, Bull. Environ. Contam. Toxicol., 24, pp. 684-691 (1980).
52. M. M. MacLean, K. G. Doe, Environment Canada, EE-111, p. 64 (1989).
53. J. H. Canton, D. M. M. Adema, Hydrobiologia, 59, pp. 135-140 (1978).
54. Z. Tong, Z. Huailin et al., Bull. Environ. Contam. Toxicol., 57, pp. 655-659 (1996).
55. L. Guilhermino, T. Diamantino et al., Ecotoxicol. Environ. Saf., 46, pp. 357-362 (2000).
56. P. S. Ziegenfuss, W. J. Renaudette et al., "Methodology for assessing the acute toxicity of chemicals sorbed to sediments: Testing the equilibrium partitioning theory", in Aquatic Toxicology and Environmental Fate, T. M. Poston and R. Purdy, Ed., ASTM, pp. 479-493 (1986).

57. Research was undertaken with funding from the following sources: U.S. National Oceanic and Atmospheric Administration (Grant #NO04OAR4600076, #NA05OAR4601153); U.S. Department of Education's GAANN Program (Graduate Assistance in Areas of National Need, Grant #P200A010448); State of Indiana 21st Century Science and Technology Fund (Grant #909010455). We also thank Joan F. Brennecke, JaNeille K. Dixon, and the staff of the University of Notre Dame's Center for Environmental Science and Technology (CEST) for their assistance throughout this project.

KONRAD J. KULACKI[1]*, DOMINIC T. CHALONER[1], DAVID M. COSTELLO[1], KATHRYN M. DOCHERTY[2], JAMES H. LARSON[3], RANDALL J. BERNOT[4], MICHAEL A. BRUESEKE[1], CHARLES F. KULPA JR.[1], GARY A. LAMBERTI[1]

*Corresponding author

1. Department of Biological Sciences
University of Notre Dame, Notre Dame, IN 46556, USA

2. Center for Ecology and Evolutionary Biology
University of Oregon, Eugene, OR 97403, USA

3. Environmental Services Section
Kansas Department of Wildlife and Parks
Pratt, KS 67124, USA

4. Department of Biology
Ball State University, Muncie, IN 47306, USA