# Transformation of Organic Chemicals in Aquatic Sediment Systems (OECD 308) under Simulated Natural Sunlight

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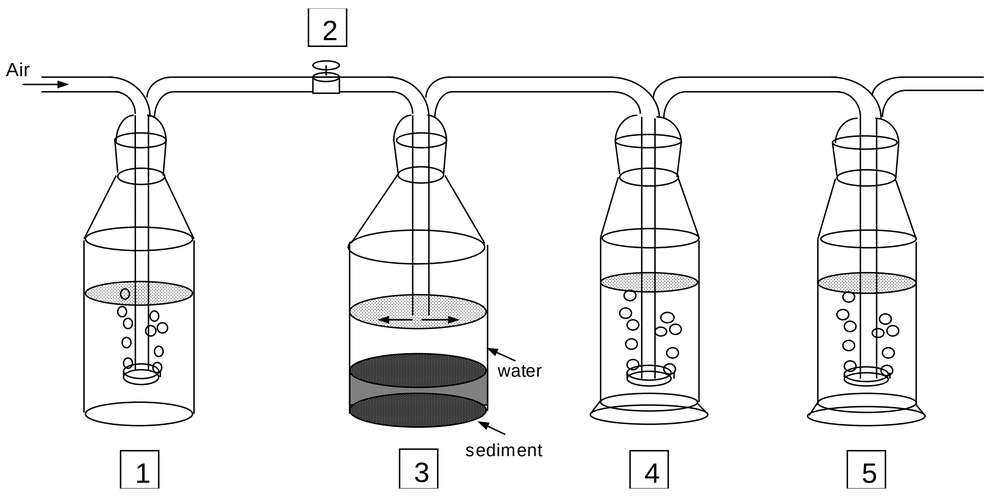
## Introduction

Chemicals that are directly applied to surface water or that are likely to reach the aqueous environment by routes such as run-off, drift and agricultural effluents, are required to pass environmental risk assessments. In order to further simulate environmental conditions and eventually providing an additional potential degradation mode for test compounds, requests for including natural sunlight and/or Algae in aquatic sediment (OECD 308) study designs have been emerging. Hence, IES Ltd has successfully been performing OECD 308 studies under simulated natural light and developed a respective test design containing Algae.

## Materials and Methods

**Table 1:** Data on OECD 308 studies performed at IES Ltd.

| Characteristics of OECD 308 studies performed at IES Ltd (2008-2019) | |
|---|---|
| No. of studies / clients | 59 / 27 |
| $^{14}$C-labels | 1 to 3 |
| Water solubility of test substances | 0.023 – 25000 mg/L |
| Specific activity | 0.78 - 11.90 MBq/mg |
| Test concentrations | 0.010 - 36.6 mg/L |
| Source of water and sediment | Lake, pond or river with ~pH 8 and 0 - 8 mg oxygen/L water |
| Temperature/light | Incubation at 20 - 22°C in the dark or under continuous irradiation |
| Test duration | 100 to 150 days (dark)<br>30 days (irradiated, without Algae)<br>Up to 63 days (irradiated, with Algae) |




(1) Water to moisten incoming air. (2) Valve to adjust the air flow. (3) Metabolism flask containing sediment and overlaying water. (4) Ethylene glycol trap for org. volatiles. (5) 2N NaOH trap for $^{14}CO_2$

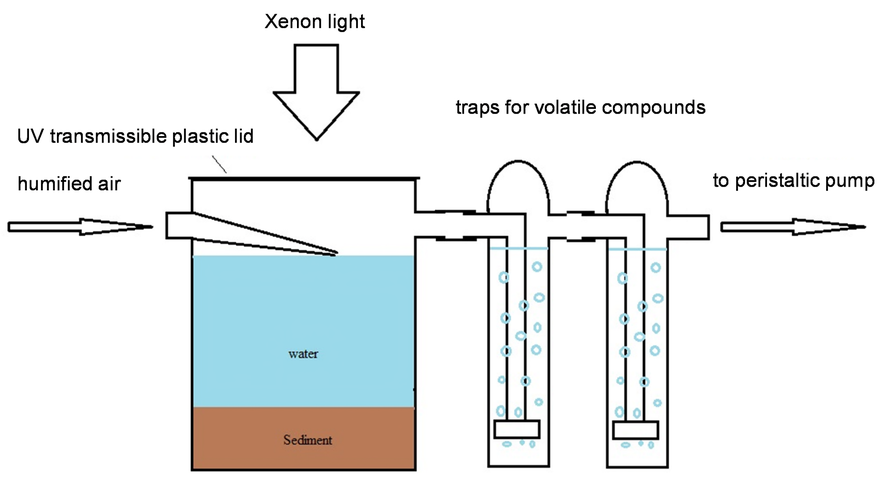



**Figure 1:** Typical OECD 308 test apparatus (top: dark, bottom: irradiated).

## Development of OECD 308 Test Design containing Algae

Goal: Controlled constant Algae population & growth, to be used in surface water, water-sediment and paddy soil studies.

