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SETAC

# An Effective Approach to Maintain Homogeneity of Test Item in Soil for Earthworm (*Eisenia fetida)* Toxicity Test

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# Introduction

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**OECD/OCDE** **222**

Adopted:
29 July 2016

**OECD GUIDELINE FOR THE TESTING OF CHEMICALS**

**Earthworm Reproduction Test (*Eisenia fetida/ Eisenia andrei* )**

- Guideline suggests analysis for volatile, unstable, and readily degradable substances

**Q1. How about insoluble and soluble compounds?**

**Q2. How to maintain homogeneity in the absence of analysis?**

# Introduction (Continued)

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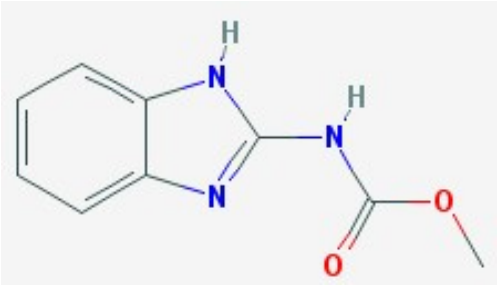
- Carbendazim




- Solubility in water is 8 mg/L

# Introduction (Continued)

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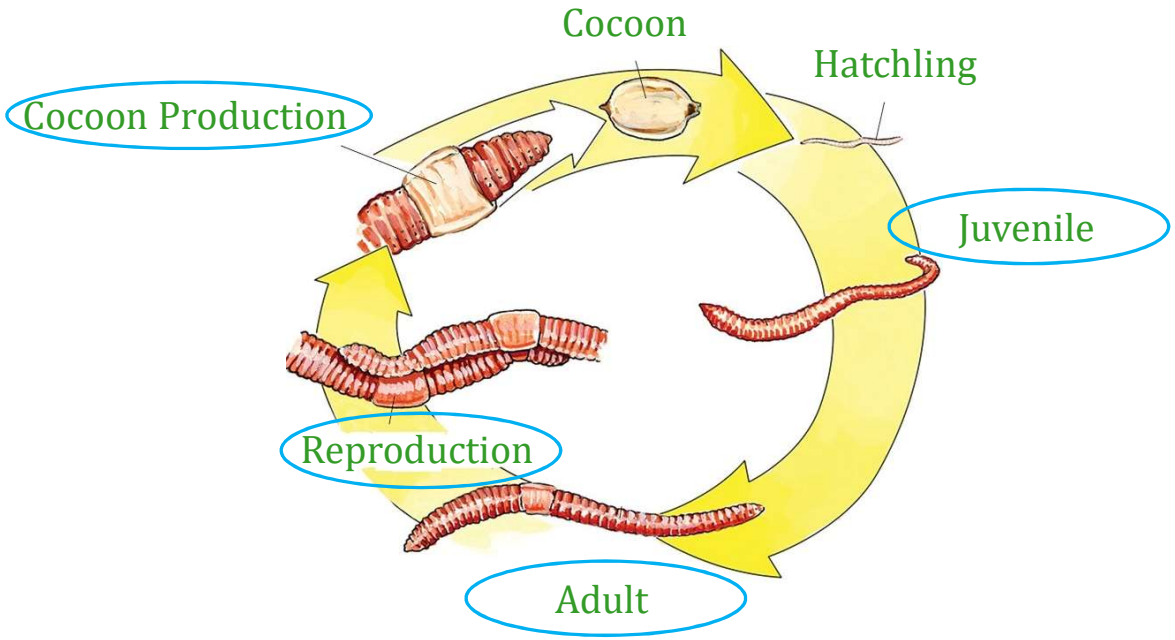
- *Eisenia fetida* has been extensively used as a standard test organism for the risk assessment of pesticides and is widely used to assess its sensitivity to chemical pollution




