Benefits from hazards, benefits from nothing, and benefits from benefits: the combined effects of five quaternary ammonium compounds to *Vibrio qinghaiensis* Q67

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**Abstract**

**Background**: Co-exposure of multiple quaternary ammonium compounds (QACs) is widely present in the aquatic environment. The knowledge about their toxicities to microorganism at low concentration is lacking. This study aims to test the toxic response of *Vibrio qinghaiensis* Q67 (12-h exposure) to individual and mixtures of QACs by the long-term microplate toxicity analysis method (L-MTA).

**Results**: Hormetic effects were observed for five individual QACs at 12-h exposure of *Vibrio qinghaiensis* Q67. The maximum stimulation effect of $-339.66\%$ was present in the $0.00561$ mol/L tetraethylammonium bromide solution. A stimulation effect ($-23.55\%$) was detected when each of QACs in the five-component mixture was present at 50% effect concentration (“benefits from hazards”). In addition, significant stimulation effects ($-96.28\%$) were observed for five QACs combined at the zero-effect point concentration (“benefits from nothing”). Further, a significant stimulation effect ($-406.16\%$) was observed when each of QACs was co-exposed at its maximum stimulation effect concentration. This phenomenon was termed “benefits from benefits”.

**Conclusions**: The results suggest that both single and mixture of QACs presented hormetic effects. Benefits from hazards, benefits from nothing, and benefits from benefits were observed for the QACs mixtures.

**Keywords**: Quaternary ammonium surfactant, Hormesis, Combined toxicity, Independent action

**Background**

The toxicity of pollutants in realistic environment is controlled not only by their concentration or dosage, but also by their exposure time. Under long-term exposure conditions, low-dose pollutants may exhibit the so-called hor metric effect [1–6]. This phenomenon implies that high-concentration chemicals show an inhibition effect on organisms, while low-concentration ones have a stimulation effect [7, 8]. The shapes of the concentration–response curves (CRC) of the pollutants are either J-shaped or inverted U-shaped [9, 10]. Nowadays, the hormetic effect concept has been thoroughly studied in the environmental science and toxicology [1–3, 11]. For example, the hormetic effect was revealed for 1-butyl-3-methylimidazolium chloride ([bmim]Cl) with an exposure time of 12 h [4, 6]. Currently, the hormetic effect has become a hotspot in environmental and toxicological research, because it challenges the linear thresholds used in conventional pollution risk assessments [12]. The hormetic model of dose response is vigorously debated and the notion that
hormesis is important for chemical risk regulations is not widely accepted.

Quaternary ammonium compounds (QACs) have characteristics of surface modification, decontamination, and sterilization, and thereby they are commonly used in textile softeners, disinfectants, personal care products, and other fields. About 75% of QACs consumed each year are discharged into the sewage treatment system. The rest is directly released into the environment [13, 14]. Due to the positive charges of nitrogen ions in their molecules, these compounds can be highly adsorbed by the surfaces of negatively charged media. QACs are widely found in industrial wastewater, agricultural wastewater, domestic sewage, surface water, and water sediment, thus threatening the aquatic ecosystems [15, 16]. Currently, S-shaped CRCs are commonly used in the toxicity evaluation for mixtures [17–19] and the hormetic effect of mixture has been ignored. This effect of QACs mixtures was observed at cellular levels [20, 21], so the hormetic effect of QACs should be considered for environmental protection.

Whether hormetic effect presented in a mixture when its components induce hormetic effect, and the amplitude and range of stimulation in a mixture are larger than its compound? These key problems have been confirmed in hormetic effect of mixture with non-equivalent effect concentration ratio. For example, Sun et al. [22] and Sui et al. [23] revealed that there was a stimulation effect when pollutants were mixed with non-equivalent effect concentration, and the amplitude and range of stimulation effect were greater than a single pollutant, which was the so-called “benefits from benefits” phenomenon. However, further discussion and verification are needed in the mixture with equivalent effect concentration ratio, especially zero-effect point (ZEP), to verify the phenomenon of “benefits from noting”.

