Reproductive toxicities of tetradecyltrimethylammonium chloride and tetradecyltrimethylammonium bromide on Caenorhabditis elegans over four consecutive generations

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Abstract

Quaternary ammonium compounds (QACs) become emerging pollutants and their toxicities earn increasing attentions. So far, their reproductive toxicities were poorly investigated, and their effects over generations were even less explored. In the present study, reproductive toxicities of two QACs, i.e., tetradecyltrimethylammonium chloride (TTAC) and tetradecyltrimethylammonium bromide (TTAB) were studied on *Caenorhabditis elegans* with a consecutive exposure over four generations (from F1 to F4). The effects of TTAC on total reproduction showed stimulation and inhibition which oscillated from F1 to F4, and such oscillation was also observed in the effects on initial reproduction. The effects of TTAB on the total reproduction commonly showed stimulation over generations. The greatest stimulation was in F2, and it was accompanied with inhibition on the initial reproduction but stimulation on the reproduction duration. Further mechanisms exploration demonstrated that both TTAC and TTAB significantly disturbed the levels of SPE8, SPE9, Vg, MSP and VAB-1 and the expressions of *vab-1*, *ceh-18*, *set-2*, *met-2* and *mes-4* over generations. Such disturbances demonstrated that both QACs impacted the reproductive processes in multiple aspects including oocyte meiosis, gonadal support and germline development. Further analysis also showed that the effects of both TTAC and TTAB in parents were connected with those in the offspring, which highlighted the conjunctive roles of reproduction in responses of adjacent generations. In addition, the differences the effects of TTAC and TTAB also demonstrated the anionic influences on the QACs' toxicities.

1. Introduction

Quaternary ammonium compounds (QACs) have wide application in disinfectants, biocides, environmental remediation and even carbon capture (Abe and Kishimura 2022, Roy and Ahmaruzzaman 2022, Mai et al. 2023). Their discharge into the environment during application and through effluents and sludge earned increasing attentions on their toxicities (Jardak et al. 2016, Mohapatra et al. 2023). It was reported that QACs showed mutagenic effects in bioluminescence assays (Dmochowska et al. 2016), growth inhibition and respiratory damage in beagles, mice and rats (Luz et al. 2020, Park et al. 2023), and even promotion on resistance gene transfer (Han et al. 2019, Liu et al. 2023). However, their toxicities still need more studies to fully demonstrate their environmental impacts on ecosystem and human health.

The reproductive toxicities are very important in ecological risk and hazard evaluation. It was reported that QACs significantly decreased reproduction, caused birth defects and impacted the development of ovaries in mice and rats (Jiao et al. 2017, Hrubec et al. 2021, Matsumoto et al. 2021). In the yeast assays and *in vitro* cell line studies, QACs impacted hormones that are important in reproduction (Hrubec et al. 2021, Liwarska-Bizukojc et al. 2021). Notably, the reproduction process connects adjacent generations and its changes by pollutants can represent intergenerational influences. As expected, QACs (e.g., didecyldimethyl ammonium chloride and alkyl dimethyl benzyl ammonium chloride) indeed impacted the body weight of the rat offspring in the exposure over generations (Luz et al. 2020). However, it remained poorly reported the reproductive toxicities of QACs over generations and also urged systematic exploration on the mechanisms.

Reproduction involves a series of subtle processes with the various regulations. In *Caenorhabditis elegans*, the reproduction includes (1) spermiogenesis, which is triggered by spermatocyte protein 8 (SPE8) and promoted by vitellogenin (Vg) (Han et al. 2010, Ellis and Stanfield 2014); (2) oogenesis, which includes mitogen-activated protein kinase (MAPK) activation, binding of major sperm protein (MSP) to VAB-1 Eph receptor protein-tyrosine kinase, POU-homeoprotein (*ceh-18*) in gonadal sheath cells and also $G\alpha_{o/i}$ and $G\alpha_{s}$ signaling pathways (Govindan et al. 2006, Yamamoto et al. 2006); and (3) the sperm-oocyte interactions and fertilization which are ensured with SPE9 (Marcello and Singson 2010). The process also requires epigenetic regulations by histone methylation at H3K4 (*set-2*), H3K9 (*met-2*) and H3K36 (*mes-4*) sites (González-Aguilera et al. 2013, Herbette et al. 2017). The reproduction of *C. elegans* was widely to employed to investigate toxicities of environmental pollutants, e.g., (Wang et al. 2018, Huang et al. 2023, Liu et al. 2024). Changes in aforementioned processes explained the reproductive toxicities of ionic liquids (ILs) (Yue et al. 2021, Wang et al. 2023). Therefore, they are expected to explain the reproductive toxicities of QACs.

