Development and Synthesis of a Native Freshwater Mussel Toxicity Database

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ABSTRACT

BRYAN, REBECCA. MASTER OF ENVIRONMENTAL ASSESSMENT. DEVELOPMENT AND SYNTHESIS OF A NATIVE FRESHWATER MUSSEL TOXICITY DATABASE

A database of native freshwater mussel toxicity test results was developed based on a literature search and review of 125 published articles and other available data. The database includes quantitative results from toxicity studies published from 1991 to 2014. The number of data results compiled in the database for metals were 10 for mercury, 22 for lead, 138 for zinc, 169 for cadmium, and 199 for copper. An extended analysis of the copper data indicates that there were 99 acute LC_{50}/EC_{50} results conducted according to American Society of Testing and Materials (ASTM) toxicity testing guideline recommendations (E2455-06; reapproved 2013), with 62 acute 24-hour glochidia (larvae) test results and 37 acute 96-hour juvenile mussel results. The number of data results compiled in the database for pesticides were 2 for glyphosate, 3 for diazinon, 8 for chlorpyrifos, 9 for permethrin, 13 for 2,4-D and pentachlorophenol, 15 for carbaryl, 26 for atrazine, and 35 for nonylphenol. The test results observed for acute glochidia and juvenile tests with copper were variable between different freshwater mussel species, indicating more than one freshwater mussel species should be used in laboratory toxicity testing to provide dependable toxicity data based on different habitat requirements. However, restricting the number of species recommended for toxicity test types will provide more reproducible laboratory data for comparisons and effect determinations. For continued expansion of the database, toxicity results from tests with the emerging contaminants of major ions, pharmaceuticals, and chemicals from pollution events in freshwater need additional representation. Additional quantitative database endpoints that follow ASTM guidelines are needed for the current database toxicants and additional chemicals of concern. The toxicity test results in the database provide guidance on freshwater mussel toxicants based on survival and effects so that appropriate water quality criteria and standards can be derived in the future.

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INTRODUCTION

The North American freshwater mussel (superfamily Unionidae) populations are in decline and are threatened by various factors, including decreased habitat quality due to pollution (Augspurger et al. 2007, Cope et al 2008, Williams et al. 1993). More than 70% of the 300 native unionid species are listed as endangered, threatened, or of special concern (Augspurger 2003, Cope 2007, Williams et al. 1993, Williams and Neves 1995). The study of anthropogenic pollution sources and the resulting chemical contaminants are important for the protection and conservation of native freshwater mussel species. Based on toxicological data published in the early 1990's, early life stages of freshwater mussels are among the most sensitive aquatic organisms when exposed to various toxicants, including metals (Keller and Zam, 1991; Jacobson et al. 1993), chlorine (Goudreau et al. 1993), and ammonia (Goudreau et al. 1993). Early published toxicity studies conducted with a subset of organic solvents and pesticides indicated freshwater mussels were relatively tolerant to some chemical contaminants (Augspurger 2003; Keller 1993; Keller and Ruessler 1997).

An accurate and updated database of mussel toxicity results from laboratory tests of specific pollutants is critical to the risk analysis and protection of freshwater mussels. The amount of reliable quantitative data from toxicity tests with freshwater mussels has increased over time since the approval of the American Society of Testing and Materials (ASTM) guideline "Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels" in 2006 (E2455- 06; reapproved 2013; https://www.astm.org/Standards/E2455.htm). This guideline provides methods for acute and chronic laboratory studies with freshwater mussels, but there are currently no United States Environmental Protection Agency (EPA) Office of Chemical Safety and Pollution Prevention (OCSPP) FIFRA (Fungicide, Insecticide Rodenticide Act) guidelines for the review or submission of freshwater mussel toxicity data for pesticide registration. Prior to 2006, data captured from published literature and other sources included variable amounts of useful quantitative toxicity data, in which some, but not all, aspects of the ASTM guideline requirements were met in published articles. Using ASTM method E2455-06 as the standard, additional reliable toxicological information is available from more recent tests conducted with early life stages of freshwater mussels to a variety of toxicants, including ammonia, chlorine, major ions, and pesticides (Cope 2007; Bringolf et al. 2007; Farag and Harper 2014; Fritts et al. 2014; Harper 2014; Wang et al. 2007a, 2007 b, and 2010).

The purpose of this project was to conduct an extensive literature search for published journal articles containing any available freshwater mussel toxicity data, to develop a database from these articles that includes reported laboratory methods and quantitative toxicity test results, and to synthesize and summarize the findings.