The long-term exposure to low-dose pollutants is a common phenomenon, and the corresponding toxicity assessment is an essential aspect of the environmental risk assessment of pollutants. To date, the conventional prediction models for toxicity assessment include concentration addition (CA) and independent action (IA) models [24–26]. Although the CA and IA models have been widely used in toxicity prediction for mixtures, these models were mainly applied to predict mixtures with S-shaped CRC. Multiple data proved that hormetic effect is a widespread phenomenon [27], and the prediction performances of CA and IA models for hormetic effect mixtures were questioned. The comparative analysis revealed that CA models were inefficient in the prediction of the toxicity of hormetic effect mixtures. They either overestimated or underestimated the toxicity level. Given this drawback of CA models, IA models were adopted to evaluate the toxicity of hormetic effect mixtures.

In this study, Vibrio qinghaiensis Q67 was used as an indicator. Five QACs, including benzalkonium bromide (BLB), tetraethylammonium bromide (TLB), Benzyli triethylammonium chloride (BLC), N,N,N-trimethyl-1-tetradecyl ammonium bromide (CTE), and dodecyl trimethyl ammonium chloride III (DTC), were used in the toxicity test. Four mixtures with fixed ratios (EE-EC_{L}, EE-EC_{min}, EE-ZEP, and EE-EC_{50}) were designed and their toxicities were determined by the long-term microplate toxicity analysis method (L-MTA) [28].

**Methods**

**Chemicals**

Five sorts of QACs were selected as the pollutants in this study. Their molecular structures are shown in Fig. 1.
and their physical and chemical properties are listed in Table 1. These compounds were of analytical purity (97%). The stock solution of QACs was prepared using pure water, which was produced with a Milli-Q system and stored at 4 °C.

### Toxicity to test

Q67 was purchased from Beijing Binsong Photon Technology Co., Ltd, China. The cultivation and preservation of strains were based on the previous study [29]. The L-MTA method [28, 30] was applied to determine the chronic photo-inhibition toxicity (12-h exposure) of five pollutants and their five-component mixture to Q67. The toxicity of individual surfactant or the mixture was represented by luminous inhibition efficiency ($E$), which was calculated as follows [31]:

$$E = \frac{l_0 - l}{l_0} \times 100\%$$  \hspace{1cm} (1)

where $l_0$ and $l$ are the average values of the relative light unit (RLU) in the control group and of tested samples measured three parallels, respectively, and $E$ is the luminous inhibition efficiency of a pollutant or mixture against Q67.

### Fitting of concentration–response curves

The fittings of J-type non-monotonic CRC were carried out by setting the value of left asymptote to 0 and 1 in the 7-parameter Logistic equation [32].

$$E = E_{\min} - \frac{E_{\min}}{1+10^{\beta_{dn}(c-c_{dn})}} + \frac{1-E_{\min}}{1+10^{\beta_{up}(c_{up}-c)}}$$  \hspace{1cm} (2)

where $E$ and $E_{\min}$ are the response and the maximal stimulation response concentration, respectively; $c_{dn}$ and $c_{up}$ are concentrations amid the declining and rising parts of the curve, respectively; $\beta_{dn}$ and $\beta_{up}$ represent slopes of the declining and rising parts, respectively; and $c$ is the concentration. The regression analysis of concentration–response data was performed via the nonlinear least square method. The higher the coefficient of determination ($R^2$) and the smaller the root mean square error (RMSE) indicate a better the fitting result.

### Mixture design and toxicity assessment

The equivalent-effect concentration ratio ray (EECR) method was employed to design four fixed ratios of the median stimulation effective concentration (left) (mixture was named as EE-$EC_L$), minimum effect concentration $EC_{min}$ (named as EE-$EC_{min}$), zero-effect point (named as EE-ZEP), and 50% effect concentration (named as EE-$EC_{50}$). According to these concentrations, the detailed concentrations or mixing ratios $p_i$ of all components are obtained in Table 2. Each ratio corresponded to 12 different combinations of concentrations.

The purpose of fixed ratios (EE-$EC_{L}$, EE-$EC_{min}$, EE-ZEP, and EE-$EC_{50}$) was to compare the toxicity of QACs with J-type CRCs. Moreover, these four ratios were used to validate a hypothesis that, based on the IA model, the mixture of $n$ components composed of $1/n$ of corresponding $EC_{x,i}$ will cause a response of $x\%$.

