



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

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222

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OECD GUIDELINE FOR THE TESTING OF CHEMICALS

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

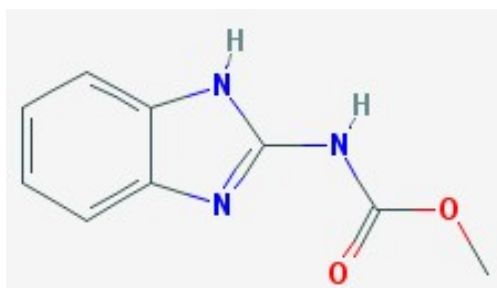
- Guideline suggests analysis for volatile, unstable, and readily degradable substances

Q1. How about insoluble and soluble compounds?

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

Introduction (Continued)

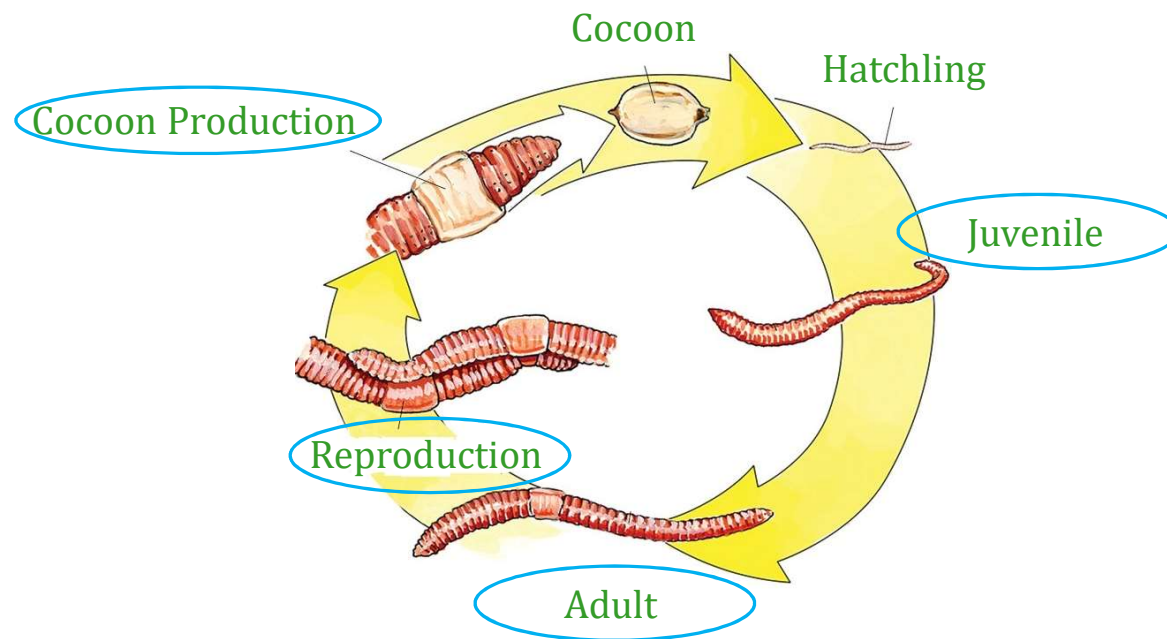
- Carbendazim



- Solubility in water is 8 mg/L

Introduction (Continued)

- *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



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)

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



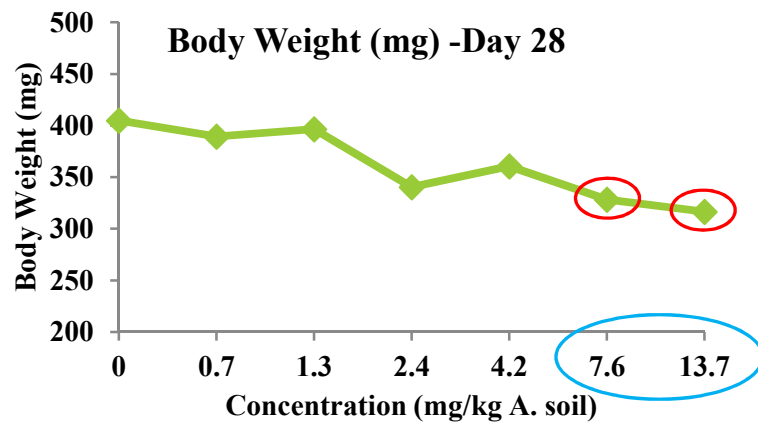
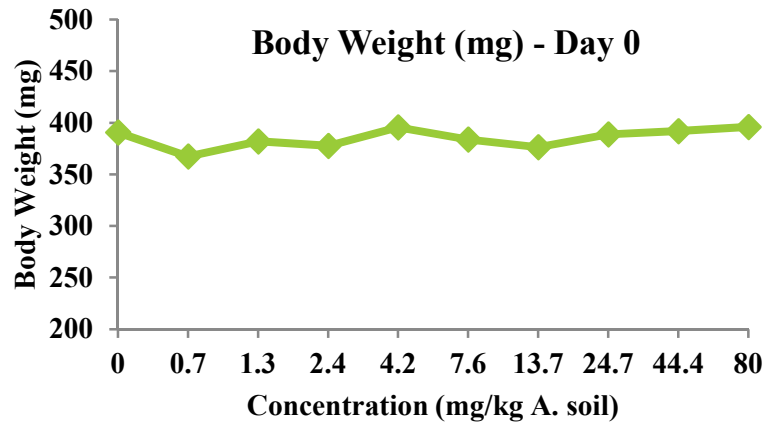
Mixing in artificial soil in replicate