**Life Cycle of Earthworm**

# Experimental Design

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Artificial soil preparation

Acclimatisation

Mixing and Exposure of Test Item

Addition of water to achieve moisture content

Distribution in test vessels

Observation

# Experimental Design (Continued)

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## Conventional Method

Mixing of Test Item

Mixing in artificial soil in bulk

Distributed in replicates

## Individual Replicate Exposure Method

Mixing of Test Item

Mixing with sand

Mixing in artificial soil in replicate

Transfer into test vessels

# Results

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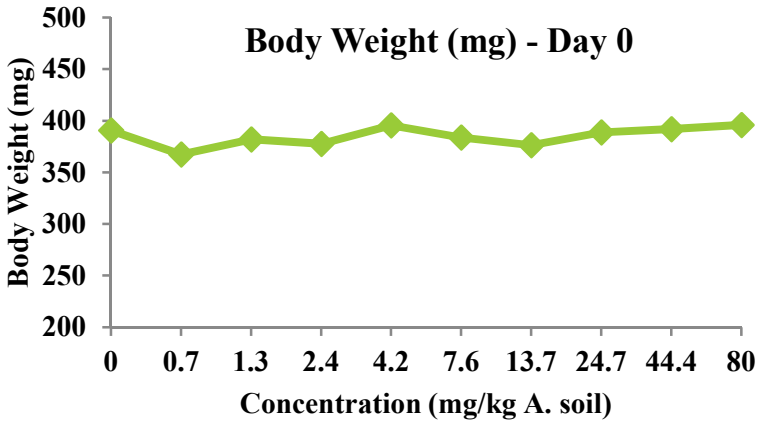
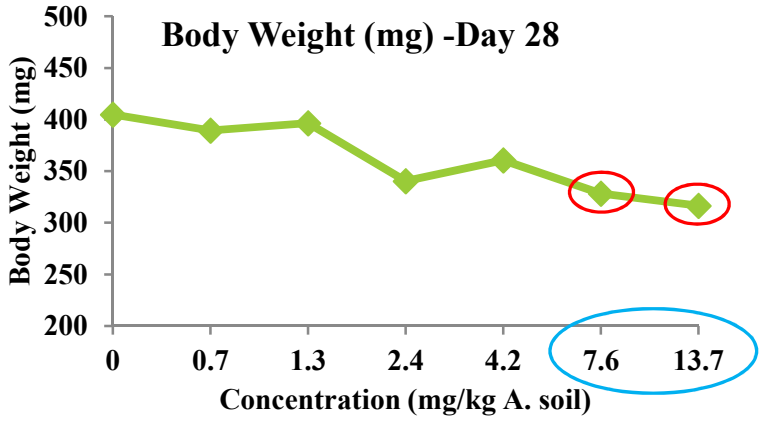
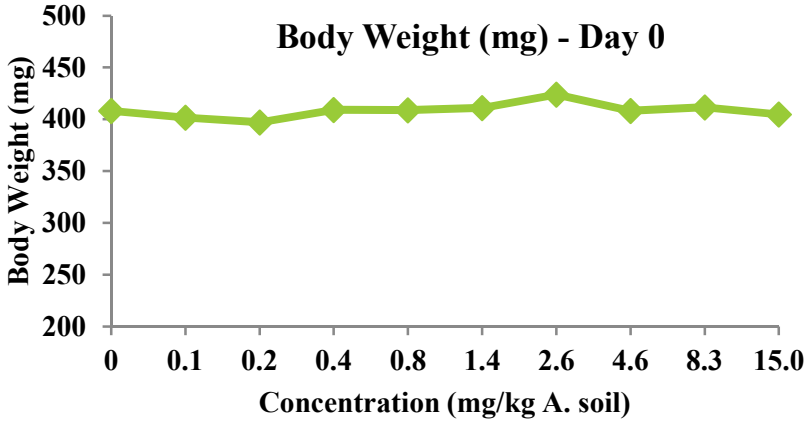
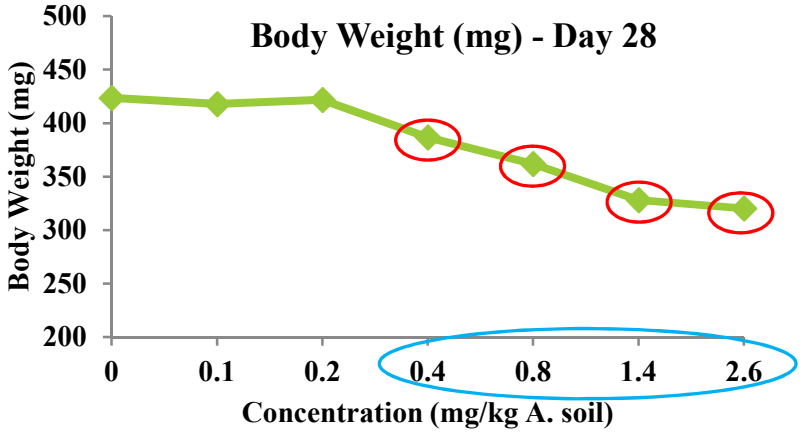
## Conventional Method




