



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

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**OECD/OCDE**

**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

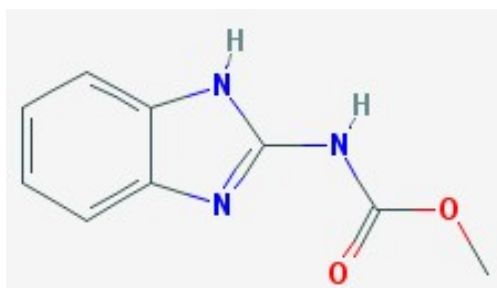
**Q1. How about insoluble and soluble compounds?**

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

# Introduction (Continued)

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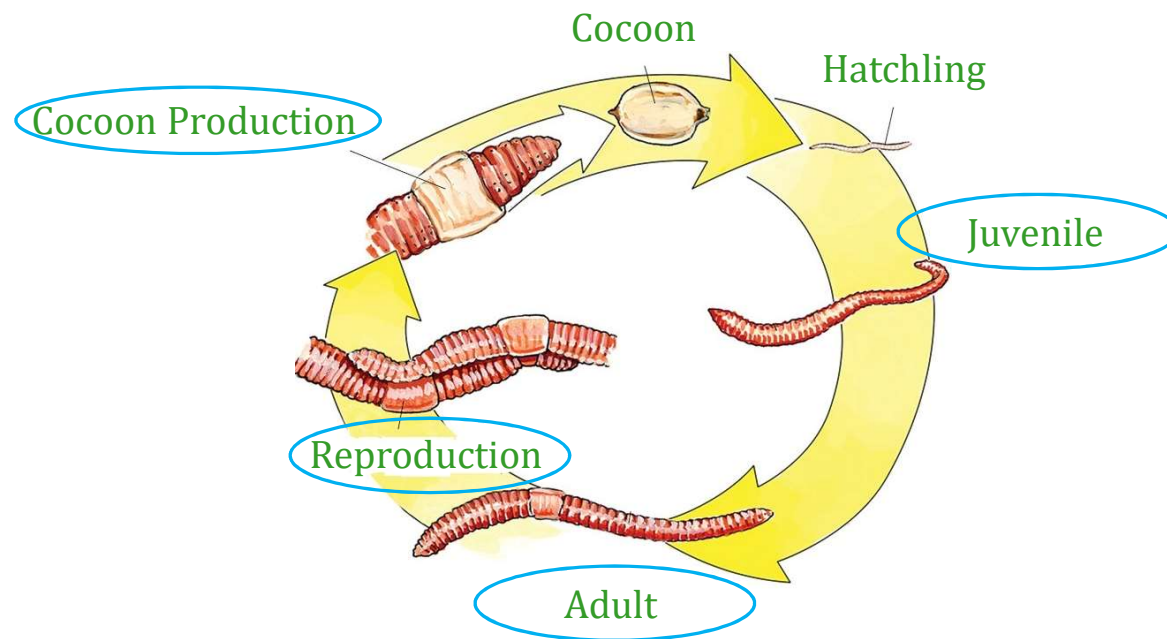
- Carbendazim



- 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



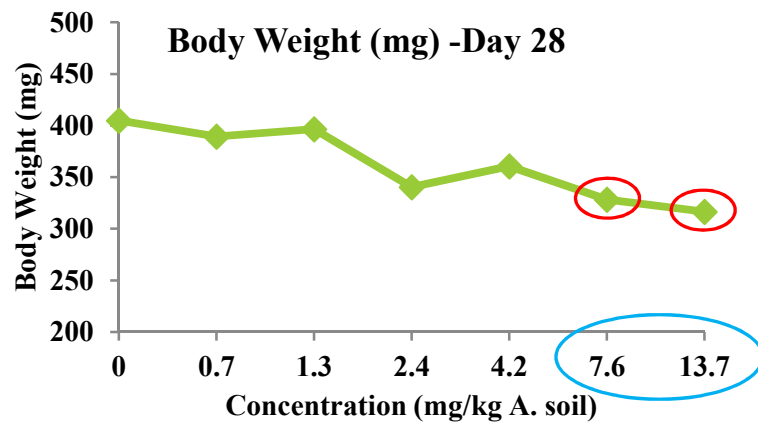
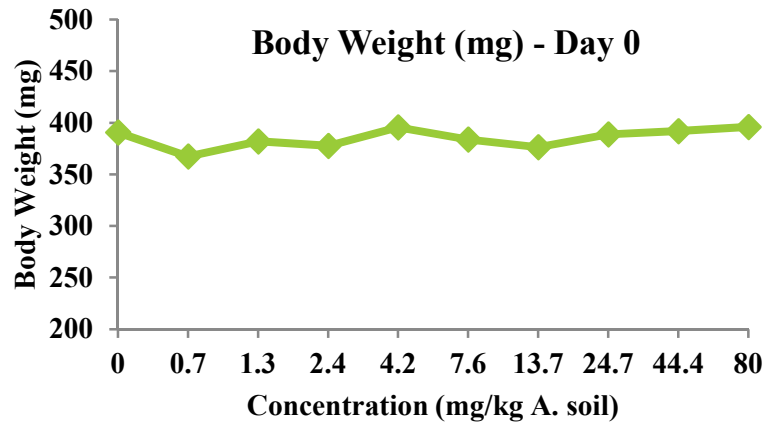
Mixing in artificial soil in replicate



