## Supplementary Materials

# Ecotoxicological effect of disinfected wastewater effluent: a short review of *in vivo* toxicity bioassays to aquatic organisms

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**1. Toxicity indicators**

Toxicity indicator in table 1 are represented with average inhibition rate (μ), toxic units (TU), $EC_{50}$ and etc. They was calculated by:

(1) μ is the average growth rate of algae used in two studies,[1, 2] which can be calculated by two equations as following.

$$\mu = \frac{lnN_2 - lnN_1}{\Delta t}$$

where μ is the average growth rate of cells, 1/d; $N_1$ is the cell density at the beginning of the bioassay, cell/mL; $N_2$ is the cell density at the end of the bioassay, cell/mL; Δt is the test duration, d.

$$\mu = \frac{lnC_2 - lnC_1}{\Delta t}$$

where μ is the average growth rate of chlorophyll-A, 1/d; $C_1$ is the chlorophyll-A concentration at the beginning of the bioassay, mg/m$^3$; $C_2$ is the chlorophyll-A concentration at the end of the bioassay, mg/m$^3$; Δt t is the test duration, d.

(2) SOD activity was analyzed according to the method established by Beauchamp et al.[3] and modified by Zhang et al.[1] in bioassays to algae. In brief, the reaction mixture of sample group contained $5\times10^3$ mol/L sodium phosphate buffer, $1.3\times10^{-3}$ mol/L D-Methionine, $7.5\times10^{-5}$ mol/L nitroblue tetrazolium, $1\times10^{-5}$ mol/L EDTA-$Na_2$, $2\times10^{-6}$ mol/L riboflavin and enzyme extract. Sodium phosphate buffer was used instead of enzyme extract in two control groups. After homogenizing, the mixtures were illuminated by a fluorescent lamp (4000 lx) for 20 min, and then the absorbance was determined at 560 nm against the control from darkness. The activity of SOD is calculated with the following equation:

$$SODA = \frac{2(A_{CK} - A_S) \times V_T}{A_{CK} \times V_S \times V_r}$$

where SODA is SOD activity, U/mL; $A_{CK}$ and $A_S$ are the optical densities of control and sample groups at 560 nm, respectively; $V_T$ is the total volume of enzyme; $V_r$ is the enzyme volume used in reaction; and $V_s$ is the volume of algal culture.

(3) Cell membrane integrity was an indicator in the bioassay to algae.[1] It was determined by cell

viability test which uses propidium iodide (PI) fluorescence staining and flow cytometric analysis. In brief, Algal cells were collected by centrifuging and then algal pellets were re-suspended with PI solution. In flow cytometer, cells were excited with an argon excitation laser (488 nm) and the fluorescence emission of 564–606 nm was detected. PI only enters cells with damaged membranes and intercalated with double-stranded nucleic acids to emit much stronger fluorescent so the integrity rate can be calculated.

(4) $EC_{50}$ is the median e□ective concentration. In studies we reviewed, $EC_{50}$ is represented with the percentage of sample dilution which induces a response halfway between the baseline and the maximal effect. There are two scenarios: 1) The effluent is diluted with Milli-Q water directly and the percentage is the dilution multiple[4]. 2) The effluent is diluted with river water when the dilution equation should be used. Firstly, toxicity unit of upstream and effluent is tested based on the method in the first scenario. Secondly, assuming completely mixed conditions, the percentage C is calculated by the following equation[5]:

$$C = \frac{C_s Q_s + C_e Q_e}{Q_e + Q_s}$$

where $C$ is the downstream toxicity unit (TU); $C_s$ is is the upstream toxicity v (TU); $Q_s$ is the upstream mean flow; $C_e$ is the effluent toxicity unit (TU); $Q_e$ is the effluent mean flow.

(5) TU is the toxic unit.[6] It is considered an another expression of $EC_{50}$ besides concentration:

$$TU = \frac{1}{EC_{50}} \times 100$$

where $TU$ is the toxic unit; $EC_{50}$ is the median e□ective concentration which is represented with the percentage of sample dilution. A hazard classification system for wastes discharge into aquatic environment described by Persoone et al.[7] as shown in Table S1.

## 2. References


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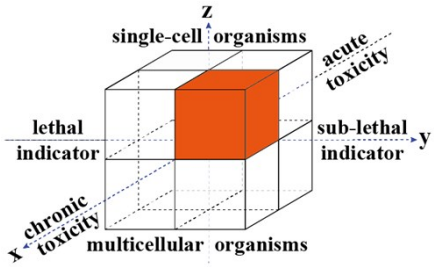


**Figure S1. Combinations of different exposure time, toxicity indicators and organisms of *in vivo* bioassays to aquatic organisms.** Each small cube represents one bioassay combination with three parts: exposure time (read from x axis, with chronic toxicity as the positive direction), toxicity indicator (read from y axis, with sub-lethal as the positive direction) and organisms (read from z axis, with single-cell organisms as the positive direction). The cube in orange (the combination of chronic sub-lethal bioassays with single-cell organisms) is considered the most sensitive one.

**Table S1. Hazard classification system using TU as indicatior[7]**

| TU | Class | Toxicity |
|---|---|---|
| < 0.4 | Class Ⅰ | No acute toxicity |
| 0.4 < TU < 1 | Class Ⅱ | Slight acute toxicity |
| 1 < TU < 10 | Class Ⅲ | Acute toxicity |
| 10 < TU < 100 | Class Ⅳ | High acute toxicity |
| > 100 | Class Ⅴ | Very high acute toxicity |