The present study aimed to explore the reproductive toxicity of two representative QACs, i.e., tetradecyltrimethylammonium chloride (TTAC) and tetradecyltrimethylammonium bromide (TTAB) with an exposure over four consecutive generations. The toxicity mechanisms were explored changes in SPE8, Vg, vitellogenin (Vn), MAPK, MSP and VAB-1, and the expressions of genetic and epigenetic regulating genes. Further hierarchical cluster analysis (HCA) and correlation analysis were employed to explore the connections among indicators underlying the reproductive toxicities over generations.

2. Method and Materials

2.1. Chemicals

Tetradecyltrimethylammonium chloride (TTAC, CAS No.: 4574-04-3, 99%) and tetradecyltrimethylammonium bromide (TTAB, CAS No.: 1119-97-7, 99%) were purchased from Adamas-beta. Stock solutions were prepared with sterile K-medium (0.051 M NaCl and 0.032 M KCl) and their nominal concentrations were arranged as 0.88 mg/L.

2.2. Preparation of organism

Wild-type N2 *C. elegans* were cultured on nematode growth medium (NGM) agars at 20 ºC temperature with *Escherichia coli* OP50 as food (Yu et al. 2017). Gravid nematodes were lysed with fresh-made clorox solution (1% NaClO and 0.5 mol/L NaOH) to obtain age-synchronized eggs which were used in subsequent experiments (Yue et al. 2021).

2.3. Multi-generational reproduction

For the exposure, 200.0 mL warm NGM containing 2.0 mL stock solutions or sterile K-medium solutions (served as the control) was aliquoted into 10 petri dishes as replicates in each group (Yu et al. 2017, Yue et al. 2021). The exposure concentration was 0.0088 mg/L, at which TTAC and TTAB did not provoke

significant lethality (less than 5%). The NGM agars further received same amounts of *E. coli* OP50 to form a bacterial lawn. At last, the NGM agars received same amounts of age-synchronization nematode eggs to start the F1 exposure.

On the 3rd day in F1, adult nematodes were used for reproduction measurement, preparation for next generation exposure and sample collection. Briefly, 48 adult nematodes in every group were transferred daily onto 24 new NGM agars (i.e., two adults per agar) containing the same TTAC/TTAB or K-medium solutions to measure initial reproduction (offspring within the first 72 h), total reproduction and reproduction duration (Yu et al. 2017, Liu et al. 2020). Another 100 adult nematodes in each group including the control were transferred onto ten replicate agars containing the same TTAC/TTAB or K-medium solutions. With the adults discarded after 24 h, the newly laid eggs started the F2 exposure (Yue et al. 2021). The rest nematodes were collected as samples for subsequent assays. Through the same way, the multi-generational exposure lasted for 4 consecutive generations (i.e., F1 to F4).

2.4. Biochemical assays

Nematode samples were homogenized in ice bath and centrifuged at 4 ºC, and the supernatants were aliquoted into new centrifuge tubes for subsequent bioassays (Liu et al. 2020, Yue et al. 2021). The contents or activities of MSP, VAB-1, SPE-9, SPE-8, Vg, vitellin (Vn) and MAPK were determined via microplate reader using the commercial enzyme-linked immune-sorbent assay (ELISA) kits (Shanghai Zhanshi Biotechnology Co. LTD). They were normalized as their portions against the total protein (TP, measured via BCA protein assay kits) in the same sample.

2.5. Gene expression analysis

The total RNA was isolated using TRIzol reagent and functioned as templates to synthesize cDNA according to the instruction of M-MLV, Promega (Li et al. 2020). Subsequent real-time polymerase chain reaction (RT-PCR) with SYBR Green Master Mix was performed on Applied Biosystems 7900HT Fast Real-Time PCR System (USA). The primers (Table S1 in Supporting information) were designed with Primer Premier (6.0, Premier Biosoft Inc., CA). The gene expressions were quantified using the $2^{-\Delta\Delta CT}$ method with *gpd-1* as the reference gene.

2.6. Data presentation and statistical analysis

Data in each sample were presented as fold-change against those in the concurrent control in the same generation (Liu et al. 2020, Yue et al. 2021). Significant differences between control and treatment and among generations were analyzed via two-way ANOVA with Tukey *post hoc* test at the level of 0.05 ($p < 0.05$). The hierarchical component analysis (HCA) and Pearson's rank correlation statistics were performed to explore further connection among the indicators (Yu et al. 2019, Yue et al. 2021).