MATERIALS AND METHODS

Description of Literature Search: Articles and source materials dated from 1974 to 2016 were included in the literature search references and/or used in the associated database. The literature search of published freshwater mussel toxicity studies was conducted from various reliable sources. Initially, the North Carolina State University libraries (http://www.lib.ncsu.edu/) and

Google Scholar (http://scholar.google.com/) web search engines were used to locate, download, and evaluate articles for database criteria. The search terms and keywords utilized were combinations of the following: *freshwater mussels, freshwater mollusks, Unionidae, acute toxicity, chronic toxicity, ecotoxicology, LC_{50} values, and NOEC values.* Toxicity data were collected from downloaded articles, as well as from Dr. W. Gregory Cope's office files of published articles stored at North Carolina State University (Raleigh, NC) and from digital files supplied by Dr. Tom Augspurger from his research at U.S. Fish and Wildlife Service (Raleigh, NC). For most recent available freshwater mollusk literature, the "2015 Freshwater Mollusk Bibliography" written by Kevin S. Cummings from Illinois Natural History Survey (Champaign, IL) was searched from the Unionoida subject heading list for articles containing toxicity data (Cummings K. 2016. http://molluskconservation.org/PUBLICATIONS/ELLIPSARIA/EllipsariaJune2016.pdf). A total of 125 freshwater mussel toxicity references were reviewed in the literature search portion of this project and are presented in **Appendix A**.

Database Development Method: The publications and sources containing quantitative toxicity data for freshwater mussels were included in the database and were dated from 1991 to 2014. A template for the database had been created and maintained at U.S. Fish and Wildlife Service, Raleigh Ecological Services Field Office using Microsoft® Excel® spreadsheets, and initially included tests with chlorine, cadmium, copper, mercury, and zinc. The template had not been updated in recent years. This template database was compiled from the published peer-reviewed literature, university theses and dissertations, and other gray literature. For the database developed for this project, additional chemicals were added to the existing spreadsheets based on the current literature search results, and the database design with all information collected for each toxicity test result is presented in **Appendix B**.

Data Included in Database: The database includes the chemical name and purity of the test chemical (if reported). The test species name is reported in the database and 25 different Unionidae freshwater mussel species of the almost 300 North American species were used in the toxicity tests (see **Table 1**). The test method information collected included life stages of glochidia or juvenile, age in days, transformation type of *in vivo* or *in vitro* for juvenile tests, study type (acute, chronic, or other), and test duration in hours (or days if chronic), and water type (i.e., ASTM reconstituted hard water).

Table 1: List of Freshwater Mussel Species in Database
Actinonaias ligamentia
Actinonaias pectorosa
Andonta cygnea zellensis L.
Anodonta grandis
Epioblasma capsaeformis
Elliptio complanata
Lampsilis cardium
Lampsilis fasciola
Lampsilis rafinesqueana
Lampsilis siliquoidea
Lampsilis straminea
Lampsilis teres
Lasmigona costata
Leptodea fragilis
Ligumia subrostrata
Medionidus conradicus
Megalonaias nervosa
Potamilus purpuratus
Ptychobranchus fasciolaris
Ptychobranchus occidentalis
Utterbackia imbecillis
Villosa ebulosi
Villosa nebulosa
Villosa iris

Based on available data provided in journal articles, the parameters added to the template database spreadsheets included chemical purity (%); the laboratory environmental endpoints of temperature (°C), hardness (mg/L), pH, and alkalinity (mg/L) of test water; and determination if the test generally followed current ASTM guidelines for acute or chronic toxicity tests (see **Table 2**). Some of the database toxicity tests conducted prior to the 2006 ASTM guideline met most of the acceptability requirements based on reported test data, and were, therefore, considered acceptable. The "ASTM followed" database column was reported as "Yes" based on recommendations of test durations, age at study start, and control survival. The environmental conditions of laboratory test water based on ASTM were optional requirements for the database and reported if provided in published articles.

Table 2. ASTM Acceptability Requirements for Database
For **acute toxicity tests with glochidia** isolated from adult mussels:
• Test duration of 24 hours recommended
• Age <24 hours old at study start (viability ≥80%, ≥90% preferred)
• Control survival average at end of test ≥90% (and >90% for 24 hours after test)
• Optional (if provided): Hardness, alkalinity, pH in dilution water should not vary by more than ±10% during exposure
• Optional (if provided): Temperatures of individual chambers within 1°C; concurrent temperatures between chambers within 2°C
For **acute or chronic toxicity tests** with **juvenile** freshwater mussels:
• Acute test duration of 96 hours recommended, chronic test durations of 10-28 days
• *In vivo* transformation
• Control survival average at end of 96-hour test ≥90% (and >90% for 24 hours after 96-hour test); control survival average at end of 10- or 28-day test ≥80%
• Optional (if provided): Hardness, alkalinity, pH in dilution water should not vary by more than ±10% during exposure
• Optional (if provided): Temperatures of individual chambers within 1°C; concurrent temperatures between chambers within 2°C

The toxicity test results included in the database were control survival (%); observation endpoint of survival, length, or other-genotoxicity; and the results endpoints of median lethal or median effective (LC_{50} or EC_{50}) concentration, no observed effect concentration (NOEC), lowest observed effect concentration (LOEC), inhibition concentrations (IC10, IC20, or IC25), chronic value (ChV), or species mean acute value (SMAV). There is one quantitative result endpoint for each database row entry, and one toxicity test could have multiple rows based on number of reported results.

