Designation: E1203 – 98(Reapproved 2012)

# Standard Practice for

# Using Brine Shrimp Nauplii as Food for Test Animals in Aquatic Toxicology[1]

This standard is issued under the fixed designation E1203; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This practice describes procedures for hatching, harvesting, and testing the acceptability of brine shrimp nauplii for use as a food for certain fish and invertebrate species that are used in aquatic toxicity tests. The term "brine shrimp" refers to all species in the genus *Artemia* (**1**) [2] although this practice specifically deals only with those species for which cysts (encysted embryos) are commercially available.

1.2 These procedures are applicable to all brine shrimp nauplii that are obtained by incubating commercially available cysts. With appropriate processing, cysts collected by noncommercial harvesters can be subjected to these same procedures.

1.3 Modification of these procedures might be justified by special needs or circumstances.

1.4 This practice is organized as follows:



1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements are given in Section 6.

## 2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1191 Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids

E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes

## 3. Terminology

3.1 *Definitions:*The words "must," "should," "may," "can," and "might" have very specific meanings in this practice. "Must" is used to express an absolute requirement, that is, to state that the test ought to be designed to satisfy the specified condition, unless the purpose of the test requires a different design. "Must" is only used in connection with factors that directly relate to the acceptability of the test. "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although violation of one "should" is rarely a serious matter, violation of several will often render the results questionable. Terms such as "is desirable," "is often desirable," "might be desirable" are used in connection with less important factors. "May" is used to mean "is (are) allowed to," "can" is used to mean "is (are) able to," and "might" is used to mean "could possibly." Thus the classic distinction between "may" and "can" is preserved, and "might" is never used as a synonym for either "may" or "can."

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *brine shrimp cyst*—a gastrula-stage embryo that is enclosed in an envelope and cuticle for resistance to desiccation. Dried brine shrimp cysts are often incorrectly referred to

[1] This practice is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this practice.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

as eggs. Upon hydration, embryonic development proceeds until a nauplius emerges from the encysting shell.

3.2.2 *brine shrimp nauplius*—a newly hatched, freely swimming, instar I stage larva. Nauplii are incapable of exogenous feeding until the instar II stage that occurs approximately 24 h after hatch at 25°C. The reddish-brown color of the nauplii is due to the presence of yolk on which they rely for endogenous food.

3.2.3 *feeding assay*—a test in which one or more life stages of an aquatic animal species is raised on a diet to determine the adequacy of the diet for the life stage(s) of the species.

3.2.4 *Reference Artemia cysts*—a homogeneous collection of cysts that has been tested and declared a Reference sample. Formerly two repositories of reference cysts existed,[4] but both supplies have been exhausted without replacement.

3.2.5 *strain*—a geographical population of brine shrimp that is genetically distinct from other brine shrimp populations of the same species. Genetic distinction is determined electrophoretically (**2**).

3.2.6 *time to 90 % hatch*—the amount of time that expires between the immersion of dried brine shrimp cysts in water and the hatching of 90 % of the hatchable cysts. Time to 90 % hatch is usually abbreviated $T_{90}$.

## 4. Summary of Practice

4.1 One can of cysts is purchased. Samples are hatched in aerated salt water and nauplii are collected on a small-mesh screen and rinsed. Nauplii are fed to a species of aquatic animal to determine if the nauplii are an acceptable complete food for that life stage of that species. Chemical and physical measurements might also be performed on the cysts or on the nauplii, or on both. If the nauplii are acceptable, a large number of cans from the same lot are purchased, appropriately stored, hatched, and fed to that life stage of that species. An alternative is to feed algae in addition to the brine shrimp to the test organisms.

## 5. Significance and Use

5.1 In certain toxicity tests during which small aquatic animals must be fed, the food of choice is often live brine shrimp nauplii because of their availability, ease of use, and presumed good nutritional quality. In addition, many test species that are cultured or held in the laboratory must be fed prior to toxicity testing, even if they are not fed during the test.

5.2 Brine shrimp nauplii can be readily hatched in the laboratory from cysts that are obtained naturally from many geographical areas around the world. Cysts from a few of those areas are available commercially, but might represent two or more species of brine shrimp (**1**).

5.3 Nauplii of different strains of the same brine shrimp species from different geographical areas can differ substantially in their nutritional value, contaminants, and acceptability as a complete food for the life stage of the species to which they are fed (**3-8**).

5.4 The results of a toxicity test can depend on the brine shrimp nauplii used as food (**9**). It is desirable to determine prior to use whether a particular batch of brine shrimp is of adequate quality for the test organisms.

5.5 The primary requirements of acceptable brine shrimp are that they are an appropriate size for the test organisms, have adequate nutritional value, and do not contain excessive concentrations of contaminants. Whether a given lot of brine shrimp meets these requirements for a given species can only be determined by a feeding assay.

5.6 Standardization of the hatching, harvesting, testing, and using of brine shrimp will probably increase the reproducibility of results of toxicity tests with some test species by decreasing the use of unhealthy organisms caused by feeding them poor quality brine shrimp.

## 6. Hazards

6.1 Because water is a good conductor of electricity, use of ground fault systems and leak detectors should be considered to help avoid electrical shocks. Salt water is such a good conductor that protective devices are strongly recommended.

## 7. Obtaining Cysts

7.1 *Selecting a Source*—Selection of a given lot of cysts for a feeding assay can be based on information concerning the geographical origin of the cysts, the way the cysts were dried and stored, and possibly the size of the cysts and resulting nauplii. However, commercial suppliers might not divulge this information, and information that is provided might not be correct. The preferred processes for drying and storing brine shrimp cysts are, respectively, fluidized bed drying and storage under vacuum or nitrogen (**10**). Cysts processed and stored in other ways might be acceptable, but hatchability will probably be reduced. Size of nauplii at hatch varies from strain to strain but is relatively constant within a strain (**11**). Mortality of some larval fish increases with increasing naupliar size (**12**) because some larvae simply cannot ingest the larger nauplii. Nutritional value and levels of contaminants vary both among and within strains, and depend mostly on the quality of the water and the algae in the water from which the cysts were harvested (**13**). The only way to determine the acceptability of cysts is to perform a feeding assay. Therefore, it is desirable to purchase and test a small sample (for example, one can) of cysts from one lot before purchasing a large quantity.

7.2 *Storage*—Properly sealed cans of cysts can be stored for several years at room temperature, under refrigeration, or frozen. If they are frozen, they should be removed from the freezer at least 48 h, and preferably about 1 week, prior to use to ensure adequate hatching. After a can has been opened, the optimal conditions for long term storage are, of course, lost. If the contents will be used up in 1 to 2 months, it is only necessary to close the can tightly (for example, with a plastic lid) after each use and store it in a cool (about 4°C), dry place, such as in a refrigerator. If the contents will not be used up within 2 months, it is best to distribute the cysts among smaller containers that hold about a 1 to 2 month supply of cysts and seal the containers under nitrogen or vacuum or freeze them. One simple method is to place the cysts in a sealable plastic

[4] Artemia Reference Center, State University of Ghent, Ghent, Belgium, or Quality Assurance Research Division, U.S. Environmental Protection Agency, Cincinnati, OH 45268.