3. Results

3.1. Multi-generational reproductive effects of TTAC and TTAB

Results showed that TTAC caused significant stimulation on the total reproduction in F1, inhibition in F2, stimulation again in F3 and inhibition in F4 (Fig. 1(A)). Moreover, the effects of TTAC on the initial reproduction were generally similar to those on the total reproduction. Notably, the effects of TTAC on the reproduction duration showed inhibition in F1 and stimulation in F2 to F4. The stimulation on the reproduction duration in F2 and F4 spared more time for reproduction but it was not enough to compensate the inhibition on initial reproduction, and therefore resulted in the inhibition in the total reproduction. Summing up, the multi-generational reproductive effects of TTAC showed oscillatory changes between stimulation and inhibition over generations. Moreover, the effects were mainly resulted from the influences on the initial reproduction which was not compensated by the prolonged reproduction duration.

(Fig. 1 around here)

In the aspect of TTAB, it showed stimulation on the total reproduction in all generations with the greatest stimulation in F2 (Fig. 1(B)). It showed stimulation on the initial reproduction in F1, F3 and F4, but inhibition in F2. It also commonly stimulated the reproduction duration in all generations. In other words, the reproductive increases by TTAC in F1, F3 and F4 were resulted from both initial reproduction and reproduction duration. Meanwhile, the reproductive increases in F2 were mainly resulted from longer reproduction duration instead of the initial reproduction. The different results of TTAC and TTAB showed the influences of anions on the reproductive toxicities of QACs.

3.2. Multi-generational effects of TTAC and TTAB on essential reproduction proteins

Multi-generational effects of TTAC on essential reproduction proteins are showed in Fig. 2(A). In F1, TTAC inhibited MSP and Vg, and stimulated VAB-1, SPE9 and SPE8. In F2, it stimulated MSP and SPE8, and inhibited SPE9, Vg and MAPK. In F3, it inhibited MSP, VAB-1, SPE9 and Vn, and stimulated Vg and MAPK. In F4, it stimulated MSP and Vn, and inhibited VAB-1 and Vg. Summing up, effects of TTAC oscillated between stimulation and inhibition on all proteins over generations. Combining the biochemical effects, the IBR values were 2.21, 2.17, 2.10 and 2.34 in F1 to F4, respectively. Compared with the IBR value in the control (2.60), the overall effects of TTAC commonly showed inhibition Fig. 2(C).

Effects of TTAB on the proteins also showed oscillation between stimulation and inhibition over generations (Fig. 2(B)). The IBR values were 2.43, 2.12, 2.18 and 2.34 in F1 to F4, respectively. The overall effects of TTAB also commonly inhibition.

(Fig. 2 around here)

3.3. Multi-generational effects of TTAC and TTAB on genetic regulations

In F1, TTAC upregulated all gene expressions (Fig. 3(A)). In F2, it upregulated the expressions of *set-2*, *met-2*, *mes-4* and *vab-1* and downregulated those of *ceh-18*. In F3, it upregulated the expressions of *set-2*, *mes-4* and *vab-1*, and downregulated those of *ceh-18* and *gsa-1*. In F4, it upregulated expressions of *set-2*, *met-2*, *mes-4*, *vab-1* and *gsa-1*, and downregulated those of *ceh-18*. The IBR values were 13.4, 3.72, 2.43 and 2.60 in F1 to F4, respectively. Compared with the IBR value of the control (2.74), the overall effects of TTAC on gene expressions were stimulatory in F1 and F2, and inhibitory in F3 and F4 (Fig. 3(C)).

(Fig. 3 around here)

In F1, TTAB did not show significant influences on all gene expressions (Fig. 3(B)). In F2, it upregulated the expressions of *set-2*, *met-2*, *mes-4*, *vab-1* and *gsa-1*, and downregulated those of *ceh-18*. In F3, it upregulated the expressions of *set-2*, *mes-4*, *vab-1* and *gsa-1*, and downregulated those of *ceh-18*. In F4, it upregulated expressions of *set-2*, *mes-4*, *vab-1* and *gsa-1*, and downregulated those of *met-2* and *ceh-18*. Collectively, the expression levels showed oscillation between upregulation and downregulation over generations. The IBR values were 2.33, 9.51, 2.86 and 2.60 in F1 to F4, respectively (Fig. 3(C)). The overall effects of TTAB on gene expressions were inhibitory in F1 and F4 and stimulatory in F2 and F3.