Exclusion criteria: All articles were reviewed during the literature search, but not all were included in database. Reasons for exclusion were the quantitative acute or chronic result endpoints were not reported (only qualitative data), no original data were presented (review article), data were duplicated results from another article, and QA/QC of reported data was inadequate. Freshwater mussel toxicity studies conducted with boron (Soucek et al. 2011; US EPA 2010), perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) (Hazelton et al. 2012), polycyclic aromatic hydrocarbons (PAHs) (Prochazka et al. 2012; Wang 2013), pharmaceuticals (Bringolf et al. 2012; Gilroy et al. 2014; Hazelton et al. 2013 and 2014), pesticides (Boogaard et al. 2011; parts of Bringolf et al. 2007a, 2007b and 2007c; Köprücü et al. 2008), and herbicides specifically (Archambault et al. 2014) were found during the literature search of articles published after the approval of the 2006 ASTM guidelines, but were excluded from the database for one or more of the above-mentioned reasons.

RESULTS

Database Development Results:

A total of 30 published references dated from 1991 to 2014 were used in the development of the freshwater mussel toxicity database (see **Table 3**).

Table 3: Database References		
Assigned Reference Number	**Chemicals in Database**	**Associated Citations**
1	Ammonia, Cadmium, Copper, Chlorine, nonylphenol	Black MC. 2001
2	Atrazine	Bringolf RB, et al. 2007a
3	Atrazine, chlorpyrifos	Bringolf RB, et al. 2007c
4	Zinc	Cherry DS, et al. 1991.
5	Chlorine	Cherry DS, et al. 2005b.
6	Aluminum, Cadmium, Iron, Manganese, Zinc	Clem SA. 1998.
7	Copper, Glyphosate, Carbaryl, Diazinon	Conners DE, Black MC. 2004
8	Sodium bicarbonate	Farag AM, Harper DD. 2014.
9	Sodium chloride	Fritts AK, et al. 2014.
10	Chlorine	Goudreau SE, et al. 1993.
11	Sodium bicarbonate	Harper DD, et al. 2014.
12	Copper	Jacobson PJ, et al. 1993
13	Copper	Jacobson PJ, et al. 1997.
14	Atrazine	Johnson IC, et al. 1993.
15	Cadmium, Zinc, Mercury	Keller AE. Unpublished. USEPA. 1999.
16	Cadmium, Copper, Mercury	Keller AE, Zam SG. 1991.
17	Cadmium	Klaine SJ, et al. 1997.
18	Cadmium	Lasee BA. 1991.
19	Copper, Zinc	McCann MT. 1993.
20	Carbaryl, PCP, 4-nonylphenol, Permethrin, 2,4-D, Copper	Milam CD, et al. 2005.
21	Ammonia, Nitrate, Nitrite, Potassium, Copper, Zinc, Atrazine	Myers-Kinzie M. 1998.
22	Cadmium, Copper, Zinc	Pynnönen K. 1995.
23	Cadmium	USEPA. 2001.
24	Chlorine	U.S. Geological Survey. 2004.
25	Chlorine, Copper	U.S. Geological Survey. 2005.
26	Mercury	Valenti TW, et al. 2005.
27	Copper, Ammonia, Chlorine	Wang N, et al. 2007a.
28	Copper, Ammonia	Wang N, et al. 2007b.
29	Lead, Cadmium, Zinc, Copper	Wang N, et al. 2010.
30	Copper	Warren LW. 1996.

There were 18 total chemicals included in the database (see **Table 4**): the toxicants of ammonia and chlorine (1993-2007); the metals of cadmium, copper, lead, mercury, and zinc (1991-2010); the major ions of sodium bicarbonate and sodium chloride (2014); and the pesticides 2,4-D, atrazine, carbaryl, chlorpyrifos, diazinon, glyphosate, nonylphenol, pentachlorphenol (PCP), and permethrin (1993-2007) (see **Figure 1**). Most database references contained data associated with multiple chemicals and multiple toxicity studies (acute and/or chronic) with different freshwater mussel species. Based on all 18 chemicals reviewed, a total of 824 toxicity endpoints were compiled in the database. These results correspond to totals of 157 endpoints for toxicants, 538 endpoints for metals, 5 endpoints for ions, and 124 endpoints for pesticides (see **Figure 2**).

