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

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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 72h-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 24h-microcrustacean immobilization and the 96h-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.

Highlights

• *Daphnia magna* is sensitive to TMAH.

• TMAH and KI have synergistic toxic effects in *D. magna*.

• Larger quarternary ammonium compounds are more toxic to *D. magna* than TMAH.

Keywords: Tetramethylammonium hydroxide, potassium iodide, aquatic toxicity, synergism, *D. magna*, semiconductor wastewater

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.

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 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, 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

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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, 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., 2005), 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 hours. 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 days and then acclimatized to the test environment into the dilution solution (ISO 6341-1982) for 7 days prior to a semi-static exposure at 23 ± 1 °C for 96 hours. 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 hours 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:

$$M = \Sigma_i TU_i = \Sigma_i (C_i / EC_{50i}) \quad \text{(Eq 1)}$$

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:

$$MTI = 1 - (logM/logM_0) \quad \text{(Eq 2)}$$

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).

3. Results

3.1. *Aquatic toxicity of tetramethylammonium hydroxide (TMAH)*

A battery of tests was carried out to estimate the aquatic toxicity of TMAH. The acute toxicity tests comprised 4 aquatic species that represent major ecological strata, i.e. 96-h fish mortality test, 24-h microcrustacean immobilization test, 72-h algal growth inhibition test and 15-min *Vibrio fischeri* bioluminescence inhibition test (Microtox® test).

Toxicity of TMAH to the bacterium *V. fischeri* and the alga *P. subcapitata* was very low (Fig. 1B and C). Half maximal inhibition concentration (IC_{50}) for the Microtox® test was not obtained in the tested concentration rage (up to 3 g L^{-1}), and the NOEC was 0.5 g L^{-1}. Interestingly, a low concentration of TMAH (200 mg L^{-1}) resulted in the promotion of algal growth (36%, Fig. 1C). Half maximal effect concentration (EC_{50}) and NOEC of the algal growth inhibition test were 360 mg L^{-1} and 150 mg L^{-1}, respectively. Conversely, an enhancement of bioluminescence by low concentrations of TMAH was not observed in the tested concentrations (Fig. 1B). Mortality was not observed in *O. latipes* within the 24-h exposure at any of the tested concentrations (Appendix Fig. 1) with a sudden increase of mortality at 48-h exposure at 200 and 300 mg L^{-1}. The half maximal lethal concentration (LC_{50}) for *O. latipes* (Fig. 1D) and NOEC were 154 and 50 mg L^{-1}, respectively. Some fish individuals showed dyschezia as estimated by abdominal morphology at sublethal concentrations of TMAH e.g. 100 mg L^{-1} (data not shown). Toxicity of TMAH to *D. magna* was relatively high (Fig. 1A) when compared to the other species tested. The EC_{50} and NOEC for the immobilization were determined as 32 mg L^{-1} and 10 mg L^{-1}, respectively.

3.2. *Acute toxicity of KI and DMSO*

Toxicity of KI and DMSO was examined individually against the same test organisms since both compounds are also routinely used in the semiconductor manufacturing process together with TMAH and discharged to the environment in large amounts (Park et al., 2001; Lee et al., 2008; Han et al., 2009; Lei et al., 2010). No adverse effects on the respiration of *V. fischeri* were observed up to 10 g L^{-1} KI (Table 1). Sensitivity of *P. subcapitata* to KI was subtle (EC_{50} = 4.1 g L^{-1}). Likewise, the LC_{50} could not be obtained for 96-h Medaka fish mortality test, due to low toxicity (up to 1 g L^{-1}). On the other hand, the EC_{50} for the 24-h *D. magna* immobilization test was determined to be as low as 1 mg L^{-1} KI, indicating a significant toxicity. Toxicity of DMSO to *D. magna*, *V. fischeri* and *P. subcapitata* was very low (Table 1). The toxicity values obtained for these tests were further utilized in the subsequent assays for joint toxicity.

3.3. *Synergistic effects of TMAH and KI*

The joint toxicity of binary mixtures of TMAH, KI and DMSO in the *D. magna* immobilization test was investigated. We examined the immobilization rate as the function of the sum of TU (M) of binary 1:1 mixture of the compounds (Fig. 2A, B and C). The observed M of the binary mixtures of TMAH and DMSO, and KI and DMSO were 0.79 [95% confidence interval (CI): 0.65-1.05] and 1.07 (95% CI: 0.85-1.32), respectively. These results indicate that the joint action of these combinations had an apparent additive effect. On the contrary, the observed M of the mixture of TMAH and KI was 0.33 (95% CI: 0.04-0.66) (Fig. 2C), indicating an increase in toxicity, namely synergistic effect.

