# OECD Test No 218, 2004

# Sediment-water Chironomid toxicity test using spiked sediment.

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# Sediment-water chironomid toxicity test using spiked sediment

- This test is designed to assess the effects of prolonged exposure of chemicals to the sediment-dwelling larvae of the freshwater dipterian Chironomus sp.

- Possible species to use: *Chironomus riparius* and *Chironomus tentans*; other well documented chironomid species may also be used, for example *Chironomus yoshimatsui*.
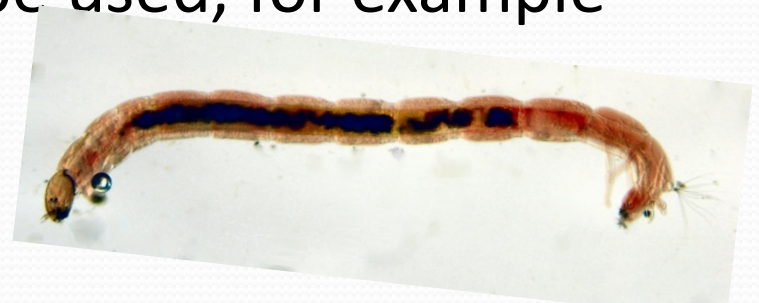
# Principle of the test.

Spiked sediment

Water

Chironomid emergency and development rate

ECx

LOEC
NOEC
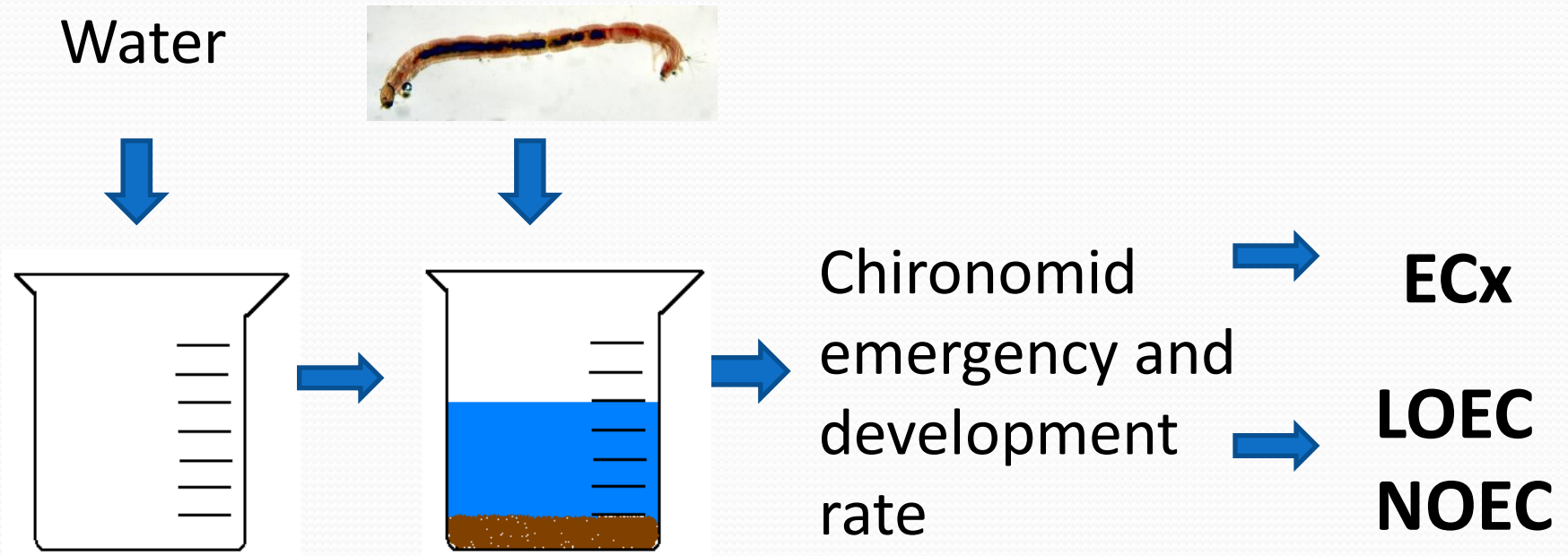
# Test duration

- *C. riparius, C. yoshimatsui* : 20 - 28 days
- *C. tentans:* 28 - 65 days
- In case if additional short-term data are required- measure of the larval survival and weight after 10 days

# Test validity

- the emergence in the controls must be at least 70% at the end of the test

- Emergence to adults from the control should occur between 12 and 23 days for C. riparius and C. yoshimatsui; from 20 to 65 days for C. tentans

- pH of water in all test vessels should be in range from 6-9, oxygen concentration at least 60%

# Materials

- Test vessels: glass beakers with volume of 600 ml and diameter 8 cm

- Sediment: formulated (artificial) sediment, based on artificial soil – quartz sand (75-76%), kaolin clay (20%), peat (4-5%)

- Water: at the beginning of the test pH 6 -9, total hardness not higher than 400 mg/l as $CaCO_3$

- Test substance: known vapour pressure, measured or calculated partitioning into sediment and stability in water and sediment

- Spiked sediments: prepared by addition of a solution of the test substrate directly to the sediment.

