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Toxicity of tetramethylammonium hydroxide to aquatic organisms and its synergistic action with potassium iodide

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HIGHLIGHTS

- *Daphnia magna* is sensitive to TMAH.
- TMAH and KI have synergistic toxic effects in *D. magna*.
- Larger quaternary ammonium compounds are more toxic to *D. magna* than TMAH.

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ABSTRACT

The aquatic ecotoxicity of chemicals involved in the manufacturing process of thin film transistor liquid crystal displays was assessed with a battery of four selected acute toxicity bioassays. We focused on tetramethylammonium hydroxide (TMAH, CAS No. 75-59-2), a widely utilized etchant. The toxicity of TMAH was low when tested in the 72 h-algal growth inhibition test (*Pseudokirchneriellia subcapitata*, EC_{50} = 360 mg L^{-1}) and the Microtox® test (*Vibrio fischeri*, IC_{50} = 6.4 g L^{-1}). In contrast, the 24 h-microcrustacean immobilization and the 96 h-fish mortality tests showed relatively higher toxicity (*Daphnia magna*, EC_{50} = 32 mg L^{-1} and *Oryzias latipes*, LC_{50} = 154 mg L^{-1}). Isobologram and mixture toxicity index analyses revealed apparent synergism of the mixture of TMAH and potassium iodide when examined with the *D. magna* immobilization test. The synergistic action was unique to iodide over other halide salts i.e. fluoride, chloride and bromide. Quaternary ammonium ions with longer alkyl chains such as tetraethylammonium and tetrabutylammonium were more toxic than TMAH in the *D. magna* immobilization test.

1. Introduction

In the last two decades, the production of thin film transistor liquid crystal displays (TFT-LCDs) has rapidly increased. TFT-LCDs are currently utilized in a myriad of electronic devices, such as televisions, monitor displays, laptop computers, mobile phones and other commonplace mobile gadgets. The recent increase in the demand for TFT-LCDs resulted in a heightened production of indium-tin oxide (ITO) glass wafer (Takano et al., 1992; Katayama, 1999; Service, 2001). A large quantity of organic solvent is used in the manufacturing process of the wafer.

Tetramethylammonium hydroxide (TMAH, CAS 75-59-2) is the most common chemical compound used in the anisotropic wet etching of the ITO glass wafer. Anisotropic wet etching is an essential step to make fine patterns of circuit on silicone wafer in an orientation dependent manner. High concentrations of TMAH

Abbreviations: TMAH, tetramethylammonium hydroxide; TFT-LCDs, thin film transistor liquid crystal displays; ITO, indium tin oxide; MTI, mixture toxicity index; TU, toxic unit; QAH, quaternary ammonium hydroxide.

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are discharged to the environment from TFT-LCD manufacturing in spite of efforts to reduce the amount of solvent used (Tabata et al., 1992; Kawabata and Sugawara, 2002). The average concentration of TMAH in a biological treatment plant for TFT-LCD wastewater was recorded as 1528 mg L^{-1} (Hu et al., 2012). Efficient and cost-effective technologies for TMAH removal from wastewater are being explored and yet to be introduced (Hu et al., 2010; Lei et al., 2010; Nishihama et al., 2010).

In occupational settings, TMAH is of relatively low hazard due to its low volatility compared with the structurally-related, non-methylated alternative etchant, ammonium hydroxide (NH_4OH), which is also a skin corrosive (Schnakenberg et al., 1990; Tabata et al., 1992; Lee et al., 2011). However, fatal poisoning with TMAH has been reported in TFT-LCD manufacturing facilities and the mechanism of action is attributed to neuromuscular toxicity caused by the tetramethylammonium ion (TMA^+) (Wu et al., 2008; Lin et al., 2010). TMA^+ inhibits acetylcholine esterase (AChE) and causes fatal cardiovascular disorders and respiratory failure in experimental animals (Kennedy et al., 1995; Akk and Steinbach, 2003; Wu et al., 2012). Lessons from the occupational and animal model studies raise questions whether TMAH could also have a detrimental effect on aquatic organisms via the same mechanism of toxicity when discharged into the environment.

In addition to TMAH, other quaternary ammonium hydroxides (QAHs) such as tetraethylammonium and tetrabutylammonium hydroxides can also be used as etchants (Schnakenberg et al., 1990; Tabata et al., 1992). Although previous research on similarly structured compounds such as tetraalkyl bromides suggests that alkyl chain length is related to toxicity to aquatic organisms (Couling et al., 2006), the toxic hierarchy amongst QAHs in the aquatic environment remains to be determined.