To further explore the synergism of the mixture of TMAH and KI, an isobologram analysis was conducted (Fig. 2D). We prepared 4:1, 3:2, 1:1, 2:3 and 1:4 mixtures of

TMAH and KI to give a theoretical M = 1. All plots of the mixtures located below the lower 95% CI of the theoretical additive effect line, indicating that the combination of TMAH and KI rendered a detrimental synergistic effect on *D. magna* mobility. The synergistic action of the mixture was further supported by the MTI analysis (Table 2). All mixture ratios of TMAH and KI (4:1, 3:2, 1:1, 2:3, 1:4) showed MTI > 1. Collectively, it is evident that the toxic joint action of the TMAH and KI mixture was synergistic.

3.4. *Synergistic action is specific to KI*

The specificity of KI for synergistic action with TMAH over the other potassium halide salts was examined. Firstly, each EC_{50} of KF, KCl, KBr and KI was determined by the 24-h *D. magna* immobilization test (Appendix Fig. 2). KI showed a dominating toxicity when compared to the other compounds (EC_{50} = 1.45 mM [95%CI, 0.649-2.92]). KF had slightly higher toxicity, EC_{50} = 6.5 mM (95% CI, 5.8-7.3) compared to KCl and KBr, EC50 = 9.0 mM (95% CI, 8.5-9.9) and 8.5 mM (95% CI, 8.1-9.2), respectively. Secondly, equal (1:1) mixtures of TMAH and the halide (M = 1) were tested. KI showed a higher immobilization rate when it coexisted with TMAH (Table 3) in agreement with the results stated above (Fig. 2 and Table 2). In contrast, KF, KCl and KBr did not show an enhancement of the toxic effect (~50% immobilization), indicating a seemingly additive effect (Table 3). This result suggests that the synergism with TMAH is not a common characteristic of potassium halide salts, but is unique to the iodide ion.

3.5. *Effects of other quaternary ammonium compounds*

A comparative analysis of other quaternary ammonium compounds was conducted (Fig. 3). The compounds with larger alkyl groups, tetrabutylammonium hydroxide and tetraethylammonium acetate showed higher toxicity to *D. magna* (Figs. 3

A and C). Ammonium chloride demonstrated the least toxicity. Logarithmic regression analysis of EC_{50} against the molecular mass of the quaternary ammonium compounds implies a strong negative linear correlation (r = -0.994) (Fig. 3B). The octanol water partition coefficient (K_{OW}) showed a negative linear trend with EC_{50}, as well (Appendix Fig. 3).

4. Discussion

4.1. Ecotoxicity

The production of TFT-LCD has increased in the last few decades (Takano et al., 1992; Katayama, 1999; Service, 2001; Lee et al., 2011). TMAH is the most utilized etchant in TFT-LCD manufacturing. Toxicity of TMAH has been intensely investigated in animal models for the determination of human toxicity (Tsubaki et al., 1986; Hallek and Szinicz, 1988; Akk and Steinbach, 2003). However, its effects on the aquatic ecosystem are currently not well understood.

In this study, we focused on the estimation of the potential impact of TMAH on freshwater organisms. To assess the aquatic ecotoxciity of TMAH, 4 test species, namely fish *O. latipes*; microcrustacean *D. magna*; alga *P. subcapitata*; and bacterium, *V. fischeri*, were used. The results indicate that TMAH is potentially toxic to *D. magna* (24-h EC_{50} = 32 mg/L), of low toxicity to *O. latipes* (96-h LC_{50} = 154 mg/L) and *P. subcapitata* (72-h growth EC_{50} = 360 mg/L) and negligibly toxic for *V. fischeri* (IC_{50} > 3 g/L) (Table 1). These TMAH results are loosely comparable with previously obtained endpoints for similar and different species, such as daphnids, with 48-h EC_{50} of 3 and 1.3-1.5 mg/L for *D. magna* and *Ceriodaphnia dubia*, respectively; a 72-h growth EC_{50} of 96 mg/L for *P. subcapitata*; and a 96-h LC_{50} of 462 mg/L in fish *Pimephales promelas* using tetramethylammonium chloride (OECD, 2012).