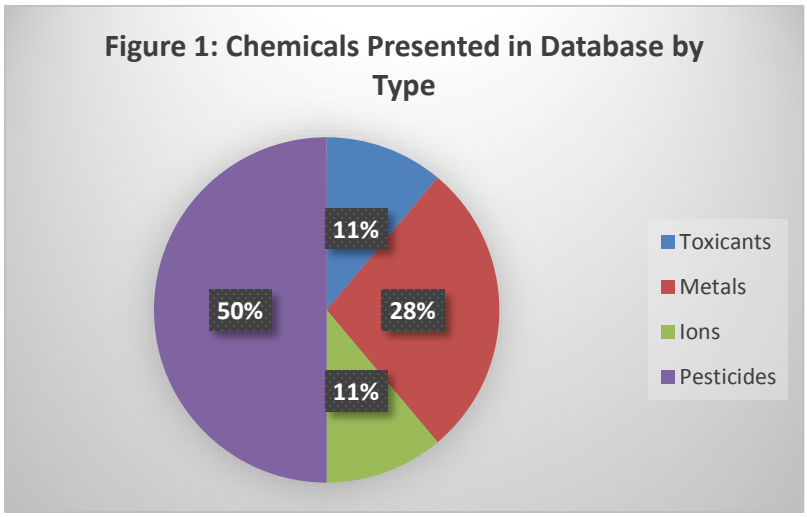


Figure 1: Chemicals Presented in Database by Type
11%
28%
11%
50%
Toxicants
Metals
Ions
Pesticides

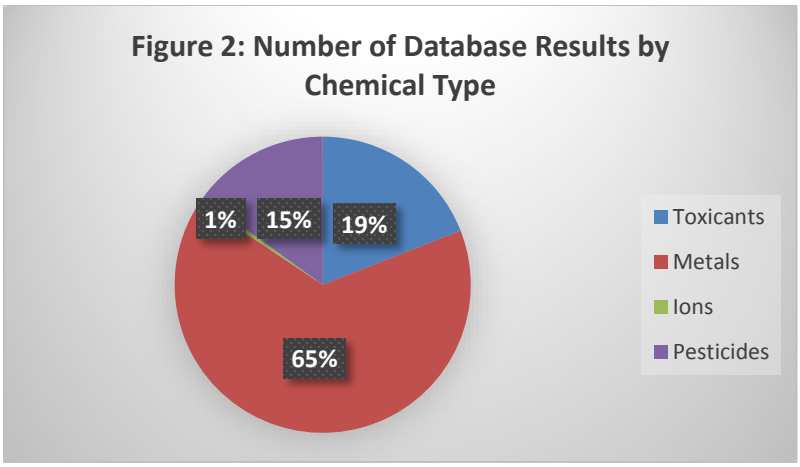


Figure 2: Number of Database Results by Chemical Type
1%
15%
19%
65%
Toxicants
Metals
Ions
Pesticides

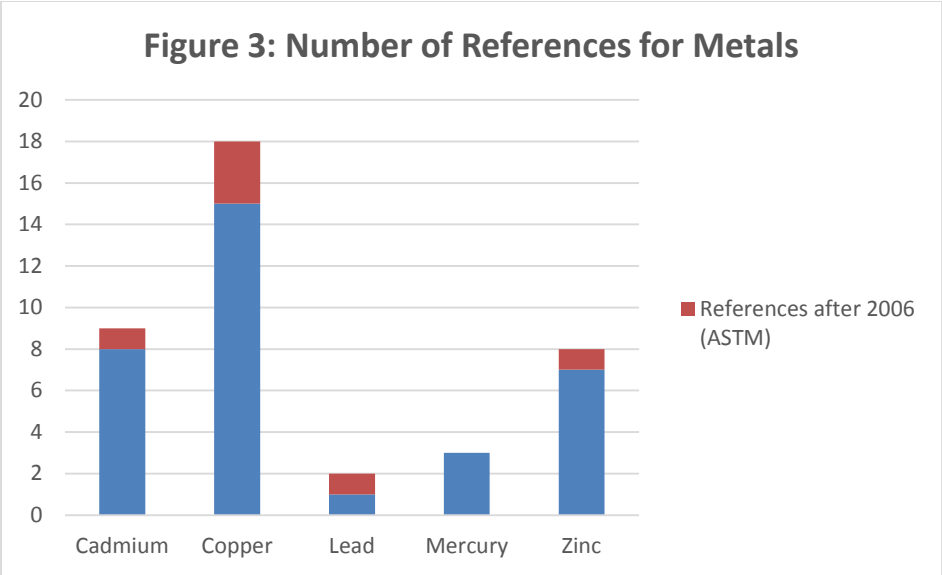
Table 4: Freshwater Mussel Toxicity Database Totals					
Assigned Chemical Number	**Chemical Name**	**No. of References**	**Years of References**	**Number of Endpoints [1]**	**Number of References Published after 2006 ASTM**
Toxicants					
1	Ammonia	2	2007	42	2
2	Chlorine	6	1993-2007	115	1
Metals					
3	Cadmium	8	1991-2010	169	1
4	Copper	15	1991-2010	199	3
5	Lead	1	2010	22	1
6	Mercury	3	1991-2005	10	0
7	Zinc	7	1991-2010	138	1
Ions					
8	Sodium bicarbonate	2	2014	3	2
9	Sodium chloride	1	2014	2	1
Pesticides					
10	2,4-D	1	2003	13	0
11	Atrazine	4	1993-2007	26	2 (duplicate acute results)
12	Carbaryl	2	2004	15	0
13	Chlorpyrifos	1	2007	8	1
14	Diazinon	1	2004	3	0
15	Glyphosate	1	2004	2	0
16	nonylphenol	2	2001-2005	35	0
17	Pentachlorophenol (PCP)	1	2003	13	0
18	Permethrin	1	2003	9	0