3.4. Overall analysis on multi-generational effects of TTAC and TTAB

In the HCA results, the effects of TTAC on reproduction (including initial reproduction, total reproduction and reproduction duration) and reproduction-related proteins (including MSP, VAB-1, SPE9, SPE8, Vg and Vn) in F1 were closed clustered showing close connection (Fig. 4(A)). Interestingly, the effects of TTAC on the gene expressions in F1 were more closely connected with the effects on reproduction in F2. Moreover, the effects on expressions of *vab-1*, *ceh-18* and *gsa-1* in F2 were also more closely connected with those on reproduction in F3. In addition, the effects on SPE-8, Vg, Vn and MAPK and those on the gene regulation in F3 were also more closely connected with the effects on reproduction in F4. Summing up, the effects of TTAC on parents were closely connected with those on the offspring. The effects of TTAB in parent generations were also closely related with those on the offspring (Fig. 4B). Moreover, the effects of TTAC in F1 were even connected with those in F3 and F4, and the effects of TTAB in F1 and F2 also showed close connection with those in F4. That is to say, the close connection among indicators expanded over adjacent generations.

(Fig. 4 around here)

(Tables 1 & 2 around here)

Table 1 Pearson's correlation between reproduction and biochemicals, between reproduction and gene expressions, and between biochemicals and gene expressions in the toxicities of

tetradecyltrimethylammonium chloride (TTAC).

TTAC	IR	TR	MSP	VAB-1	SPE-9	SPE-8	Vg	Vn	MAPK	*set-2*	*met-2*	*mes-4*	*vab-1*	*ceh-18*	*gsa-1*
IR	-	1.000	0.612	0.733	0.741	0.740	0.758	0.651	0.813	0.695	0.701	0.751	0.756	0.836	0.718
TR		-	0.618	0.739	0.746	0.747	0.763	0.656	0.815	0.698	0.704	0.756	0.762	0.838	0.721
MSP			-	0.964*	0.948*	0.973*	0.976*	0.988*	0.941*	0.539	0.585	0.724	0.766	0.553	0.413
VAB-1				-	0.992*	0.998*	0.964*	0.980*	0.959*	0.737	0.770	0.876	0.907*	0.751	0.638
SPE-9					-	0.985*	0.949*	0.980*	0.969*	0.771	0.808	0.900*	0.918*	0.779	0.670
SPE-8						-	0.978*	0.981*	0.964*	0.699	0.733	0.849	0.884*	0.725	0.602
Vg							-	0.968*	0.978*	0.567	0.608	0.744	0.783	0.627	0.471
Vn								-	0.964*	0.636	0.683	0.800	0.828	0.640	0.512
MAPK									-	0.660	0.704	0.810	0.828	0.715	0.570
set-2										-	0.997	0.971*	0.950*	0.973*	0.982*
met-2											-	0.981*	0.959*	0.967*	0.966*
mes-4												-	0.994*	0.956*	0.922*
vab-1													-	0.940*	0.897*
ceh-18														-	0.978*
gsa-1															-

*, at 0.05 level.

Table 2 Pearson's correlation between reproduction and biochemicals, between reproduction and gene expressions, and between biochemicals and gene expressions in the toxicities of tetradecyltrimethylammonium bromide (TTAB).

TTAB	IR	TR	MSP	VAB-1	SPE-9	SPE-8	Vg	Vn	MAPK	*set-2*	*met-2*	*mes-4*	*vab-1*	*ceh-18*	*gsa-1*
IR	-	0.846	0.968*	0.990*	0.980*	0.972*	0.976*	0.983*	0.980*	0.635	0.628	0.431	0.801	0.929*	0.717
TR		-	0.940*	0.895*	0.922*	0.945*	0.926*	0.926*	0.911*	0.931*	0.946*	0.836	0.980*	0.856	0.616
MSP			-	0.990*	0.982*	0.998*	0.996*	0.996*	0.982*	0.806	0.780	0.637	0.925*	0.893*	0.774
VAB-1				-	0.990*	0.991*	0.996*	0.997*	0.994*	0.718	0.702	0.531	0.867	0.919*	0.756
SPE-9					-	0.992*	0.991*	0.994*	0.997*	0.735	0.753	0.565	0.875	0.960*	0.664
SPE-8						-	0.997*	0.998*	0.990*	0.796	0.788	0.630	0.918*	0.921*	0.730
Vg							-	0.998*	0.994*	0.771	0.756	0.599	0.905*	0.913*	0.756
Vn								-	0.994*	0.762	0.753	0.586	0.896*	0.927*	0.735
MAPK									-	0.727	0.735	0.555	0.873	0.946*	0.697
set-2										-	0.966*	0.967*	0.968*	0.609	0.622
met-2											-	0.951*	0.937*	0.697	0.440
mes-4												-	0.881*	0.455	0.451
vab-1													-	0.758	0.717
ceh-18														-	0.436
gsa-1															-

*, at 0.05 level.