Microcrustaceans and fish share important ecological niche in aquatic environments as predators. Selective reduction of the population of consumers with the

discharge of TFT-LCD wastewater may cause an ecological imbalance. The concentration of TMAH in a wastewater treatment facility of TFT-LCD manufacturing factory was estimated to be 1528 mg L^{-1} (Hu et al., 2012). It is deduced that the discharge of TMAH-rich wastewater from LCD factories to the environment without appropriate corrective measures for the residual TMAH concentration may acutely disrupt the aquatic ecosystem predominantly through the detrimental effects on daphnids and secondarily on fish. In Japan, annual production of TMAH in 2004 is about 3,000 t. The chemical is also produced in the United States and Korea, while no production amount data are available for these countries (J-CHECK, 2010). Accounting for its large amount of production, the toxicity to fish should be aware as well as to microcrustaceans albeit the relatively high LC_{50}. Our data suggests that undertaking biomonitoring with a significant attention to behavior and population of microcrustaceans and fish should be effective to preserve aquatic ecosystem receiving TFT-LCD wastewater.

TMAH and KI are utilized in the wet etching process in the same facility and discharged to the same water bodies. It is expected that a simultaneous discharge of an equimolar mixture of TMAH and KI would cause an increase of approximately 3-fold higher toxicity to microcrustaceans than the release of single chemical (Fig. 2C). On the other hand DMSO, which is also utilized in the same representative facility in a large quantity, did not show a synergistic effect. The strict synergistic effect of the two chemicals discharged from TFT-LCD manufacturing is a noticeable finding. Both chemicals are highly water-soluble and may exert a synergistic effect to planktonic species. The determination of the concentrations of these chemicals in receiving water bodies is an important task to address the question whether the synergic toxicity is rendered in real situations.

Larger quaternary ammonium hydroxides exhibited higher toxicity (Fig. 3) confirming a correlation between toxicity and alkyl chain length of room-temperature

ion liquids in *V. fischeri* and *D. magna* (Couling et al., 2006). TMAH can be replaced by other QAHs with longer alkyl chains, e.g. TEAH and TBAH in the TFT-LCD production process (Schnakenberg et al., 1990; Tabata et al., 1992; Lei et al., 2008). However, the use of QAHs with larger alkyl groups as a replacement of TMAH is not recommended due to their greater potential for ecotoxicological impact as mentioned above.

4.2. Toxic mechanism

Very limited data is available on the specific toxicity mechanism of TMAH to invertebrates, and none to fish to our best knowledge. However, animal tests and occupational incidents suggest that TMA^+ is a neurotoxic chemical acting by inhibiting AChE activity potentially leading to mortality from suffocation and cardiovascular dysfunction (Kennedy et al., 1995; Wu et al., 2012). It is important to note that crustaceans and fish have a highly developed nervous system. In fact, AChE inhibitors, including carbamates and organophosphates, are known to induce death in *D. magna* (Carvalho et al., 2003). It is well known that inhibition of AChE of fish by pollutants results in acute toxicity to fish (e.g. de Bruijn and Hermens, 1993). We believe that our data and preceding studies indicate that TMAH inhibits AChE and results in the perturbation of cholinergic transmission of daphnids and Medaka fish. We do not necessarily deny other toxic mechanism of TMAH to aquatic animals.

Apparent synergism with TMAH was not observed among potassium halides, except KI (Table 3). The mechanism of this synergism remains unresolved. Whereas TMAH had an adverse effect on *D. magna* and Medaka fish (Table 1) KI was toxic only to *D. magna* (Table 1). This difference may indicate that the toxicity targets and very likely the modes of action of TMAH and KI are not identical. Although sensitivity to iodide by *D. magna* has been previously mentioned (Laverock et al., 1995), further research focusing on the effect of KI is needed to establish the mode of synergistic

action of the two chemicals.

5. Conclusions

(1) *Daphnia magna* is sensitive to tetramethylhydroxyammonium hydroxide (TMAH).

(2) Microcrustaceans may be amongst the most sensitive organisms in the aquatic environment where semiconductor wastewater is released.

(3) The coexistence of KI and TMAH results in a deleterious synergistic effect to *D. magna*.

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Figure captions

Fig. 1. Acute toxicity of TMAH.

(A) Microcrustacean 24-h immobilization test.