# Test design

- **ECx**: at least **5** concentrations with **3** replicates in each concentration; the factor between concentrations should not be greater than two.

- **LOEC/NOEC**: at least **5** concentrations with **4** replicates in each concentration; the factor between concentrations should not be greater than two.
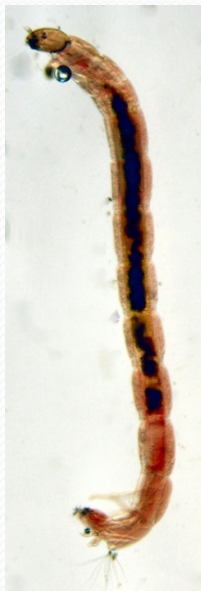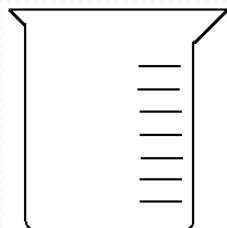
# Procedure

1. Preparation of spiked sediment-water system
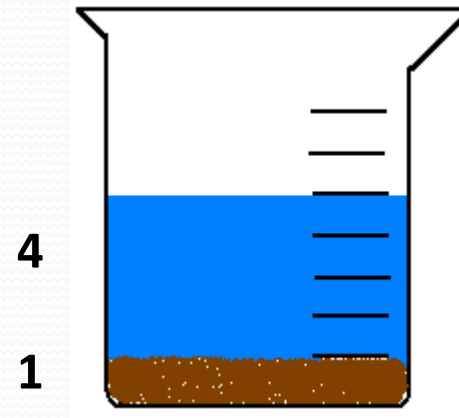


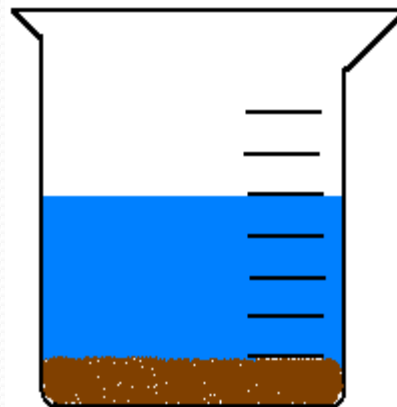2. Stabilization – **48** hours recommended

# Procedure

3. Addition of test organisms

**Chironomid emergency**



x**20**

Control vessels without any test chemical but including sediment should be included in the test with the appropriate number of replicates.

Maximum exposure duration is 28 days for *C. riparius* and 65 days for *C. tentans*

# Observations

The development time and the total number of fully emerged male and female midges are determined.
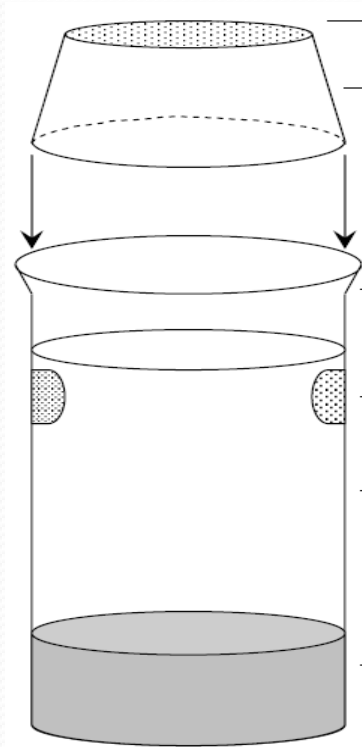
# Treatment of results

- Emergency rate

$$ER = \frac{n_e}{n_a}$$

where:

| | | |
|---|---|---|
| $ER$ | = | emergence ratio |
| $n_e$ | = | number of midges emerged per vessel |
| $n_a$ | = | number of larvae introduced per vessel |

ECx  - regression analysis
NOEC/LOEC – ANOVA methods (Cochran-Armitage, Fisher's exact
(with Bonferroni correction), or Mantel-Haentzal)

# Treatment of results

- Development rate

$$\overline{x} = \sum_{i=1}^{m} \frac{f_i x_i}{n_e}$$

where:

$\overline{x}$ : mean development rate per vessel
$i$ : index of inspection interval
$m$ : maximum number of inspection intervals
$f_i$ : number of midges emerged in the inspection interval i
$n_e$ : total number of midges emerged at the end of experiment $(= \sum f_i)$
$x_i$ : development rate of the midges emerged in interval i

$$x_i = 1 \Big/ \left( \text{day}_i - \frac{l_i}{2} \right)$$

where:

$\text{day}_i$: inspection day (days since application)
$l_i$ : length of inspection interval i (days, usually 1 day)

ECx  - regression analysis
NOEC/LOEC – ANOVA methods (Dunnett-test, Williams-test)

# Thank you for your attention!

Used photo sources:

http://www.deltaenvironmental.com.au/archives/vernal/invertebrates.htm

http://jeremybiggs.wordpress.com/2009/03/18/in-answer-to-matthew/

http://www.stevenanz.com/Main_Directory/Recent%20Photos/2009/09_07_02_PA/source/img_0159.htm

http://www.mzephotos.com/gallery/insects/chironomidae.html