In the Pearson's correlation results, the biochemical effects of TTAC (e.g., on MSP, VAB-1, SPE-9, SPE-8, Vg, Vn and MAPK) were well correlated with themselves and also occasionally with those on *mes-4* and *vab-1* expressions (Table 1). Meanwhile, the biochemical effects of TTAB showed correlations with themselves, but also with reproduction, and commonly with those on *vab-1* and *ceh-18* expressions

(Table 2). The results demonstrated the connection among indicators and also the differences between TTAC and TTAB.

4. Discussion

4.1. Multi-generational reproductive effects of TTAC and TTAB

The multigenerational reproductive effects showed oscillation between stimulation and inhibition over generations. The similar oscillations were also observed in multigenerational effects of imidazole-based ILs (Yue et al. 2021, Zhang and Feng 2022, Wang et al. 2023), pyridine-based ILs (Shi et al. 2021, Zhang et al. 2022), and antibiotics (e.g., sulfamethoxazole, enrofloxacin, ofloxacin and norfloxacin) (Li et al. 2020, Zhang et al. 2022, Zheng et al. 2022). Offspring in uterus prepared similar responses to their maternal experiences via fetal reprogramming for better adaptation to the environment (Li et al. 2020). However, such preparation does not guarantee the matching with the actual requirement of the offspring. Accordingly, the outcomes in the offspring can be stimulation, inhibition and even non-significant influences, therefore explaining the oscillatory changes over generations. More investigation is needed to establish models for predicting multigenerational effects in the future.

Similar to the effects of TTAB, pyridine-based ILs and antibiotics also caused consistent effects on the initial and total reproduction of nematodes (Shi et al. 2021, Zhang et al. 2022, Zhang et al. 2022). Meanwhile, the effects of TTAC in F2 showed inconsistence between effects on the initial reproduction and those on the total reproduction with compensation from longer reproduction duration. Notably, animals in the actual environment would reproduce as early as possible to ensure the population growth (Yu et al. 2017), and delayed reproduction with longer reproduction duration would be unrealistic due to accidental death or predation. Therefore, with or without compensation by longer reproduction duration, the reproductive toxicities of TTAC and TTAB would significantly influence the population growth and their ecological risks should be seriously considered in their application.

It was known that QACs are cationic surfactants, and therefore most investigations were studied on cationic toxicity on various organisms (Luz et al. 2020, Suthar et al. 2022). The present study showed the different results of TTAC and TTAB, which showed the influences of anions on the reproductive toxicities. In future studies, the anionic influences should be considered in the QACs toxicities to more accurate evaluation on their ecological risks.

4.2. Mechanisms of the multi-generational reproductive effects

In the reproductive processes, the spermiogenesis is triggered via SPE8 and the sperm movement is promoted by Vg (Han et al. 2010, Ellis and Stanfield 2014), the oocyte meiotic maturation is regulated by MAPK activation and binding of MSP to VAB-1 (Govindan et al. 2006, Yamamoto et al. 2006), and the

sperm-oocyte interactions and fertilization are ensured with SPE9 (Marcello and Singson 2010). At the molecular level, the expressions of *vab-1*, *ceh-18*, *set-2*, *met-2* and *mes-4* regulate the oocyte meiotic maturation, gonadal sheath cells and germline genome integrity (Govindan et al. 2006, Yamamoto et al. 2006, González-Aguilera et al. 2013, Herbette et al. 2017). The effects of TTAB on the reproduction showed positive correlations with those on the aforementioned biochemicals and those on the expressions of the aforementioned genes. Moreover, the effects of TTAB on the gene expressions (e.g., *vab-1* and *ceh-18*) were also positively correlated with those on the biochemicals including MSP, SPE-9, Vg and MAPK. The correlation demonstrated that the reproductive toxicities of TTAB were well related with its impacts on the whole reproductive processes including oocyte meiosis, gonadal support and germline development. However, the effects of TTAC did not show such correlations as those in the results of TTAB, and therefore the differences supported the anionic influences on QACs toxicities as well.