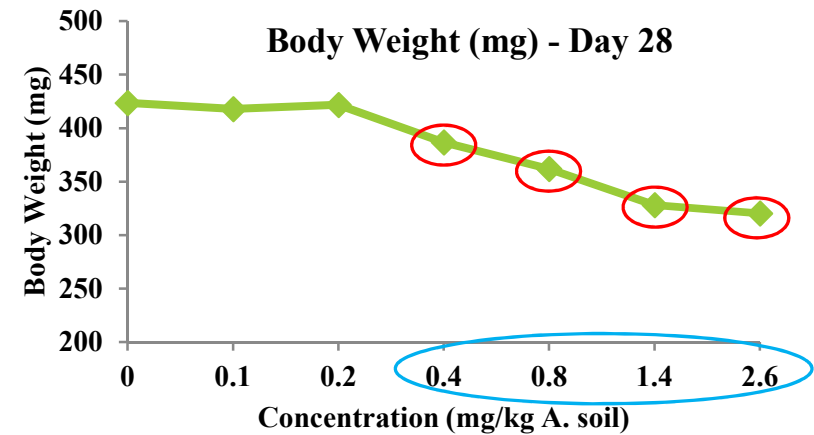
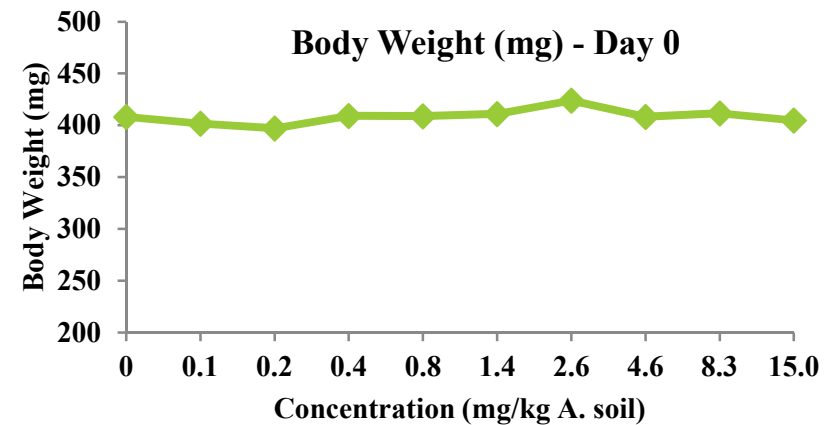
Transfer into test vessels

Results

Conventional Method



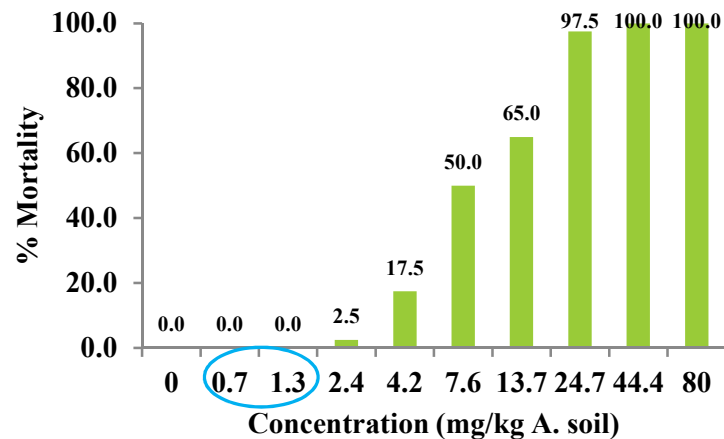
Individual Replicate Exposure Method



Results (Continued)