### 1. Four different aquatic sediment systems were assessed

| System | Sediment | Water |
|---|---|---|
| River | Rhine, Mumpf, Switzerland | Rhine, Mumpf, Switzerland |
| | | Rhine, Rheinfelden, Switzerland |
| | | Rhine, Altrhein, Wyhlen, Germany |
| Pond | Biederthal, France | Biederthal, France |

### 2. Set-up for defined indoor conditions

### 3. Influence of parameters on natural Algae growth

- Inoculation of aqueous phase with Algae (*Pseudokirchneriella subcapitata*; variation in concentrations and intervals)
- Addition of growth medium for freshwater Algae (AAP medium)
- Replacement of 50% v/v aqueous phase with tap water at time 0
- **Replacement of 80% v/v aqueous phase with fresh natural water at the end of acclimation i.e. after 27 d (648 h) of irradiation (green line):**

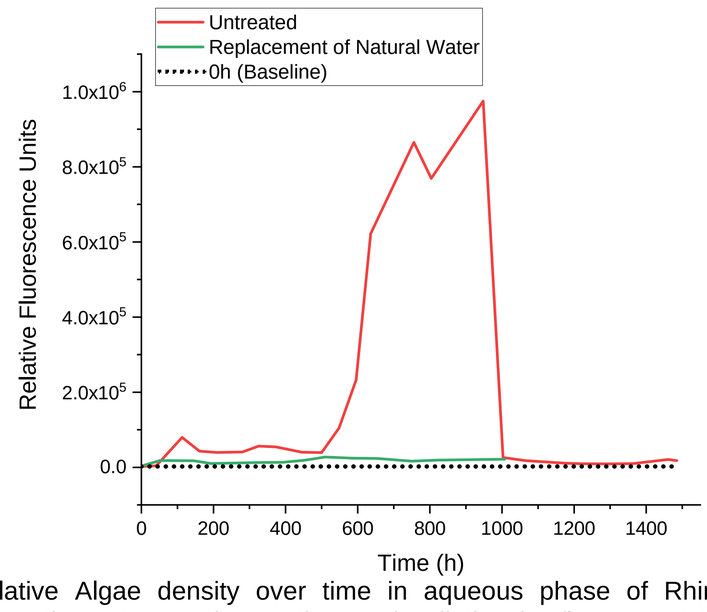



**Figure 2:** Relative Algae density over time in aqueous phase of Rhine (Mumpf, Switzerland) aquatic system under continuous irradiation by fluorescent tubes in the laboratory (T=20°C); the baseline represents the initial Algae population in aqueous phase of samples at time zero.

### 4. Quantification of Algae in water and sediment

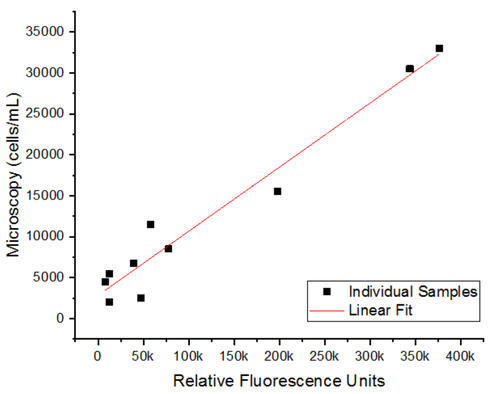



**Figure 3:** Correlation between manual (microscopy) and fluorescence (SpectraMax i3x) counting of algal density in aquatic aqueous phase.

**Table 2:** Chlorophyll a content in sediment (µg Chl.a/ 200 g sediment) measured by spectrophotometry of sediment acetone extracts.

| Chl.a a content (µg/200g) Mean of 16 samples | |
|---|---|
| Irradiated Samples | 2503 |
| Dark Controls | 794 |
| **Net Chl.a** | **1709** |

## Results

1. Rhine water and sediment (Mumpf, Switzerland) was shown to be the most suitable aquatic system for Algae growth.
2. Uncontrollable algal growth and deficient temperature and light control were observed under outdoor conditions. Stable conditions were obtained during incubation under fluorescent tubes in the laboratory at about 20 °C.
3. No significant effect was observed on algal growth by inoculation of aquatic systems with *Pseudokirchneriella subcapitata,* nor by addition of AAP growth medium, nor by replacement of 50% v/v aqueous phase with tap water at time 0. However, replacement of 80% v/v aqueous phase with fresh natural water after 27 days of irradiation, at the end of acclimation, resulted in controlled algal density in the aqueous phase, showing predominance of benthic Algae.
4. Quantification of Algae in aqueous phase is feasible by manual cell counting (microscopy) and fluorescence measurement (SpectraMax i3x), and in bethos by acetone extraction of unprocessed sediment followed by spectrophotometric measurement of Chl.a extract.

## Summary & Conclusion

- Increased interest of industry in customised OECD 308 test systems for persistent chemical compounds.
- OECD 308 test system with controllable Algae population feasible for an incubation time up to 30 days.
- Algae in OECD 308 studies might provide an additional degradation mode for test items due to a combination of increased oxygen and light, or a potential effect of Algae on chemicals.
- No significant influence of nutrient addition, inoculation with Algae cells and replacement by tap water on Algae growth in aquatic test systems.
- Quantification of Algae in water feasible by fluorescence and microscopy.
- Acetone extraction of benthic Algae without any further processing, followed by quantification through photospectroscopy.

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