The toxicity of QACs’ mixture characterized by J-type CRC was predicted by the CA model (Eq. 3) and the IA model (Eq. 4) [33].

$$EC_{x,\text{mix}} = \left( \sum_{i=1}^{n} \frac{p_i}{EC_{x,i}} \right)^{-1}$$  \hspace{1cm} (3)

$$E(c_{\text{mix}}) = 1 - \prod_{i=1}^{n} (1 - E(c_i))$$  \hspace{1cm} (4)

| Name                           | Abbreviation | Molecular formula | CAS number | Molecular weight | Source |
|--------------------------------|--------------|-------------------|------------|------------------|--------|
| Benzalkonium bromide           | BLB          | $C_{12}H_{25}BrN$ | 7281-04-1 | 384.44           | TRC    |
| Tetraethylammonium bromide     | TLB          | $(CH_2CH_2)_2NBr$ | 71-91-0    | 210.16           | TRC    |
| Benzyltrimethylammonium chloride| BLC         | $C_{12}H_{24}CN$  | 56-37-1    | 227.774          | TRC    |
| Myristyltrimethylammonium bromide| CTE       | $C_{18}H_{38}BrN$ | 1119-97-7 | 336.39           | TRC    |
| Dodecytrimethylammonium chloride| DTC        | $C_{18}H_{36}N$   | 112-00-5   | 263.89           | TRC    |

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| Mixture ray | BLB | TLB | BLC | CTE | DTC |
|-------------|-----|-----|-----|-----|-----|
| EE-$EC_L$   | 1.79E-04 | 8.94E-01 | 1.03E-01 | 2.50E-04 | 1.25E-04 |
| EE-$EC_{min}$| 1.22E-04 | 9.43E-01 | 5.69E-02 | 1.56E-04 | 1.74E-04 |
| EE-ZEP      | 8.35E-05 | 9.63E-01 | 3.71E-02 | 1.13E-04 | 1.33E-04 |
| EE-$EC_{50}$| 9.24E-05 | 9.59E-01 | 4.04E-02 | 1.25E-04 | 1.52E-04 |
where $EC_{x \text{mix}}$ is the concentration of the mixture that causes $x\%$ effect; $n$ the number of mixture components; $EC_{x i}$ the concentration of the $i$th component causing $x\%$ effect when applied individually; $p_i$ the concentration ratio of the $i$th component in the mixture; $E(c_{\text{min}})$ the predictive effect of a mixture with a total concentration of $c_{\text{min}}$; $c_i$ the individual concentration of $i$th component in the mixture; and $E(c_i)$ the effect of this concentration if a compound is applied singly.

If the observed toxicity is consistent with that predicted, then the combined toxicity shows additivity or non-interaction. If the observed toxicity is higher or lower than the toxicity predicted by CA or IA, it is deemed to be synergism or antagonism, respectively [34, 35].

**Results**

**The hormetic effects of individual QACs**

All concentration–response relationships of BLB, TLB, BLC, CTE, and DTC exposed on Q67 (12 h) exhibited hormetic effects, i.e., non-monotonic J-type CRCs. The Logistic function (Eq. (2)) could be fitted to the data. The RMSE values of < 0.069 and the $R^2$ values of > 0.977 indicated that the CRCs of hormetic effects could be accurately fitted by the Logistic function. The location parameters ($\alpha$) and the shape parameters ($\beta$, $EC_{50}$, $pEC_{50}$, ZEP, $EC_{\text{min}}$, and $E_{\text{min}}$) are listed in Table 3. The experimental data, the fitting result, and the 95% CI are depicted in Fig. 2. As showed in Fig. 2 and Table 3, the maximum stimulation response (~340%) was found for TLB. The maximum stimulation response values for the rest four compounds (BLB, BLC, CTE, and DTC) were similar (~30.23%, ~30.05%, ~23.69%, and ~20.72%, respectively). According to the molecular structures of BLB, TLB, BLC, CTE, and DTC, the hormetic effect of QACs may be caused by the “alkyl chain effect” and halogen (chlorine or bromine) anions.

According to the $pEC_{50}$ values, the toxicities of these five compounds to Q67 were ranked as follows: BLB (4.777) > CTE (4.646) > DTC (4.561) > BLB (2.136) > TLB (0.759). The difference between the $EC_{50}$ of most toxic BLB (1.67E–05 mol/L) and that of the least toxic TLB (1.74E–01 mol/L) was four orders of magnitude. In Fig. 2, the CRC profiles of these five QACs are located above ZEP (zero response), and these CRC profiles are almost parallel, indicating that the toxicity of 50% response and other inhibition effects changed in the same order. Figure 2 shows that the slope of the CRC of TLB in the inhibitory effect part is the largest, indicating that the toxicity of TLB varied to the greatest extent with the concentration.

