

# “Shaken, not stirred”: The consequences of experimental freedom in the current OECD 309 guidance

Dieter Hennecke<sup>4</sup>, Kathrin Fenner<sup>1</sup>, Mark Honti<sup>3</sup>, [Prasit Shrestha](#)<sup>4</sup>, Stefan Hahn<sup>5</sup>, Thomas Junker<sup>2</sup>

<sup>1</sup>Eawag, Überlandstrasse 133, 8600 Dübendorf, Switzerland

<sup>2</sup>ECT Oekotoxikologie GmbH, Böttgerstrasse 2-14, 65439 Flörsheim/Main, Germany

<sup>3</sup>Hungarian Academy of Sciences, Muegyetem rkp. 3, 1111 Budapest, Hungary

<sup>4</sup>Fraunhofer IME-AE, Auf dem Aberg 1, 57392 Schmallenberg, Germany, [prasit.shrestha@ime.fraunhofer.de](mailto:prasit.shrestha@ime.fraunhofer.de)

<sup>5</sup>Fraunhofer ITEM, Nikolai-Fuchs-Strasse 1, 30625 Hannover, Germany

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OECD 309 guideline is an environmental simulation study used to assess degradation of chemicals in surface water. The guideline allows either a pelagic (without sediment) or suspended sediment test with 0.01 to 1 g/L sediment. To keep the sediment in suspension, OECD 309 allows either stirring or shaking. However, it is not clear yet which method is more appropriate and what are the pros and cons associated with these approaches.

Within the scope of CEFIC LRI-ECO18 project, a series of simulated water-sediment systems were studied including OECD 309. Four different test substances with differing biodegradation and sorption potential served as reference substances to test the different characteristics of the systems. For OECD 309, a suspended sediment test was carried out with 1 g/L sediment, which was agitated using a magnetic stirrer.

With less biodegradable substances OECD 309 was criticized already to be just an expensive sorption study. However, in the ECO18 studies huge amounts of NER formation were observed within 60 days. This finding was more pronounced in coarse sediment with low OC rather than in fine sediment with high OC. Also, the concentration-time series were hard to interpret: After initial equilibration, radioactivity in the water phase was further lost and that loss seemed to be directly compensated by an increase in the NER pool. Lastly, we observed wearing of the stirrer bar, in particular with the coarse sediment, and grinding of the sediment, which was confirmed with sediment texture determination. A drastic change in the grain size distribution before and after the stirring experiments was observed. This led to the hypothesis that sediment grinding during the experiment led to an increase of accessible sediment, which would lead to both increased NER formation and increased reversible sorption. In order to investigate this hypothesis, a shaken OECD 309 study was performed with one of the test substances (celecoxib). All experimental procedures adopted for sample processing and analysis were similar as in the stirred test.

In contrast to the stirred approach, in the shaking test almost no NER formation was observed over 60 days. Instead significant formation of transformation products was observed in the shaking test whereas in the stirring approach only traces occurred.

The current test results confirm what James Bond knew since decades already: it makes a huge difference whether the system is mixed by shaking or by stirring! The test setup (stirring vs. shaking) has a major influence on the test result of the 309. Thus, guidance in the present OECD 309 guideline with regard to the experimental test set-up does not seem sufficient to generate meaningful and reproducible degradation data for the use in environmental risk assessment.