## Individual Replicate Exposure Method



# Results (Continued)

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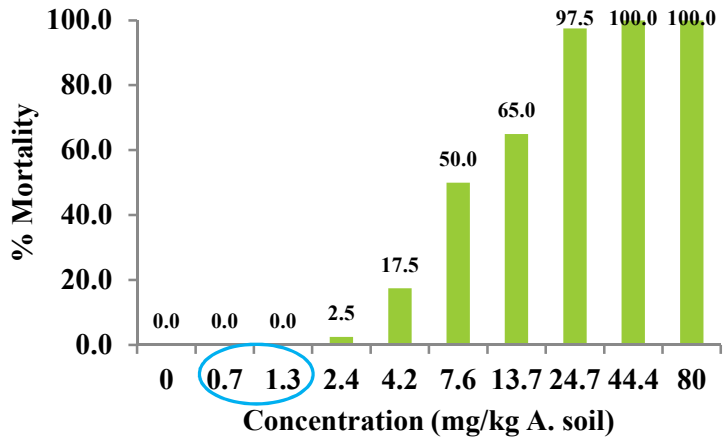
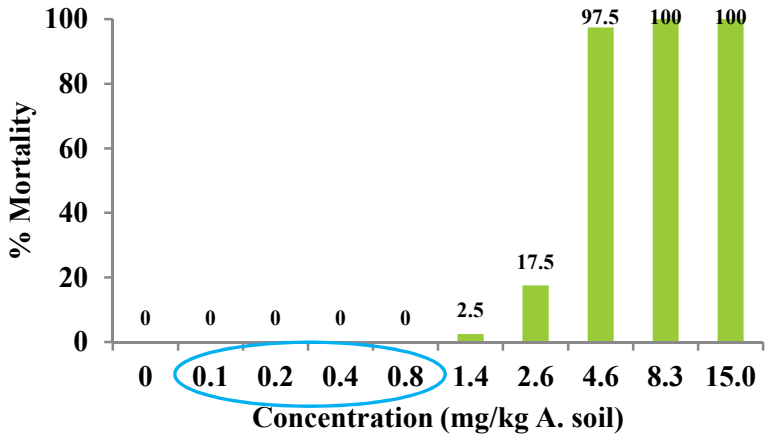
## Conventional Method

| Concentrations (mg/kg A. soil) | Sign of Toxicity |
|---|---|
| 4.2 | Sluggish, Shrunk |
| 7.6 | Sluggish, Shrunk |
| 13.7 | Sluggish, Shrunk |
| 24.7 | Shrunk |
| 44.4 | - |
| 80.0 | - |




## Individual Replicate Exposure Method

| Concentrations (mg/kg A. soil) | Sign of Toxicity |
|---|---|
| 1.4 | Sluggish |
| 2.6 | Sluggish, Shrunk |
| 4.6 | Shrunk |
| 8.3 | - |
| 15.0 | - |