The J-CRC contained three critical characteristics of the hormetic effect of the pollutants tested: (1) a concentration ($EC_{\text{min}}$) corresponding to the maximal stimulation response ($E_{\text{max}}$); (2) two zero-effect points (ZEP)—one smaller than $EC_{\text{min}}$ ($EC_{\text{L}}$) and the other larger than $EC_{\text{min}}$ ($EC_{\text{R}}$)—because of the intersection of the J-CRC and concentration axis. The default value of $ZEP$ refers to the one greater than $EC_{\text{min}}$; (3) two concentrations corresponding to the same stimulation response ($x\%$). The concentrations of smaller and greater than ZEP were denoted by $EC_{\text{L}}$ and $EC_{\text{R}}$, respectively, and the $E_{\text{L}}$ refers to the median stimulation effective concentration (left).

**Hormetic effects of QAC_{5} mixtures at specific concentrations**

The CRCs were measured by L-MTA method and fitted with the Logistic functions. Figure 3a shows the effect of 5 QACs at $EC_{50}$ and the effect of mixture that 5 QACs mixed at $EC_{50}$. In Fig. 3, b–d are similar to a.

**Table 3** Effect concentration and fitting parameters of single compound and its mixture rays

| Compound | $\alpha$ | $\beta$ | $R^2$ | RMSE | $EC_{50}$ (mol/L) | $pEC_{50}$ | ZEP | $EC_{\text{min}}$ (mol/L) | $E_{\text{c}}$ |
|----------|---------|---------|-------|-------|------------------|-----------|-----|------------------|-----------|
| BLB      | 8.587   | 0.085   | 0.987 | 0.039 | 1.67E–05         | 4.777     | 1.4E–05 | 1.04E–05         | -0.3023   |
| TLB      | 6.321   | 0.103   | 0.980 | 0.061 | 1.74E–01         | 0.759     | 1.56E–01 | 5.61E–02         | -3.3966   |
| BLC      | 10.948  | 0.167   | 0.995 | 0.033 | 7.31E–03         | 2.136     | 6.32E–03 | 5.14E–03         | -0.3005   |
| CTE      | 9.115   | 1.158   | 0.991 | 0.040 | 2.26E–05         | 4.646     | 1.91E–05 | 1.45E–05         | -0.2369   |
| DTC      | 9.447   | -0.091  | 0.977 | 0.069 | 2.75E–05         | 4.561     | 2.27E–05 | 1.61E–05         | -0.2072   |
| EE-EC_{L} | 13.248  | -0.074  | 0.999 | 0.043 | 7.93E–02         | 1.101     | 0.0746  | 0.0524           | -4.9425   |
| EE-EC_{R} | 24.979  | 1.077   | 0.960 | 0.309 | 6.93E–02         | 1.159     | 0.0670  | 0.0540           | -5.8199   |
| EE-ZEP   | 7.781   | -0.207  | 0.991 | 0.238 | 1.08E–01         | 0.968     | 0.0967  | 0.0534           | -5.5992   |
| EE-EC_{R} | 10.162  | 0.500   | 0.945 | 0.485 | 2.02E–01         | 0.695     | 0.186   | 0.120            | -4.4265   |

\(a\) ZEP refers to zero-effect concentration point

\(b\) $EC_{\text{max}}$ refers the maximum stimulation effective concentration

\(c\) $E_{\text{c}}$ refers to the maximum stimulation effect
Comparing the effects of individual QACs and its mixture, three interesting phenomena can be seen from Fig. 3 and Table 3. First, the significant stimulation effects of $-213.54\%$ and $-406.16\%$ were observed when five QACs were mixed at concentrations of $EC_L$ (Fig. 3a) and $EC_{min}$ (Fig. 3b), respectively. This phenomenon was termed “benefits from benefits”, which is defined as hormetic effect of a mixture results from the hormetic effects of components in the mixture. Second, the stimulation effect of $-23.55\%$ was observed when each QACs in the mixture was present at the 50% effect concentration (Fig. 3d), which was termed “benefits from hazards”, which is defined as hormetic effect of a mixture results from the inhibition effects of components in the mixture. Third, as shown in Fig. 3c, five QACs mixed at the ZEP concentrations yielded a significant stimulating response of $-96.28\%$. This phenomenon was termed “benefits from nothing”, which is defined as hormetic effect of a mixture results from the no observed effects of components in the mixture.

