

State of the Science of Mayfly Ecotoxicity Testing

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Why Mayflies?

- Found in almost all types of freshwater habitat throughout the world.
- Live in both flowing and static waterbodies.
- Represent a range of sensitivities with some of them being very sensitive to environmental contaminants.
- Along with stoneflies and caddisflies, they are one of the commonly-used indices of aquatic ecosystem health (EPT Index).
- Option for testing some mayfly species in both water-only and sediment assays; stoneflies and caddisflies can only be tested in water-only exposures.

Characteristics of Ideal Test Species

- Sensitive to contaminants.
- Readily available throughout the year.
- Tolerant of handling stress.
- Easy to culture and maintain.
- Ecologically, commercially, and/or recreationally important.
- Consistent and reproducible response of relevant endpoints.
- Short life cycle.
- Large database of toxicological responses available.

Mayfly Toxicity Test Review

- Literature search covered the ecology, species' ranges, toxicity test methods, and relative sensitivity of mayflies.
- Focused on main test species of *Cloeon dipterum*, *Neocloeon triangulifer*, *Hexagenia*, *Isonychia*, and *Caenis*. Included *Ephoron virgo* (Europe) and *Ephemera orientalis* (East Asia) due to ecological relevance.
- Entered search results into EndNote library.
- Search yielded over 150 articles of interest and annotated bibliographies were written for 34 of them.

Cloeon dipterum



Source: NatureSpot.



Cloeon dipterum

- Distribution: Lakes and ponds in Europe, Asia, and eastern North America.
- Univoltine or multivoltine, depending on environmental factors such as temperature.
- Cultures can be maintained for up to 8 generations in the lab; ovoviviparous.
- Test duration: 96-h to full life cycle.
- Acute and chronic test endpoints available.
- Endpoints: Survival, immobilization, growth, development rate, and fecundity.

Neocloeon triangulifer



Source: Pennsylvania Department of Environmental Protection.



Neocloeon triangulifer

- Distribution: eastern North American streams.
- Multivoltine and obligatory parthenogenetic.
- Continuous culture can be maintained in the lab; short life cycle; amenable to static conditions.
- Test duration: 96-h to full life cycle.
- Acute and chronic test endpoints available.
- Endpoints: Survival, immobilization, growth, biomass, emergence, and fecundity.
- Dietary exposure to toxicant (*e.g.*, selenium) can be measured by utilizing natural periphyton biofilms as contaminant source.

Hexagenia spp.



Source: BugGuide.Net.



Hexagenia spp.

- Distribution: Burrowing mayfly found throughout North America in freshwater aquatic sediments.
- Semivoltine; eggs collected in the field and be stored over 12 months allowing for year-round testing.
- Tested in both water-only and sediment assays.
- Test duration: 96-h to 120-d.
- Acute and chronic endpoints available.
- Endpoints: Survival, immobilization, growth, biomass, emergence, molting frequency, and behavior.
- 28-d bioaccumulation test with whole-body concentration, lipid content, survival, and growth as endpoints.

Isonychia spp.



Source: Orvis News.



Isonychia spp.

- Distribution: swiftly flowing waters in eastern North America.
- Bivoltine; field-collected mayfly.
- Test duration: 96-h to 14-d.
- Acute and sub-chronic test endpoints available.
- Endpoints: Survival and immobilization.
- Prefers lotic habitat and susceptible to handling stress.

Caenis spp.



Source: DiscoverLife.



Caenis spp.

- Distribution: Northern hemisphere; lotic and lentic habitats.
- Univoltine or multivoltine depending on environmental factors; field-collected mayfly.
- Test duration: 96-h to 28-d.
- Acute and sub-chronic test endpoints available.
- Endpoints: Survival and immobilization.
- Lack of substrate in test containers can be an additional stress factor.

Ephoron virgo



Source: Chovzvirat.

Ephoron virgo

- Distribution: Burrowing mayfly found throughout Europe in freshwater aquatic sediments.
- Eggs collected in the field and can be stored up to 3 years; allows for year-round testing.
- Univoltine.
- Tested in both water-only and sediment assays.
- Test duration: 96-h to 21-d.
- Acute and chronic test endpoints available.
- Endpoints: Survival and growth.
- Lack of comparative studies.

Ephemera orientalis



Source: Flickrriver.

Ephemera orientalis

- Distribution: Burrowing mayfly found in lowland rivers and streams of temperate East Asia in freshwater aquatic sediments.
- Univoltine or multivoltine depending on environmental factors; field-collected mayfly.
- Test duration: 24-h to 14-d.
- Acute and sub-chronic test endpoints available.
- Endpoints: Survival and egg hatching rate.
- Lack of comparative studies; potential sediment assay.