Notably, the effects of both TTAC and TTAB in parents were connected with those in the offspring. The connection was also observed in the multi-generational effects of antibiotics and ILs (Zhang et al. 2022, Wang et al. 2023). Such connection supported that reproductive processes provided one essential tie to combine health over generations. Especially, the expressions of *set-2*, *met-2* and *mes-4* involved histone methylation (e.g., at H3K4, H3K9 and H3K36 sites) to preserve germline genome integrity and normal development (González-Aguilera et al. 2013, Herbette et al. 2017). Therefore, the connection in the effects between generations also supported the involvement of epigenetic modulation on the responses of offspring to the maternal experience (Yue et al. 2021). Therefore, further studies are still needed to explore the epigenetic mechanisms underlying the multigenerational consequences of pollutants.

5. Conclusion

Both TTAC and TTAB caused reproductive toxicities with changes over generations. The reproductive effects of TTAC showed oscillation between stimulation and inhibition over generations. The toxicities of TTAB on initial reproduction can be compensated by reproduction duration. Biochemical and gene expression results showed that TTAC and TTAB impacted oocyte meiosis, gonadal support and germline development. Moreover, the reproductive toxicities also involved epigenetic regulations on histone methylation. In addition, the results also demonstrated the anionic influences on the toxicities of QACs.

Declarations

Ethical Approval:

Not applicable.

Consent to Participate and Publish:

All authors agreed with authorship and the content, and approved to submit the present version to be published.

Authors Contributions

Formal analysis, investigation and writing original draft were performed by Jing Zhang. Experiment performance and data curation were performed by Ruoqi Ding. Supervision, project administration and funding acquisition were performed by Zhenyang Yu.

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Competing Interests

The authors have no relevant financial interests to disclose.

References

1. Abe H, Kishimura H (2022) CO_2-driven surface reconstruction in quaternary ammonium ionic liquid-propanol solutions. J Ionic Liquids 2:100018
2. Dmochowska B, Sikora K, Woziwodzka A, Piosik J, Podgórska B (2016) Mutagenic activity of quaternary ammonium salt derivatives of carbohydrates. Beilstein J Org Chem 12:1434–1439
3. Ellis RE, Stanfield GM (2014) The regulation of spermatogenesis and sperm function in nematodes. Semin Cell Dev Biol 29:17–30
4. González-Aguilera C, Palladino F, Askjaer P (2013) *C. elegans* epigenetic regulation in development and aging. Brief Func Genomics 13:223–234
5. Govindan JA, Cheng H, Harris JE, Greenstein D (2006) $G\alpha_{o/i}$ and $G\alpha_s$ signaling function in parallel with the MSP/Eph receptor to control meiotic diapause in *C. elegans*. Curr Biol 16:1257–1268
6. Han SM, Cottee PA, Miller MA (2010) Sperm and oocyte communication mechanisms controlling *C. elegans* fertility. Dev Dynam 239:1265–1281
7. Han Y, Zhou Z-C, Zhu L, Wei Y-Y, Feng W-Q, Xu L, Liu Y, Lin Z-J, Shuai X-Y, Zhang Z-J, Chen H (2019) The impact and mechanism of quaternary ammonium compounds on the transmission of antibiotic resistance genes. Environ Sci Pollut Res 26:28352–28360
8. Herbette M, Mercier MG, Michal F, Cluet D, Burny C, Yvert G, Robert VJ, Palladino F (2017) Th*e C. elegans* SET-2/SET1 histone H3 Lys4 (H3K4) methyltransferase preserves genome stability in the germline. DNA Repair 57:139–150
9. Hrubec TC, Seguin RP, Xu L, Cortopassi GA, Datta S, Hanlon AL, Lozano AJ, McDonald VA, Healy CA, Anderson TC, Musse NA, Williams RT (2021) Altered toxicological endpoints in humans from common quaternary ammonium compound disinfectant exposure. Toxicol Rep 8:646–656
10. Huang P, Liu SS, Wang ZJ, Ding TT, Tao MT, Gu ZW (2023) Study on the characterization of pesticide modes of action similarity and the multi-endpoint combined toxicity of pesticide mixtures to