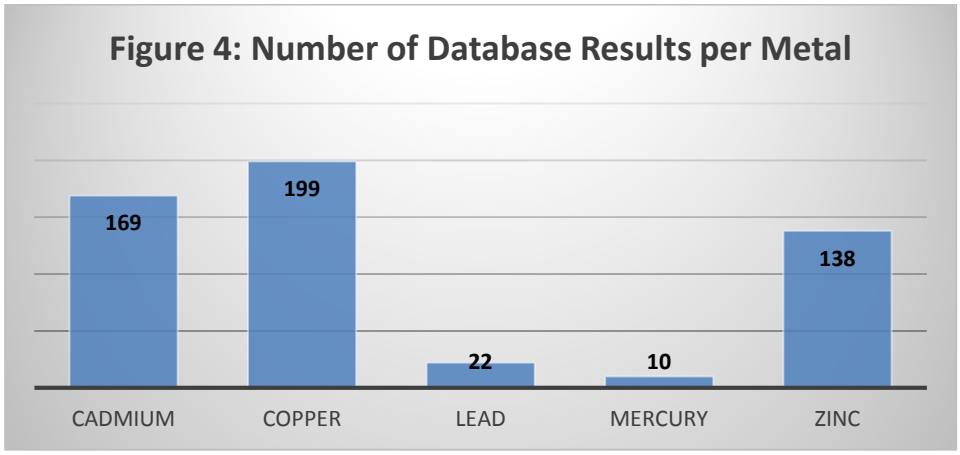
[1] Each data row corresponds to a toxicity test result value of LC_{50}, NOEC or other.

Database Analysis: The seven articles published after the 2006 ASTM guidance was developed include Bringolf et al. 2007, Farag and Harper 2014, Fritts et al. 2014, Harper 2014, and Wang et al. 2007a, 2007 b, and 2010. These studies refer to the ASTM guidelines and contain acute and/or chronic toxicity test results with various freshwater mussel species.

Metals: For the five metal contaminants included in the database, results were based on 1 lead reference, 3 mercury references, 7 zinc references, 8 cadmium references, and 15 copper references (see **Figure 3, Table 3**). Two of these articles were published after 2006, and provided acute (Wang at al. 2007a) and chronic (Wang at al. 2007b) toxicity results from ASTM laboratory tests with copper. The chronic studies with juvenile freshwater mussels in the Wang at al. 2007b article were used to develop the ASTM standards for this study type. The Wang et al. 2010 article provided acute and chronic test results with cadmium, copper, lead, and zinc using laboratory tests according to ASTM guidance, with copper as the reference toxicant for the

acute studies. Overall, the number of toxicity test results complied in the database by metal included 10 for mercury, 22 for lead, 138 for zinc, 169 for cadmium, and 199 for copper (see **Figure 4**).





Copper Analysis: The toxicity test results compiled for copper have the most entered database results with 99 acute test results conducted according to ASTM guideline recommendations (see **Table 2**). The LC_{50}/EC_{50} results are reported from 62 acute 24-hour glochidia tests and 37 acute 96-hour juvenile tests, allowing for comparisons between study types and freshwater mussel species (see **Table 5**). For acute 24-hour glochidia tests with copper, the LC_{50}/EC_{50} survival results were highly variable among the 11 species types and within the same species in tests with different environmental conditions; usually differences in water hardness or alkalinity were observed in the database. The acute 96-hour juvenile tests with copper were conducted using 5 different

freshwater mussel species, and the variability in test results appears less than in the acute glochidia tests included in the database.