(B) *Vibrio fischeri* MicroTox test. Inhibition of bioluminescence in 15 min.

(C) 72-h algal growth inhibition test.

(D) Medaka fish 96-h mortality test.

Solid lines indicate Probit-model fitting curves. Dot lines indicate 95% confidential intervals. Error bars indicate standard deviation (n=4). Regression curve was not obtained for the algal growth inhibition test due to the irregular shape of inhibition curve.

Fig. 2. Joint toxicity of TMAH, DMSO and KI on 24-h *Daphnia magna* immobilization test.

(A) Binary mixture of TMAH and DMSO (1:1).

(B) Binary mixture of KI and DMSO (1:1).

(C) Binary mixture of TMAH and KI (1:1).

Solid line curves (A, B and C) indicate Probit regression fitting. Error bars indicate standard deviation (n=4).

(D) Isobologram of TMAH and KI mixture. Solid straight line indicates theoretical additive toxicity. Dot lines indicate 95% confidential interval of the theoretical additive effect. T.U., toxic unit.

Fig. 3. Acute toxicity of quaternary ammonium cations on microcrustacean.

Acute toxicity of tetrabutylammonium hydroxide (TBAH), tetraethylammonium acetate (TEAA), tetramethylammonium hydroxide (TMAH) and ammonium chloride (NH_4Cl) was examined by 24-h *D. magna* immobilization test (Daphtox kit F).

(A) Concentration effect plots of quaternary ammonium salts on *D. magna* immobilization. Regression curve is of Probit model fitting. Error bars indicate standard deviation (n=4). Diamond: TBAH, square: TEAA, triangle: TMAH, circle: NH_4Cl.

(B) Linear regression analysis of log EC_{50} versus molecular mass of quaternary ammonium ions and ammonium ion. Error bars indicate 95% confidential intervals obtained from panel A by Probit regression analysis. *r*: Pearson product-moment correlation coefficient. P =0.01