Caenorhabditis elegans. Sci Total Environ 893:164918

11. Jardak K, Drogui P, Daghrir R (2016) Surfactants in aquatic and terrestrial environment: occurrence, behavior, and treatment processes. Environ Sci Pollut Res 23:3195–3216
12. Jiao Y, Niu L-n, Ma S, Li J, Tay FR, Chen J-h (2017) Quaternary ammonium-based biomedical materials: State-of-the-art, toxicological aspects and antimicrobial resistance. Prog Polym Sci 71:53–90
13. Li Z, Yu ZY, Cui CZ, Ai FT, Yin DQ (2020) Multi-generational obesogenic effects of sulfomethoxazole on *Caenorhabditis elegans* through epigenetic regulation. J Hazard Mater 382:121061
14. Liu C, Goh SG, You L, Yuan Q, Mohapatra S, Gin KY-H, Chen B (2023) Low concentration quaternary ammonium compounds promoted antibiotic resistance gene transfer via plasmid conjugation. Sci Total Environ 887:163781
15. Liu H, Mo LY, Hou MF, Zhang J (2020) Life stage-dependent toxicities of 1-ethyl-3-methylimidazolium bromide on *Caenorhabditis elegans*. Environ Pollut 265:114848
16. Liu Z, Hua X, Zhao Y, Bian Q, Wang D (2024) Polyethylene nanoplastics cause reproductive toxicity associated with activation of both estrogenic hormone receptor NHR-14 and DNA damage checkpoints in *C. elegans*. Sci Total Environ 906:167471
17. Liwarska-Bizukojc E, Stevens CV, Delbeke EIP, Geem KMV (2021) Endocrine disrupting potency and toxicity of novel sophorolipid quaternary ammonium salts. Ecotoxicolgy 30:658–666
18. Luz A, DeLeo P, Pechacek N, Freemantle M (2020) Human health hazard assessment of quaternary ammonium compounds: Didecyl dimethyl ammonium chloride and alkyl (C12–C16) dimethyl benzyl ammonium chloride. Regul Toxicol Pharm 116:104717
19. Mai Y, Wang Z, Zhou Y, Wang G, Chen J, Lin Y, Ji P, Zhang W, Jing Q, Chen L, Chen Z, Lin H, Jiang L, Yuan C, Xu P, Huang M (2023) From disinfectants to antibiotics: Enhanced biosafety of quaternary ammonium compounds by chemical modification. J Hazard Mater 460:132454
20. Marcello MR, Singson A (2010) Fertilization and the oocyte-to-embryo transition in *C. elegans*. BMB Rep 43:389–399
21. Matsumoto M, Takano M, Takabe M, Yamaguchi N, Iso T, Shigeta Y, Murata Y, Hirose N, Inoue K, Hirose A (2021) Initial hazard assessment of ethyl(dimethyl)(tetradecyl)ammonium ethyl sulfate: Genotoxicity tests and combined repeated-dose and reproductive/developmental toxicity screening in rats. Regul Toxicol Pharm 122:104914
22. Mohapatra S, Yutao L, Goh SG, Ng C, Luhua Y, Tran NH, Gin KY-H (2023) Quaternary ammonium compounds of emerging concern: Classification, occurrence, fate, toxicity and antimicrobial resistance. J Hazard Mater 445:130393
23. Park JE, Ryu S-H, Ito S, Song M-K, Gu EJ, Shin H, Kim Y-H, Jeon J (2023) Bioaccumulation and in vivo fate of toxic benzylalkyldimethylammonium chloride in rats via the radiotracer analysis. Chemosphere 338:139460
24. Roy S, Ahmaruzzaman M (2022) Ionic liquid based composites: A versatile materials for remediation of aqueous environmental contaminants. J Environ Manage 315:115089