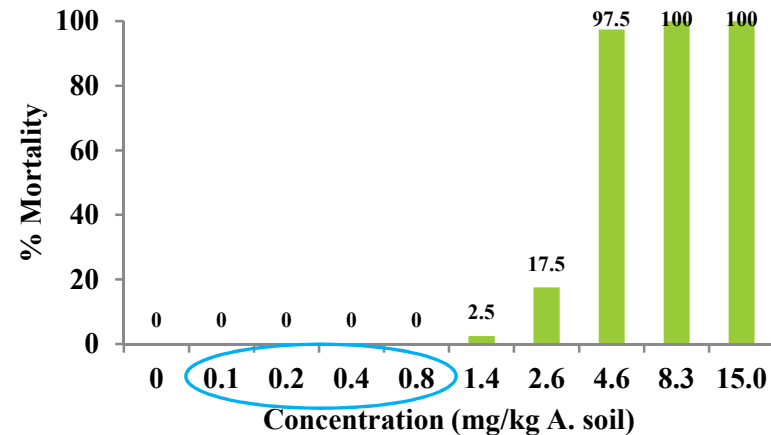
Conventional Method

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	-



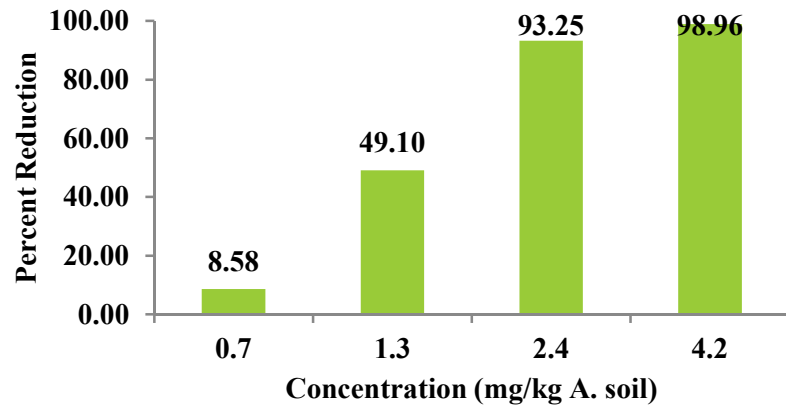
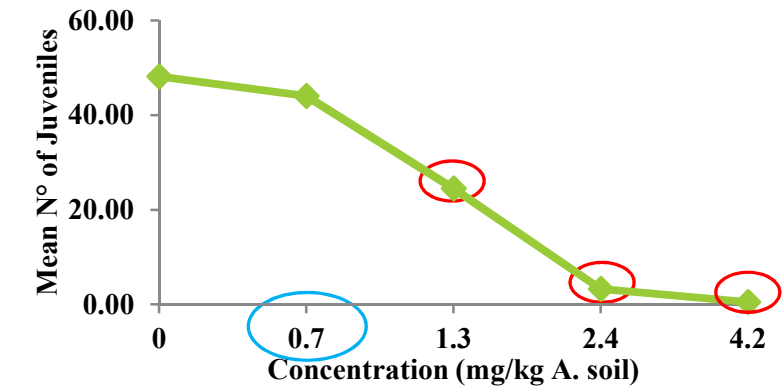
Individual Replicate Exposure Method

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

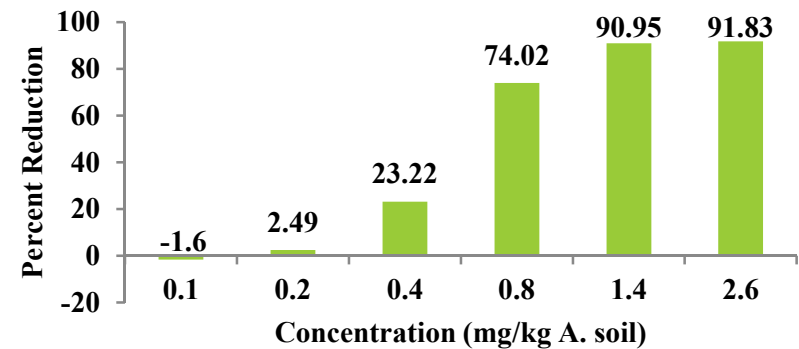
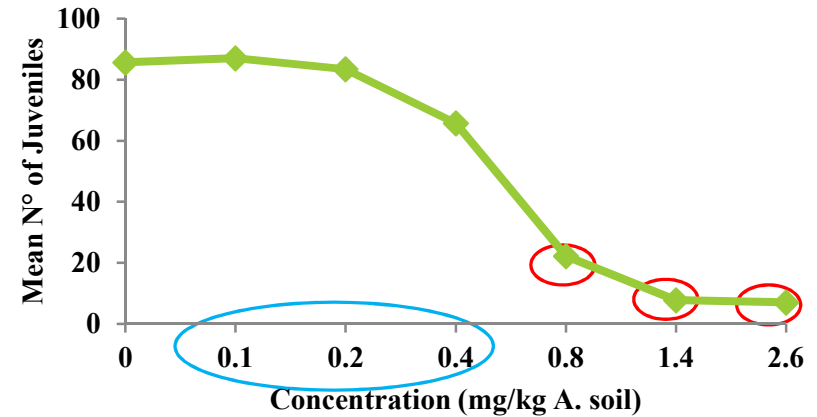


Results (Continued)

Conventional Method



Individual Replicate Exposure Method



Results (Continued)

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



End points	Garcia, 2004	Sian R Ellis et al., 2007	R. Shanmugasundaram 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



- 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



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.

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Thank You