For risk assessment, J-type CRCs may be critical in the evaluation of potential health effects of environmental pollutants and the determination of the harmful and beneficial aspects of the hormetic effect of individual components in their mixtures. For instance, as showed in Fig. 3, individual compounds that showed stimulation responses exhibited distinct combined effects in different mixtures. The combined effects included “benefits from hazards”, “benefits from noting”, and “benefits from benefits”. Exposure to mixtures of pollutants is very common in the real environment, so whether an effect is harmful or beneficial should be judged in mixing scenarios.

**Toxicity effects of QACs mixtures**

The EECR method was employed to design four fixed ratios: EE-$EC_L$, EE-$EC_{min}$, EE-ZEP, and EE-$EC_{50}$. Under the condition of exposure time of 12 h, CRCs with hormetic effects for Q67 were observed (Fig. 4). The maximum stimulation response values ($E_{min}$) were $-494.25\%$ for EE-$EC_L$ mixture, $-581.99\%$ for EE-$EC_{min}$, $-559.92\%$ for EE-ZEP and $-442.65\%$ for EE-$EC_{50}$. The Logistic function was used to fit the concentration–response data of these four mixtures. The parameters obtained and corresponding $EC_{50}$, $pEC_{50}$, ZEP, $EC_{min}$ and $E_{min}$ values are listed in Table 3. The CRCs and their 95% OCI are depicted in Fig. 4.

The CRCs of individual QACs (Fig. 2) show that among those QACs with J-shaped CRC, the stimulation response...
to TLB was the largest (−340%), and the maximum stimulation response values to the other four compounds (BLB, BLC, CTE, and DTC) were similar. The CRCs of these five QACs above ZEP (zero-effect point) were relatively parallel. The four mixtures had similar compositions and the highest stimulation response values to these four mixtures were below −400%.

Figures 4 and 5 show that the CA model was inefficient in the prediction for the mixture with hormetic effect. The CA model failed to predict the part with stimulation response and underestimated the toxicity of mixture. In contrast, the effects bellowed 0% (negative effect) could be predicted by the IA model. For example, the CRC of EE-

\( EC_{50} \) mixture in the concentration range of 0.00421–0.0123 mol/L did not significantly deviate from the IA-CRC, showing an additive effect. The values in CRC were significantly lower than those predicted by IA in the range of 0.0123–0.0503 mol/L, showing an antagonistic effect. The values in CRC were significantly higher those predicted by IA in the range of 0.0503–0.123 mol/L, showing a synergistic effect.

In the range of 0.004–0.0609 mol/L for the EE-

\( EC_{min} \) mixture, IA-CRC exhibited an additive effect within the confidence interval of measured CRC. In the range of 0.0609–0.119 mol/L, the measured values were significantly higher than those predicted by IA, showing a synergistic effect. For the EE-

\( ZEP \) mixture of 0.00841–0.0643 mol/L, the measured values were significantly smaller than those predicted by IA, showing an antagonistic effect. In the range of 0.0643–0.245 mol/L, the measured values were significantly higher than the predicted ones, showing a synergistic effect. For the EE-

\( EC_{50} \) mixture of 0.00621–0.0593 mol/L, IA-CRC exhibited a synergistic effect within the confidence interval of measured CRC. In the range of 0.0593–0.181 mol/L, the measured values were significantly higher than those predicted by IA, showing a synergistic effect. According to the above results, it could be concluded that the IA model could not accurately predict these EE-

\( EC_{L} \) and EE-

\( EC_{min} \) with hormetic effect.
A possible mechanism of hormetic effect was analyzed based on the molecular structures of these five QACs on the luciferase in Q67. The hormetic effect of these five compounds may be related to the “alkyl chain effect” and halogen (chlorine or bromine) anions. Therefore, it was assumed that low-concentration QACs could play the role of a luciferase activator, while high-concentration QACs prevented the substrate (such as luciferin) from entering the luciferase activity center and, thus, played the role of a luciferase inhibitor, after the luciferase activity center was saturated by QACs.