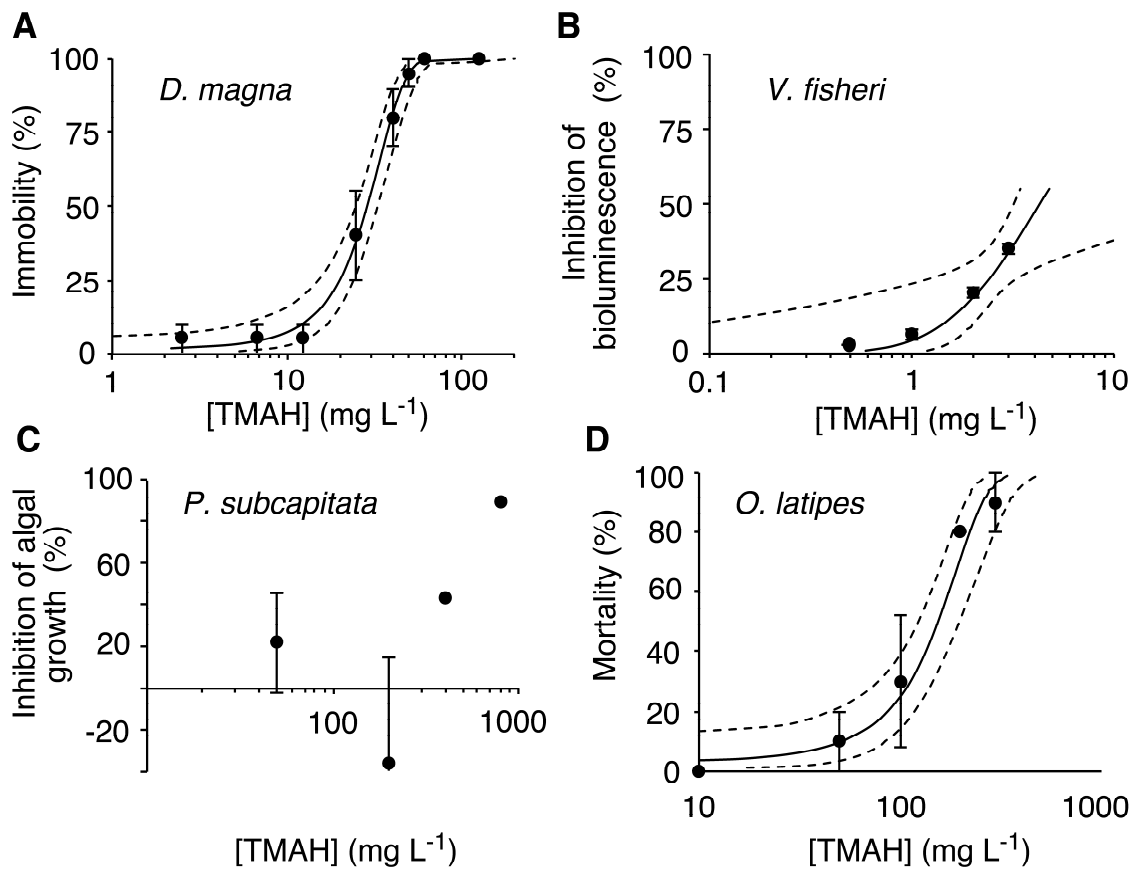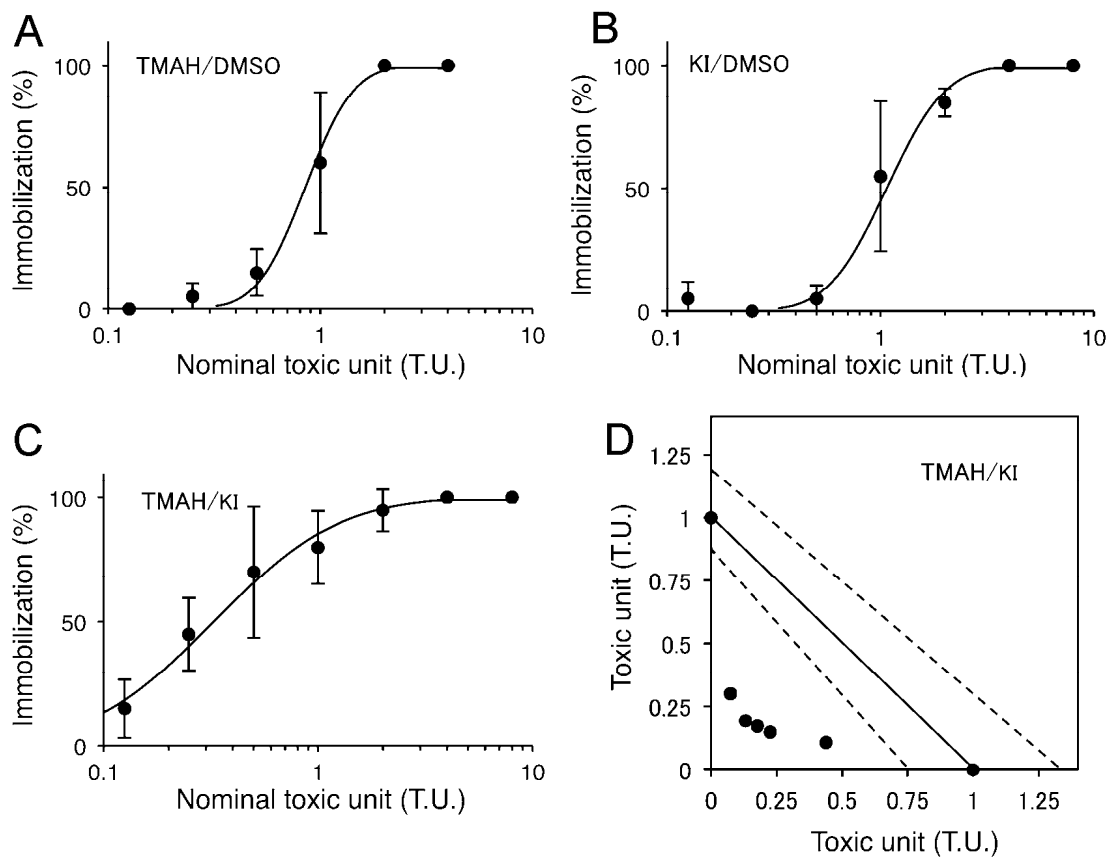(C) Chemical structure of quaternary ammonium ions.
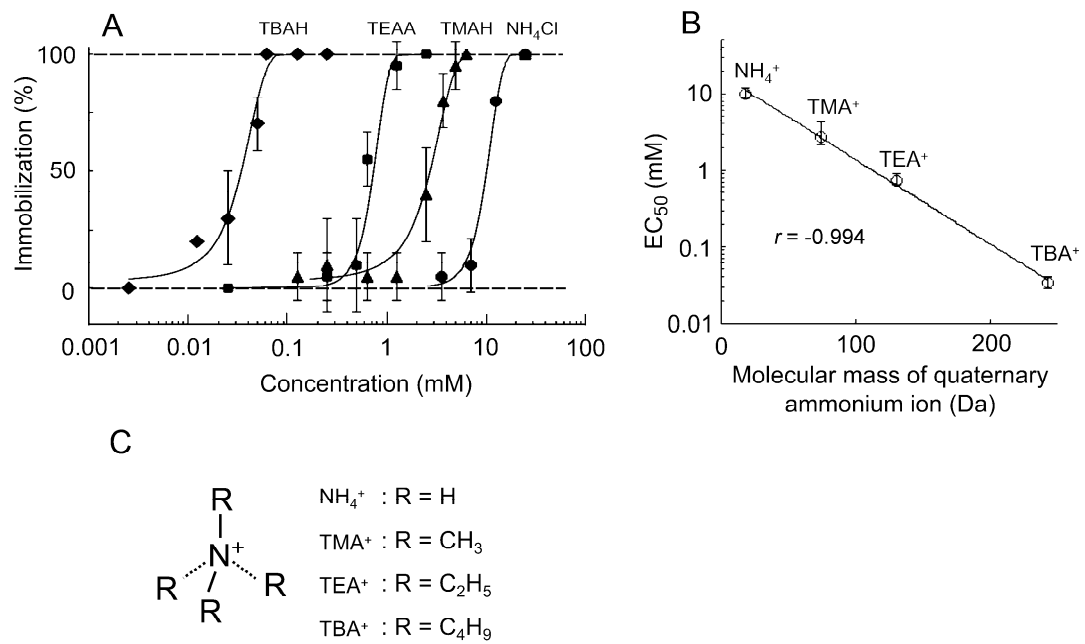
Figure 1