# Results (Continued)

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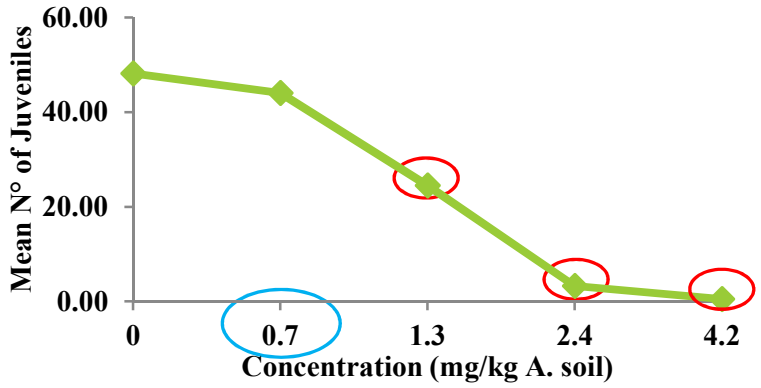
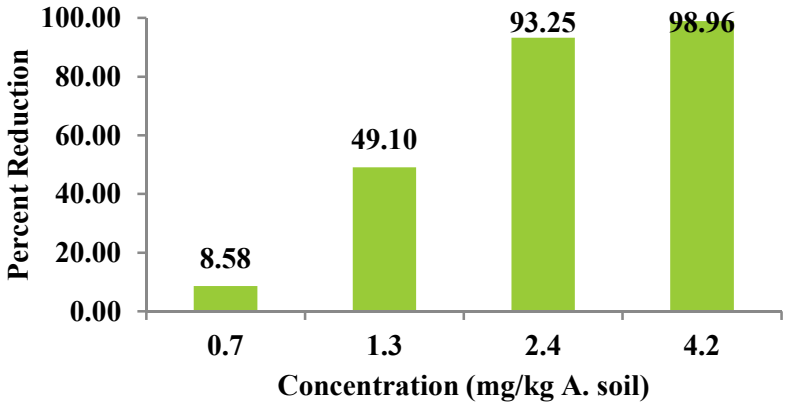
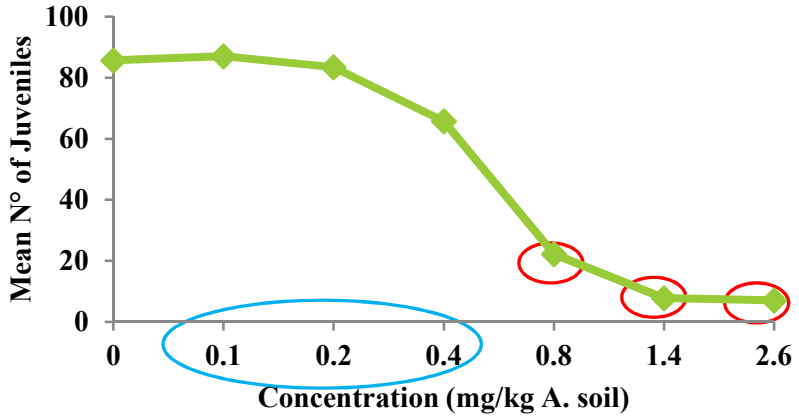
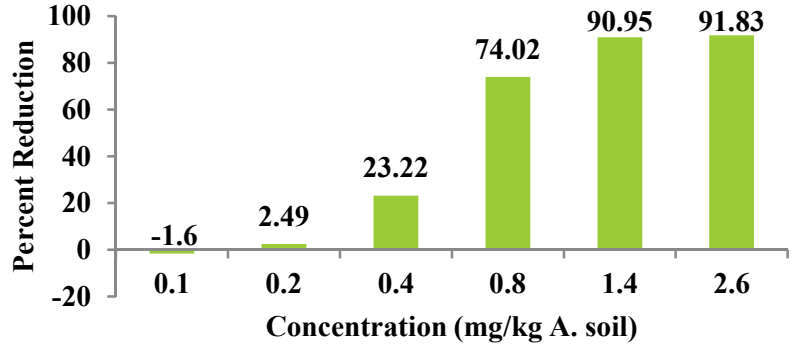
## Conventional Method