Cloeon spp. sensitivity comparison (LC50s)

Test Material	Sensitivity	More Sensitive Species/Order/Class	Less Sensitive Species/Order/Class
Acetamiprid	Intermediate	N. triangulifer, Ephemerella, Caenis, Hemiptera, Trichoptera, Coleoptera, Diptera, Crustacea, Oligochaeta	Hexagenia, Isonychia, McCaffertium, Odonata, C. dubia
Clothianidin	Intermediate	N. triangulifer, Ephemerella, Caenis, McCaffertium, Plecoptera, Hemiptera, Trichoptera, Coleoptera, Diptera, H. azteca, Oligochaeta	Hexagenia, Isonychia, Odonata, Crustacea
Dinotefuran	Intermediate	N. triangulifer, Ephemerella, Caenis, Odonata, Hemiptera, Coleoptera, Diptera, H. azteca, Oligochaeta	≈ Hexagenia, McCaffertium, Trichoptera, Crustacea
Imidacloprid	Intermediate	N. triangulifer, Ephemerella, Caenis, Isonychia, Hemiptera, Trichoptera, Coleoptera, Diptera, H. azteca, Oligochaeta	Hexagenia, McCaffertium, Odonata, Crustacea
Thiacloprid	Intermediate	N. triangulifer, Ephemerella, Caenis, Hemiptera, Coleoptera, Diptera, H. azteca, Oligochaeta	Hexagenia, McCaffertium, Odonata, Trichoptera, C. dubia
Thiamethoxam	Intermediate	N. triangulifer, Ephemerella, Caenis, Hemiptera, Trichoptera, Coleoptera, Diptera, H. azteca, Oligochaeta	Hexagenia, Isonychia, McCaffertium, Odonata, Plecoptera, Crustacea
Deltamethrin (formulation)	High	None	L. sponsa, C. aenea, D. magna, Caenis
Esfenvalerate (formulation)	High	None	L. sponsa, C. aenea, D. magna, Caenis
Malathion	High	D. magna	P. clarkia, G. affinis, C. longiareolata
Genapol OXD (surfactant)	High	≈ Chironomini	P. clarkia, G. affinis, C. longiareolata, D. magna
Chlorpyrifos	Intermediate	G. pulex	S. vetulus, C. punctata, C. obscuripes, G. aculeatus, P. pungitius

Neocloeon triangulifer sensitivity comparison (LC50s)

Test Material	Sensitivity	More Sensitive Species	Less Sensitive Species/Order/Class
Acetamiprid	High	≈ <i>C. dilutus</i> , <i>H. azteca</i>	Ephemeroptera, Odonata, Hemiptera, Trichoptera, Coleoptera, Diptera, Crustacea, Oligochaeta
Clothianidin	High	≈ <i>C. dilutus</i> , <i>H. azteca</i>	Ephemeroptera, Odonata, Plecoptera, Hemiptera, Trichoptera, Coleoptera, Diptera, Crustacea, Oligochaeta
Dinotefuran	High	None	Ephemeroptera, Odonata, Hemiptera, Trichoptera, Coleoptera, Diptera, Crustacea, Oligochaeta
Imidacloprid	High	None	Ephemeroptera, Odonata, Hemiptera, Trichoptera, Coleoptera, Diptera, Crustacea, Oligochaeta
Thiacloprid	High	≈ <i>C. dilutus</i>	Ephemeroptera, Odonata, Hemiptera, Trichoptera, Coleoptera, Diptera, Crustacea, Oligochaeta
Thiamethoxam	High	None	Ephemeroptera, Odonata, Plecoptera, Hemiptera, Trichoptera, Coleoptera, Diptera, Crustacea, Oligochaeta
NaCl	High	None	<i>C. dubia</i> , <i>D. magna</i>
CuSO ₄	High	None	<i>C. dubia</i> , <i>D. magna</i>
KCl	Low	<i>C. dubia</i> , <i>D. magna</i>	None

Conclusions

- Mayflies meet some, but not all, of the characteristics of ideal test species.
- Ephemeroptera are a fairly sensitive order, but not the most sensitive to all chemicals.
- Allow for testing in both water-only (lotic and lentic) and sediment assays, as well as dietary exposure and bioaccumulation assays.
- Research needs going forward include standardization of protocols and culture methods, ring testing, evaluation of physiological requirements, and identification of new species.

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