The results depicted in Fig. 3 may have important implications for the ecological risk assessment of mixtures. If all chemicals in wastewater have hormetic effect but do not show toxic interactions, the discharge of non-toxic wastewater can be theoretically realized through adjusting their concentrations to $ZEP/n$, where $n$ is the number of components. However, it has been reported that long-term exposure to pollutants with concentrations not exceeding ZEP could produce a strong effect of mixtures [5, 23], so that the ZEP concentration does not guarantee a zero effect. This result may be useful in the control of the discharge of toxic wastewater. The second result was “Benefits from hazards”. For example, Sun et al. [22] studied the time toxicity of quorum sensing inhibitors and sulfonamides to *Vibrio fischer*, the research results showed that the pollutants were mixed according to $EC_{50}$ and other effective concentrations, and the mixture had obvious hormetic effect. The third result has been observed when individual compound was present at the level that did not induce measurable effects and well below the individual ZEP. For example, Rajapakse et al. [36] found that combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhanced steroid hormone action. Therefore, we mixed five QACs with their real zero-effect concentrations and found that these mixtures could produce...

![Fig. 4 CRC, CA, and IA prediction lines of 4 mixture rays (EE-EC$L$, EE-EC$\text{min}$, EE-ZEP, and EE-EC$50$). (black circle indicates the experimental point, black dashed lines the CRC fitting line, dotted lines the 95% confidence interval OCI, blue dashed lines the CA prediction line, and red dashed lines the IA prediction line)](image)
hormetic effect. This proved that even if the components of ZEP (zero-effect) concentrations were mixed, the respective concentrations of these mixtures can be regarded as safe levels in the ecological risk assessment.

Since the concentration–response relationships of five QACs with an exposure time of 12 h showed hormetic effects and the relevant CRCs were parallel above ZEP, it could be expected that luciferase molecules would show a similar toxic response to QACs through the competition for luciferase active sites. The exposure of luciferase molecules to a sufficiently high concentration of QACs’ mixture could transform the low-dose beneficial effects of individual chemicals into zero effects of mixtures after accumulation. A good agreement between the measured and the IA-predicted responses implied that the IA model could be used to predict the hormetic effect of mixtures effectively; although there were still some deviations in the prediction of the IA model (EE-EC_50 and EC_{min}) for the maximum stimulation effect of QACs, the hormetic effect of the mixture of EC_{min} and EC_{L}, which was still a problem to be solved in the future.

Conclusions

The hormetic effects of five individual QACs with an exposure time of 12 h were observed on Q67. Mixtures of these five QACs with ratios of EC_{L}, EC_{min}, ZEP, and EC_{50} also exhibited hormetic effects. The possible mechanism of these hormetic effects may be explained by the low concentration of QACs that can play the role of luciferase activator, while high concentration of QACs starts to play the role of luciferase inhibitor by preventing substrate (such as luciferin) from entering luciferase active center, which is a possible mechanism of QACs’ hormetic effect on luciferase.

The combined effects of the mixtures showed that the mixture had hormetic effect when the five QACs were...
mixed at ZEP, the so-called “benefits from noting” phenomenon. When the five QACs were mixed at $E_{C_{67}}$, the corresponding mixture also showed hormetic effect, the so-called “benefits from hazards” phenomenon. When the five QACs were mixed at $E_{C_{1}}$ and $E_{C_{min}}$, the corresponding mixtures showed hormetic effects, the so-called “benefits from benefits” phenomenon. These three phenomena have important implications for ecological risk assessment of mixtures with hormetic effects.

The result showed that the CA model had a blind area in predicting the mixtures with hormetic effects. Although the IA model could predict the mixtures with hormetic effects, the prediction results have certain deviation.

Abbreviations
QACs: Quaternary ammonium cationic surfactants; Q67: Vibrio qinghaiensis sp. Q67; CRC: Concentration–response curve; EECR: Equivalent effect concentration ratio ray; $E_{C_{1}}$: The maximum stimulation effective concentration (left); $E_{C_{67}}$: The maximum stimulation effective concentration, ZEP: Zero-effect point where the effect is 0 and the concentration is ZEP; CA: Concentration addition; IA: Independence action; BLB: Benzalkonium bromide; TLB: Tetraethylammonium bromide; BLC: Benzyltrimethylammonium chloride; CTE: Myristyltrimethylammonium bromide; DTC: Dodecyltrimethylammonium chloride.

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Authors’ contributions
LM made the study design, wrote the manuscript and is the PI of the NSFC project and NSFG. YL and JZ managed part of the chemical and data analysis. LQ involved in study design and the revision of the manuscript. HZ gave suggestions on experimental design. All authors read and approved the final manuscript.

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