## Individual Replicate Exposure Method



# Results (Continued)

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| End points | Conventional Method | Individual Replicate Exposure Method | Comparison |
|---|---|---|---|
| | mg/artificial soil | | |
| $LC_{50}$ for adult mortality (28 day) | 8.26 | 2.81 | 2.9 times |
| $EC_{50}$ for reproduction | 1.32 | 0.68 | 1.9 times |
| NOEC | 0.7 | 0.4 | 1.8 times |
| LOEC | 1.3 | 0.8 | 1.6 times |

# Published results

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| End points | Garcia, 2004 | Sian R Ellis et al., 2007 | R. Shanmuga-sundaram et al., 2013 | Mcshane et al. 2012 | Method 1 | Method 2 |
|---|---|---|---|---|---|---|
| $LC_{50}$ | - | 8.03 | 6.33 | - | **8.26** | **2.81** |
| $EC_{50}$ | 2.7 | - | 2.45 | 3.3 | **1.32** | **0.68** |
| NOEC | 0.1 | - | 1.14 | - | **0.7** | **0.4** |
| LOEC | - | - | 2.06 | - | **1.3** | **0.8** |

# Summary

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- Individual replicate exposure method leads to deduction of the end points: survival, growth, and reproduction to higher level of accuracy, with compressed range of concentrations.
- While the $LC_{50}$, $EC_{50}$, and LOEC results of our study are substantially lower than the published data.

# Conclusion

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*The Individual Replicate Exposure Method*:

- Helps in maintaining the homogeneity of the test item in soil
- Fulfills the objective of test to get enhanced accuracy for the end points.

# References


- Allen, H.E., 2002. Bioavailability of Metalsin Terrestrial Ecosystems: Importance of Partitioning for Bioavailability to Invertebrates, Microbes, and Plants. SETAC, NewYork.
- Boudina, C. E., Baaliauamer, A., Grenier-Loustalot, M.F., Choven, J.M., 2003. Photochemical behaviour of carbendazim in aquatic solution. Chemosphere. 50, 649-655.
- Edwards, C.A., 1998. Earthworm Ecology. St. Lucie Press, New York.
- Edwards, C.A., Bohlen, P.J., 1992. The effects of toxic chemicals on earthworms. Rev. Environ. Contam. Toxicol. 125, 23–99.
- Ellis, S.R., Hodson, M.E., Wege, P., 2007. The influence of different artificial soil types on the acute toxicity of carbendazim to the earthworm *Eisenia fetida* in laboratory toxicity tests. Eur. J. Soil Biol. 43, 239-245.
- Garcia, M., Römbke, J., de Brito, M.T., Scheffczyk, A., 2008. Effects of three pesticides on the avoidance behavior of earthworms in laboratory tests performed under temperate and tropical conditions. Environ. Pollut.153(2), 450-456.

# References (Continued)

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- Holmstrup, M., 2000. Field assessment of toxic affects on reproduction in the earthworm *Aporrectodea longa* and *Aporrectodea rosea*. Environmental toxicology and chemistry.19,1781-1787.
- ISO, 1998. Soil quality Effects of Pollutants on Earthworms (*Eisenia fetida*). Part2: Determination of Effects on Reproduction. ISO - The International Organization for Standardization, Genève, Switzerland.
- Landrum, M., Cañas, J.E., Coimbatore, G., Cobb, G.P., Jackson, W.A., Zhang, B., derson, T.A., 2006. Effects of perchlorate on earthworm (*Eisenia fetida*) survival and reproductive success. Sci. Total Environ. 363, 237–244.
- OECD, 1984. Guidelines for Testing of Chemicals. Test 207: Earthworm Acute Toxicity Tests. Organization for Economic Co-operation and Development (OECD).
- OECD, 2016. Guidelines for Testing of Chemicals. Test 222: Earthworm Reproduction Test (*Eisenia fetida*/ *Eisenia andrei*). Organization for Economic Co-operation and Development (OECD).
- Reinecke, S.A., Reinecke, A.J., 2007. The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. Ecotoxicol. Environ. Saf. 66(2), 244–251.
- Römbke, J., Jänsch, S., Didden, W., 2005. The use of earthworms in ecological soil classification and assessment concepts. Ecotoxicol. Environ. Saf. 62, 249–265.

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# Thank You