An iodine (I_2)/potassium iodide (KI) solution is utilized in the electrolyte solution (1.5–15%) in the same etching process (Han et al., 2009). Research on the removal of iodide in LCD wastewater is in progress (Lee et al., 2008) added to the list of chemicals involved in the LCD manufacturing industry, large volumes of dimethyl sulfoxide (DMSO) are used as a solvent and are found in wastewater, e.g. 800 mg L^{-1} (Muratani, 1999; Park et al., 2001; Lei et al., 2010). As a result, LCD manufacturing discharges wastewater containing high concentrations of TMAH, KI and DMSO to receiving water bodies. Single chemical analyses may underestimate the risk of the chemical mixtures in wastewater and effluents (Cairns and Scheier, 1968; Hodges et al., 2006). The joint toxicity of these chemicals remains to be determined.

In this study, we report on the toxicity of TMAH to aquatic organisms, the toxic hierarchy of QAHs and the joint toxicity actions of TMAH, KI and DMSO.

2. Materials and methods

2.1. Chemicals

Tetramethylammonium hydroxide (10% in water) was obtained from Nacalai Tesque (Kyoto, Japan). KI and DMSO were purchased from Wako Pure Chemistry (Osaka, Japan). Other chemicals were of the highest-grade products of Nacalai Tesque and Wako Pure Chemistry. pH of TMAH solution was adjusted to 7.0 with HCl prior the toxicity tests.

2.2. Microcrustacean immobilization test

Acute toxicity to microcrustaceans was examined with the 24-h *Daphnia magna* immobilization test using Daphtox kit F (Microbiotests Inc., Belgium) as previously reported (Okamura et al., 1998).

2.3. Algal growth inhibition test

The algal growth inhibition test was carried out using the Algaltox Kit F (Microbiotests Inc., Belgium) essentially as previously reported (Okamura et al., 1998). In brief, *Pseudokirchneriellia subcapitata* (strain NIES-35) was inoculated into the supplier-provided dilution medium to a cell density of 1×10^4 cells mL^{-1} in which an any given concentration of test chemicals were included, following a pre-culture for 72 h in the medium. Cells were grown in a glass test tube under continuous illumination with fluorescent tubes at a photon density of 100 μmol m^{-2} s^{-1} with 100 rpm of continuous agitation at 25 °C. Cell density of *P. subcapitata* was determined with a haemocytometer at 24, 48 and 72 h. The rate of growth inhibition was calculated according to OECD test guideline 201 (OECD, 1992).

2.4. Microtox® toxicity test

Toxicity to *Vibrio fischeri* was assessed with the Microtox® Analyzer 500, according to the manufacturer's manual (Strategic Diagnostics Inc., Newark, DE). Decrease of luminescence in a 15-min exposure was expressed in percentages as previously reported (Lei and Aoyama, 2010).

2.5. Fish mortality test

Toxicity to Medaka fish (*Oryzias latipes*) was examined according to the OECD test guideline 203 (OECD, 1992). Healthy *O. latipes* (2–2.5 cm in body length, approximately 0.3 g each) were obtained from a local aquarium shop, cultured for 5 d and then acclimatized to the test environment into the dilution solution (ISO 6341-1982) for 7 d prior to a semi-static exposure at 23 ± 1 °C for 96 h. The fish were fed daily with dried daphnids until 24 h before the test. Two individuals were loaded in a liter of the test solution in a vessel. Twenty fish were tested for each condition. A light cycle of 16-h light: 8-h dark was used. Mortality of *O. latipes* was examined at 24, 48, 72 and 96 h of exposure.

2.6. Statistical analysis and joint toxicity analyses

The toxicity endpoints (EC_{50}, IC_{50} and LC_{50}) and 95% confidence intervals (CI) were calculated by probit regression analysis with the SPSS® software ver. 21 (SPSS Japan Inc., Tokyo). No Observable Effect Concentrations (NOECs) were determined by the Dunnett's test ($\alpha = 0.05$).

To assess joint action toxicity of the binary mixtures, isobologram and mixture toxicity index (MTI) analyses were conducted (Lange and Thomulka, 1997; Koutsaftis and Aoyama, 2007). The sum of the toxic units (M) of the compounds in the mixture was calculated as follows:

$$M = \sum_i \mathrm{TU}_i = \sum_i (\mathrm{C}_i / \mathrm{EC}_{50i}) \quad (1)$$

where TU_i is toxic unit (TU) of compound i, C_i is concentration of compound i in the mixture and EC_{50i} is the experimentally obtained EC_{50} of compound i (Koutsaftis and Aoyama, 2007). The MTI was determined according to the equation:

$$\mathrm{MTI} = 1 - (\log M / \log M_0) \quad (2)$$

where M is sum of TU_i of compounds in the mixture, M_0 is M divided by the largest fraction in the mixture ($M_0 = M/\max(TU_i)$). Where MTI = 1, the interaction is regarded as an additive effect. Where MTI > 1, a synergistic effect is predicted (for more detail, see Koutsaftis and Aoyama, 2007).