18

Figure 2

A

TBAH   TEAA   TMAH   NH$_4$Cl

Immobilization (%)

100

50

0

0.001   0.01   0.1   1   10   100

Concentration (mM)

B

NH$_4^+$

TMA$^+$

TEA$^+$

$r = -0.994$

TBA$^+$

EC$_{50}$ (mM)

10

1

0.1

0.01

0   100   200

Molecular mass of quaternary
ammonium ion (Da)

C

R
|
R····N$^+$····R
|
R

NH$_4^+$: R = H

TMA$^+$: R = CH$_3$

TEA$^+$: R = C$_2$H$_5$

TBA$^+$: R = C$_4$H$_9$

Figure 3

Tables

Table 1. EC_{50}, IC_{50} and LC_{50} of acute toxicity tests for four aquatic organisms, *Daphnia magna*, *Pseudokirchneriella subcapitata*, *Vibrio fisheri* and *Oryzias latipes*.

Organism (Endpoint)	TMAH (mg L^{-1})	DMSO (g L^{-1})	KI (mg L^{-1})
D. magna (EC_{50})	32 (28-38)[a]	53 (39-56)	1.0 (0.35-1.4)
P. capitata (EC_{50})	360[b]	30 (28-32)	4100 (4000-4200)
V. fischeri (IC_{50})	N.D. (> 3000)	53 (47-59)	N.D. (> 10000)
O. latipes (LC_{50})	154 (132-197)	—	N.D. (> 1000)

[a]95% confidential limits are shown in parentheses. [b]95% confidence interval of TMAH was not able to obtain for *P. capitata* growth inhibition test. IC_{50} of TMAH of *P. capitata* was estimated from a linear handwriting line connecting two points intercepting the 50% inhibition level. N.D.: not detected. —: not examined.

Table 2. Mixture toxicity index of the mixture of TMAH and KI examined by 24-h *D. magna* immobilization test.