Intra- and inter-laboratory variability of acute toxicity results with glochidia and juvenile unionid mussels was assessed in a recent (2016) article by Raimondo et al. In their article, increased inter-laboratory variability in glochidia acute tests was reported compared to juvenile acute tests. The intra-laboratory variability for EC_{50} values averaged about 2-fold for both life stages, but the inter-laboratory variability averaged 3.6-fold for juvenile mussels and 6.3-fold for glochidia. The glochidia and juvenile EC_{50} values were within a factor of 2 of each other for 50% of paired records among chemicals assessed. The juveniles were more sensitive than glochidia by more than 2-fold for 33% of the comparisons made between life stages.

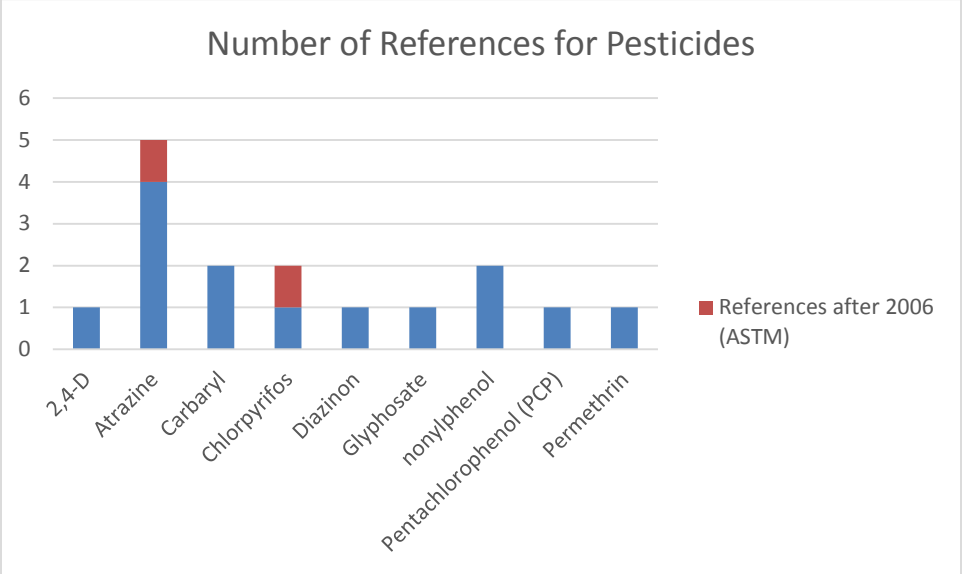
Table 5: Summary Results for Acute Freshwater Mussel Toxicity Laboratory Tests with Copper

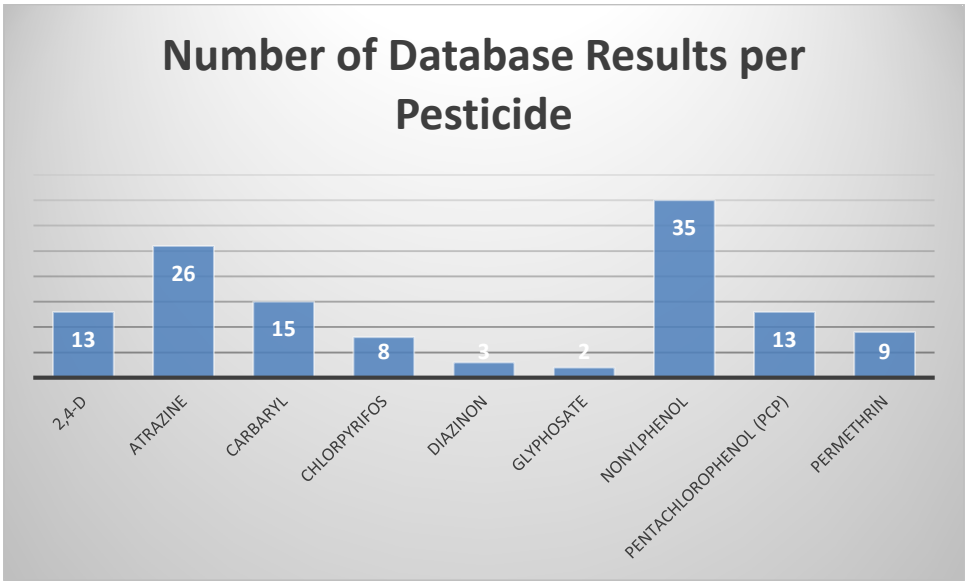
Species	n	LC_{50}/EC_{50} values (µg/L)		
		Range	Mean	SD
Acute 24-hour glochidia tests:				
Actinonaias ligamentia	4	35-66	53.3	13.3
Actinonaias pectorosa	5	23.1-132	71.4	42.9
Lampsilis cardium	1	210	NA	NA
Lampsilis fasciola	3	26-48	40.0	12.2
Lampsilis siliquoidea	15	27-130	42.1	25.6
Leptodea fragilis	2	50-90	70.0	28.3
Ligumia subrostrata	1	150	NA	NA
Medionidus conradicus	6	37-81	52.3	18.2
Megalonaias nervosa	1	180	NA	NA
Pyganodon grandis	2	46-347	196.5	NA
Utterbackia imbecillis	8	22-520	93.7	172.8
Acute 96-hour juvenile tests:				
Lampsilis rafinesqueana	1	23	NA	NA
Lampsilis siliquoidea	13	18-41	23.8	7.3
Lasmigona subvirdis	3	52.05-92.99	69.89	20.97
Utterbackia imbecillis	20	22-111	48.1	23.2