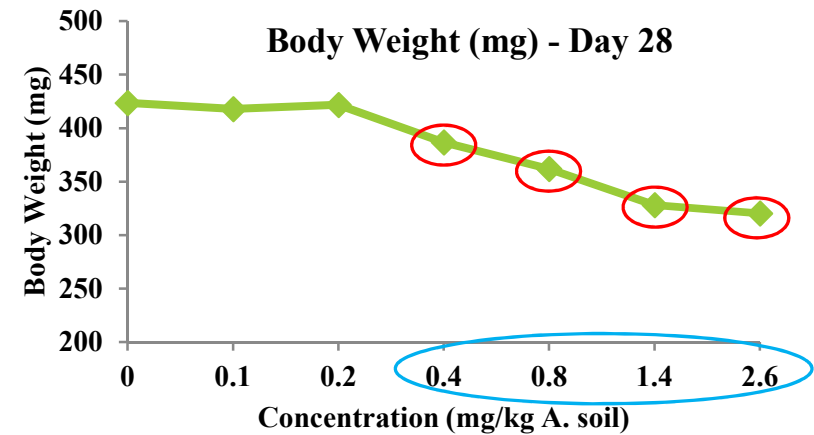
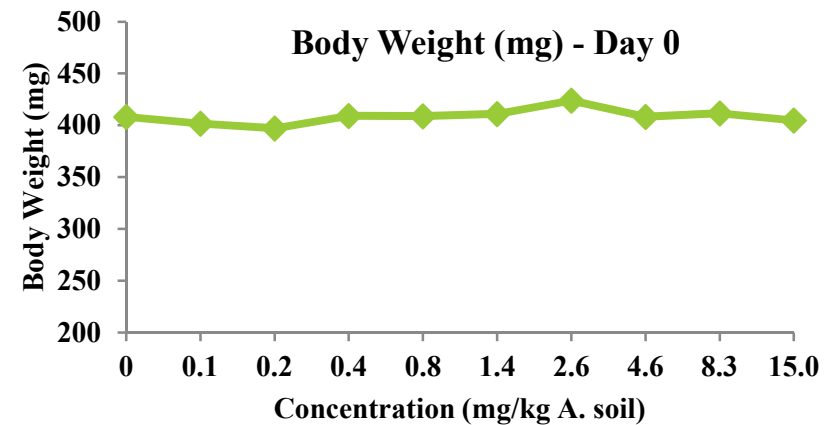
Transfer into test vessels