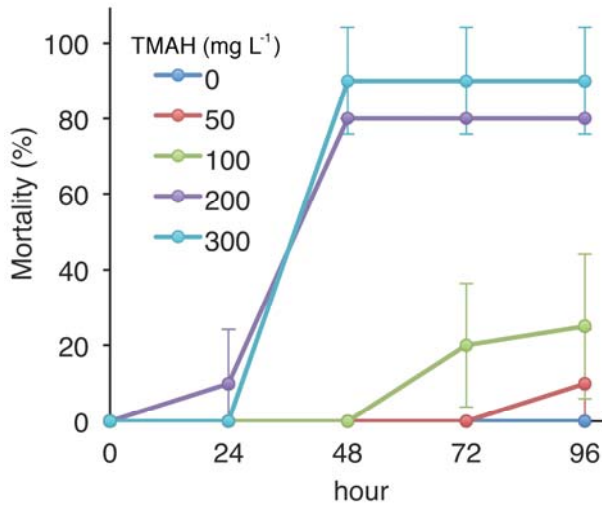| Mixture ratio (TMAH : KI)	MTI[a]
4:1	1.87
3:2	2.42
1:1	2.51
2:3	2.62
1:4	2.42

[a]Mixture toxicity index.

Table 3. Joint toxic effect of binary mixture of TMAH and potassium halide on *D. magna* mobility.

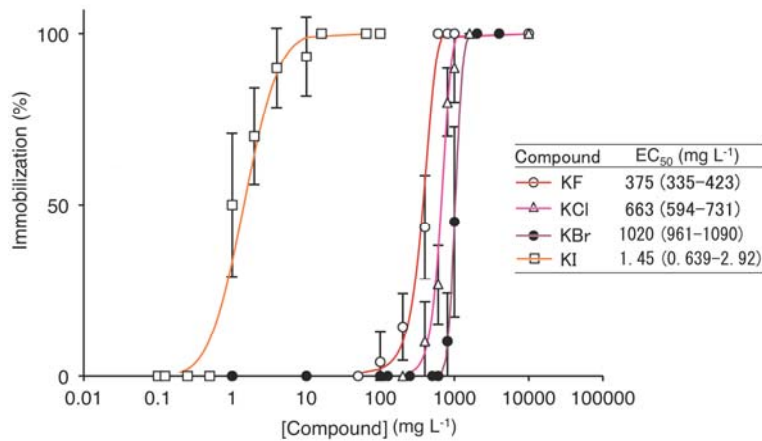| Mixture	Immobilization[b]
KF[a]	50 ± 20
KCl[a]	50 ± 26
KBr[a]	35 ± 25
KI[a]	90 ± 20[c]

[a]Mixture of TMAH (T.U. = 0.5) and designated compound (T.U. = 0.5) was added to the *D. magna* test medium. [b]Percentage of Immobilized *D. magna* neonates from 4 independent bioassays was expressed as mean ± standard deviation. [c]Lower 95% confidential interval was 65.4.
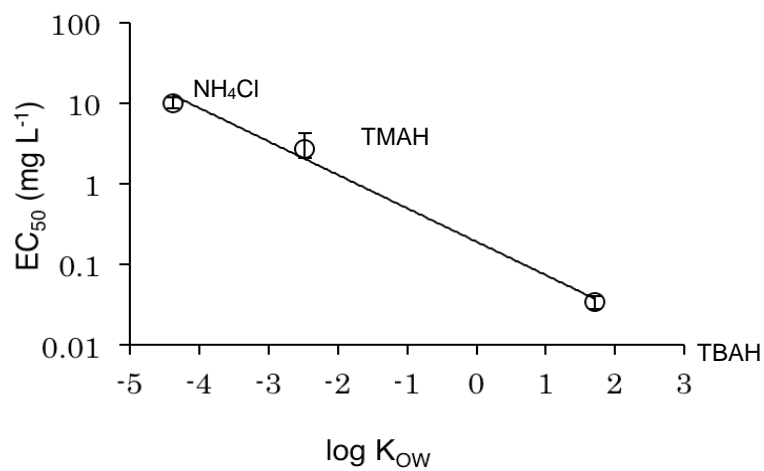
Appendix Figure 1. Mortality of *Oryzias latipes* exposed in tetramethylammonium hydroxide (TMAH) for 96 hours.

Data consists of 4 independent trials. Five individuals were used for each trial. Error bars indicate standard deviation of 4 experiments.

Appendix Figure 2. Toxicity of potassium halide salts on *D. magna* immobility.

EC50 was estimated by probit regression analysis. The obtained EC_{50}s were presented in the inset table. Data were from 4 independent bioassays. Error bars represent standard deviation.

Appendix Figure 3. Plot of EC_{50} of quaternary ammonium compounds versus octanol-water partition coefficient (K_{OW}).

EC_{50} was obtained with *D. magna* immobilization test. K_{OW} was examined by ESI suite™.

K_{OW} of TBAA was not available. r = 0.996, P = 0.056.