n=number of results (references) per species
NA=Not applicable; ≤2 test results

Pesticides: Toxicity test results for the nine pesticides included in the database were based on: one reference for 2,4-D, chlorpyrifos, diazinon, glyphosate, pentachlorophenol (PCP), and permethrin; two references for carbaryl and nonylphenol; and four references for atrazine (see **Figure 5, Table 3**). The Milam 2005 reference is an example of a single article with multiple chemicals tested (i.e., 2,4-D, carbaryl, 4-nonylphenol, PCP, and permethrin). One of these articles was published after 2006, and provided acute and chronic toxicity results from ASTM laboratory tests with atrazine and chlorpyrifos (Bringolf at al. 2007c). There were two articles that provided duplicate data for atrazine acute tests (Bringolf at al. 2007a and 2007c). The number of data results complied in the database by pesticide included 2 for glyphosate, 3 for diazinon, 8 for chlorpyrifos, 9 for

permethrin, 13 for 2,4-D and PCP, 15 for carbaryl, 26 for atrazine, and 35 for nonylphenol (see **Figure 6**).





DISCUSSION

The freshwater mussel toxicity studies identified through a literature search were included in a database that contains quantitative endpoints that will assist in future risk assessment analysis

and/or regulatory decisions. Currently, the database includes the toxicants of ammonia and chlorine; the metals of cadmium copper, lead, mercury, and zinc; the major ions of sodium bicarbonate and sodium chloride; and the pesticides 2,4-D, atrazine, carbaryl, chlorpyrifos, diazinon, glyphosate, nonylphenol, PCP, and permethrin. The toxicity data collected in the database includes published articles and sources from the gray literature dating from 1991 to 2014. The ASTM guidelines (E2455- 06; reapproved 2013) were first published starting in 2006, and provided useful guidance in the conduct of laboratory toxicity tests with freshwater mussels. The more recently published articles reference the ASTM guideline and provide more dependable results for analysis.

For metals, data results compiled in the database are most numerous for copper (199). An extended analysis of copper data indicates that nearly half represent acute LC_{50}/EC_{50} results conducted according to ASTM guideline recommendations, with 62 acute 24-hour glochidia test results and 37 acute 96-hour juvenile results. Based on the variable acute test results for copper among species in the database, the use of more than one freshwater mussel species in laboratory toxicity testing is important in determining impacts to mussels with different habitat and life history requirements. However, restricting the number of species recommended for test types will provide more reproducible laboratory data for comparisons and effect determinations. According to Raimondo et al. (2016), glochidia and juvenile mussel acute tests should be performed on a chemical-by-chemical basis, and the commonly tested mussel species of *L. siliquoidea, U. imbecillis*, and *V. iris* adequately represented mussel sensitivity in the absence of a diverse mussel toxicity dataset. However, toxicity testing with additional mussel species is needed to improve the application of safety factors. The safety factors of 2 to 5 might be applied to data for commonly tested mussel species for protective levels inclusive of inter-species and inter-laboratory variation (Raimondo et al. 2016).

For pesticides, the data results complied in the database are most numerous for atrazine (26) and nonylphenol (35). In general, fewer pesticide toxicity test references were found in the literature search, and fewer test results for pesticide toxicity in freshwater mussels were available in comparison to metals. However, the database included more individual pesticide chemicals (9) than metals (5).

For continued expansion of the database, toxicity results from tests with the emerging contaminants of major ions, pharmaceuticals, and chemicals from pollution events in freshwater need additional representation. The toxicity tests should follow ASTM guidelines, and quantitative results are needed for both the current database toxicants and additional chemicals of concern. Other recommendations for expansion of this freshwater mussel toxicity database include the following: quality control and quality assurance of all data by qualified experts in this field; addition of web links to publicly available journal articles and abstracts; making the database public with searchable data fields such as chemical, endpoints, and species; additional computer programming functions and statistical analysis of data; and annual updates based on new published toxicity articles and any new testing guideline requirements. In the future, this database could be provided as on-line toxicity information to natural resource professionals involved in freshwater mussel conservation.