# Results

## Conventional Method



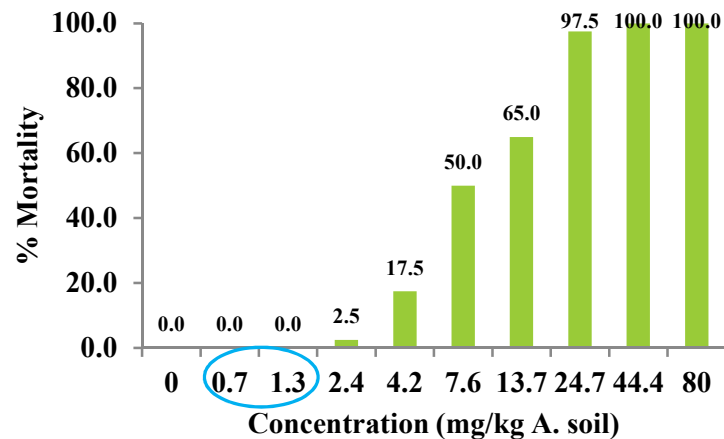
## Individual Replicate Exposure Method



# Results (Continued)

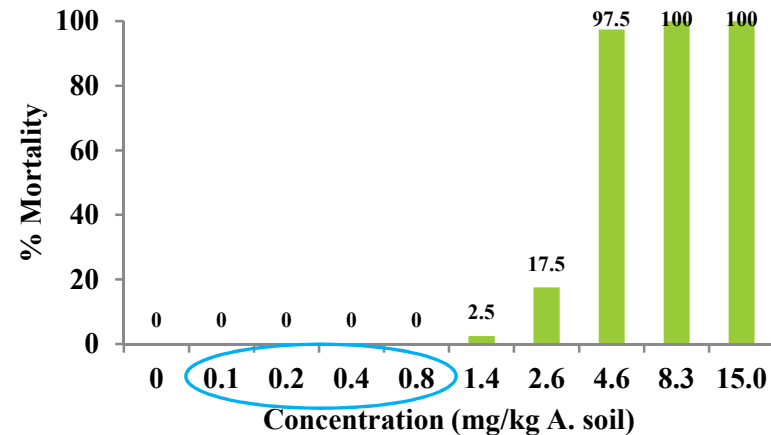
## Conventional Method

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

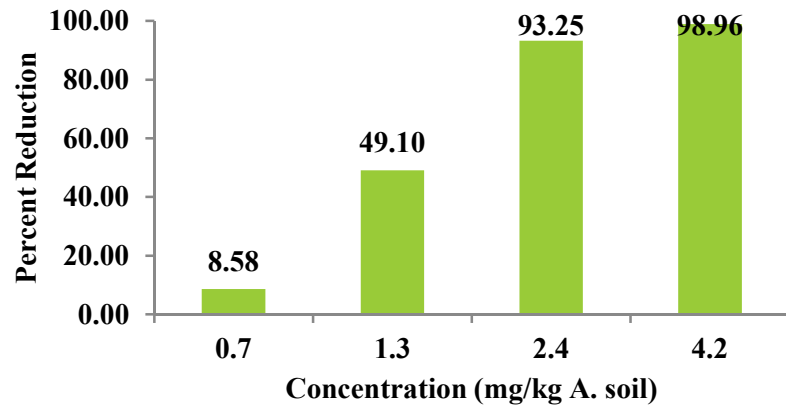
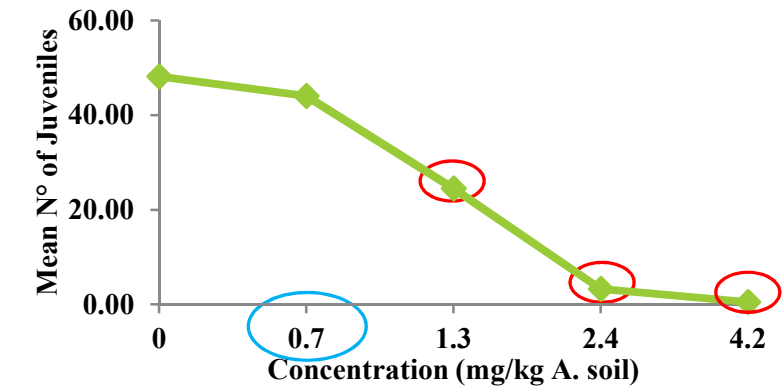
Concentrations (mg/kg A. soil)	Sign of Toxicity
1.4	Sluggish
2.6	Sluggish, Shrunk
4.6	Shrunk
8.3	-
15.0	-



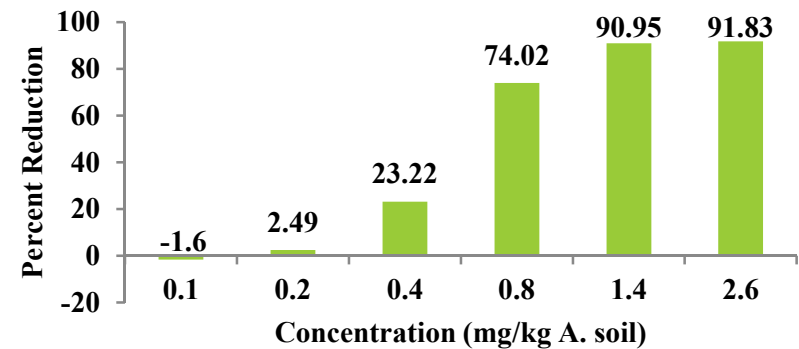
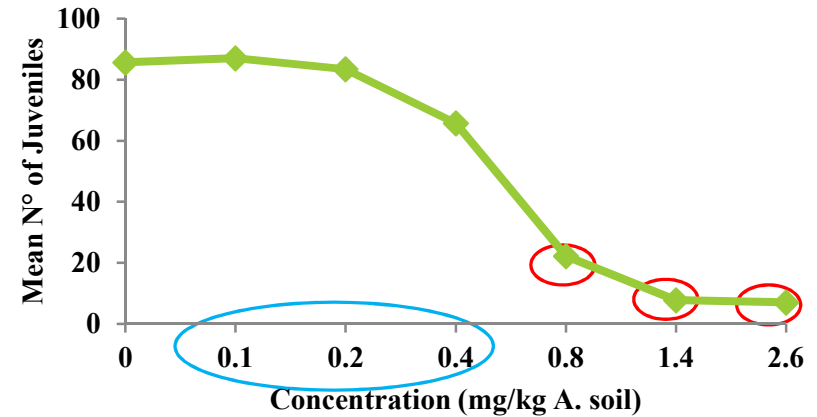


# Results (Continued)

## Conventional Method



## Individual Replicate Exposure Method



# Results (Continued)



End points	Conventional Method	Individual Replicate Exposure Method	Comparison
	mg/artificial soil		
LC <sub>50</sub> for adult mortality (28 day)	8.26	2.81	2.9 times
EC <sub>50</sub> 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 <sub>50</sub>	-	8.03	6.33	-	8.26	2.81
EC <sub>50</sub>	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

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**Thank You**