REFERENCES: (see **Appendix A** for complete list of database and literature search references).

APPENDIX A: Literature Search References for Freshwater Mussel Toxicity	
Reference Number	**Citations**
1	Akarte SR, Mane UH. 1988. Toxicity of folithion to three species of freshwater bivalve molluscs in different seasons. *J Environ Biol* 9(1):27-38
2	American Society for Testing and Materials. 2013. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06 (reapproved 2013). DOI: 10.1520/E2455-06R13; https://www.astm.org/Standards/E2455.htm
3	Archambault JM, Bergeron CM, Cope WG, Richardson RJ, Heilman MA, Corey III JE, Netherland MD, Heise RJ. 2014. Sensitivity of freshwater molluscs to hydrilla-targeting herbicides: providing context for invasive aquatic weed control in diverse ecosystems. *Journal of Freshwater Ecology*, DOI: 10.1080/02705060.2014.945104
4	Augspurger T, Dwyer FJ, Ingersoll CG, Kane CM. 2007. Editorial, Advances and opportunities in assessing contaminant sensitivity of freshwater mussel (Unionidae) early life stages. *Environ Toxicol Chem* 26:2025-2028
5	Augspurger T, Keller AE, Black MC, Cope WG, Dwyer FJ. 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. *Environ Toxicol Chem* 22:2569-2575
6	Augspurger TP, Wang N, Kunz JL, Ingersoll CG. 2014. Pollutant sensitivity of the endangered Tar River Spiny mussel as assessed by single chemical and effluent toxicity tests. U.S. Fish and Wildlife Service, Ecological Services, Raleigh, NC.
7	Bartsch MR, Newton TJ, Allran JW, O'Donnell JA, Richardson WB. 2003. Effects of pore-water ammonia on in situ survival and growth of juvenile mussels (*Lampsilis cardium*) in the St. Croix riverway, Wisconsin, USA. *Environ Toxicol Chem* 22:2561-2568
8	Beggel S, Geist J. 2015. Acute effects of salinity exposure on glochidia viability and host infection of the freshwater mussel *Anodonta anatina* (Linnaeus, 1758). Science of the *Total Environment* 502:659-665. DOI: 10.1016/j.scitotenv.2014.09.067
9	Besser JM, Brumbaugh WG, Kemble NE, Ivey CD, Kunz JL, Ingersoll CG, Rudel D. 2011. Toxicity of nickel-spiked freshwater sediments to benthic invertebrates-spiking methodology, species sensitivity, and nickel bioavailability, prepared in cooperation with the Nickel

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Reference Number	**Citations**
	Producers Environmental Research Association. US Department of Interior, US Geological Survey, Scientific Investigations Report 2011-5225.
10	Besser JM, Ingersoll CG, Brumbaugh WG, Kemble NE, May TW, Wang N, MacDonald DD, Roberts AD. 2015. Toxicity of sediments from lead zinc mining areas to juvenile freshwater mussels (*Lampsilis siliquoidea*), compared to standard test organisms. *Environ Toxicol Chem* Accepted Article. DOI: 10.1002/etc.2849
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122	Ward S, Augspurger T, Dwyer FJ, Kane C, Ingersoll CG. 2007. Contaminant sensitivity of freshwater mussels, Risk assessment of water quality in three North Carolina, USA, streams supporting federally endangered freshwater mussels (Unionidae). *Environ Toxicol Chem* 26:2075-2085
123	Warren LW. 1996. The use of juvenile mussels, *Utterbackia imbecillis* Say (Bivalvia: Unionidae), as a standardized toxicity testing organism. Thesis, Clemson University. Clemson, SC.
124	Weinstein JE. 2001. Characterization of the acute toxicity of photoactivated fluoranthene to glochidia of the freshwater mussel, *Utterbackia imbecillis*. *Environ Toxicol Chem* 20:412-419
125	Weinstein JE, Polk KD. 2001. Phototoxicity of anthracene and pyrene to glochidia of the freshwater mussel Utterbackia imbecillis. *Environ Toxicol Chem* 20:2021-2028

APPENDIX B: Database Template with Data Entry Options

Chemical	Chemical name, purity (%)
Species	Species name
Life Stage	Glochidia or Juvenile
Age (d)	value
Transformation	For juvenile tests
Study Type	Acute, chronic or other
Test Duration (h)	value
Control survival (%)	value
Temp. (°C)	value
Water type	type
Water hardness (mg/L)	value
Water pH	value
Water Alkalinity (mg/L)	value
Observation Endpoint	Survival, length, or other
Results Endpoint	LC_{50}, EC_{50}, NOEC, LOEC
Conc (mg/L)	value
Additional Info	95% CI or other
ASTM Guideline Followed	Yes or No
Reference	Name, date