25. Shi Y, Meng XZ, Zhang J (2021) Multi- and trans-generational effects of N-butylpyridium chloride on reproduction, lifespan, and pro/antioxidant status in *Caenorhabditis elegans*. Sci Total Environ 778:146371
26. Suthar A, Gopalakkrishnan A, Maji C, Dahiya RK, Kumar R, Kumar S (2022) Evaluation of the inhibitory efficacy of quaternary ammonium compounds on *in vitro* growth of *Theileria equi* parasite in MASP culture. Int J Parasitol-Drug 20:11–16
27. Wang L, Wu D, Yu Z, Huang S, Zhang J (2023) Hormone-mediated multi- and trans-generational reproductive toxicities of 1-ethyl-3-methylimidazolium hexafluorophosphate on *Caenorhabditis elegans*. Sci Total Environ 863:160958
28. Wang S, Chu Z, Zhang K, Miao G (2018) Cadmium-induced serotonergic neuron and reproduction damages conferred lethality in the nematode *Caenorhabditis elegans*. Chemosphere 213:11–18
29. Yamamoto I, Kosinski ME, Greenstein D (2006) Start me up: Cell signaling and the journey from oocyte to embryo in *C. elegans*. Dev Dynam 235:571–585
30. Yu Z, Sun G, Liu Y, Yin D, Zhang J (2017) Trans-generational influences of sulfamethoxazole on lifespan, reproduction and population growth of *Caenorhabditis elegans*. Ecotoxicol Environ Safe 135:312–318
31. Yu ZY, Yin DQ, Zhang J (2019) Sex-dependent effects of sulfamethoxazole exposure on pro-/anti-oxidant status with stimulation on growth, behavior and reproduction in the amphipod *Hyalella azteca*. Environ Pollut 244:398–404
32. Yue WY, Mo LY, Zhang J (2021) Reproductive toxicities of 1-ethyl-3-methylimidazolium bromide on *Caenorhabditis elegans* with oscillation between inhibition and stimulation over generations. Sci Total Environ 765:144334
33. Zhang J, Feng L (2022) Intermittent multi-generational effects of 1-hexyl-3-methylimidazolium nitrate on *Caenorhabditis elegans* mediated by lipid metabolism. Sci Total Environ 812:152479
34. Zhang J, Shi Y, Yu Z (2022) Balances among reproduction, antioxidant responses and lipid metabolism underlying the multi-generational effects of N-butylpyridinium bromide on *Caenorhabditis elegans*. Sci Total Environ 846:157468
35. Zhang J, Zheng Y, Yu Z (2022) Reproductive toxicities of ofloxacin and norfloxacin on *Caenorhabditis elegans* with multi-generational oscillatory effects and trans-generational residual influences. Environ Toxicol Pharm 95:103962
36. Zheng YG, Yu ZY, Zhang J (2022) Multi-generational effects of enrofloxacin on lifespan and reproduction of *Caenorhabditis elegans* with SKN-1-mediated antioxidant responses and lipid metabolism disturbances. Sci Total Environ 804:150250

Figures

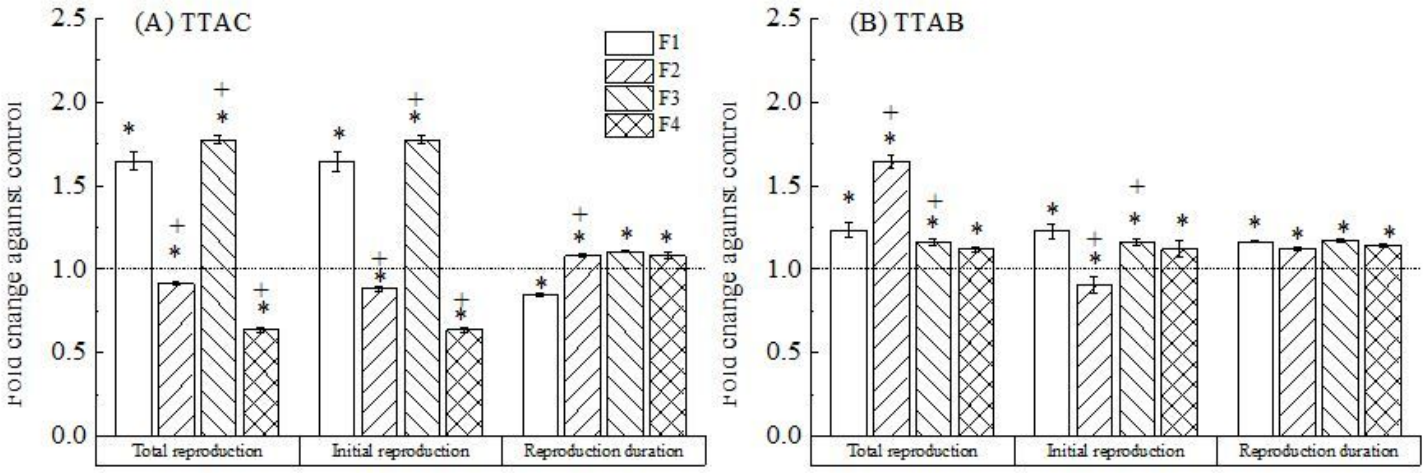

Figure 1

Effects of tetradecyltrimethylammonium chloride (TTAC) and tetradecyltrimethylammonium bromide (TTAB) on reproduction of *C. elegans* via multi-generational exposure from F1 to F4. *, significantly different from control group (1.0) at 0.05 level by ANOVA; +, significantly different from the earlier generation at the same concentration 0.05 level. The data in the figure were represented as mean ± standard deviation.

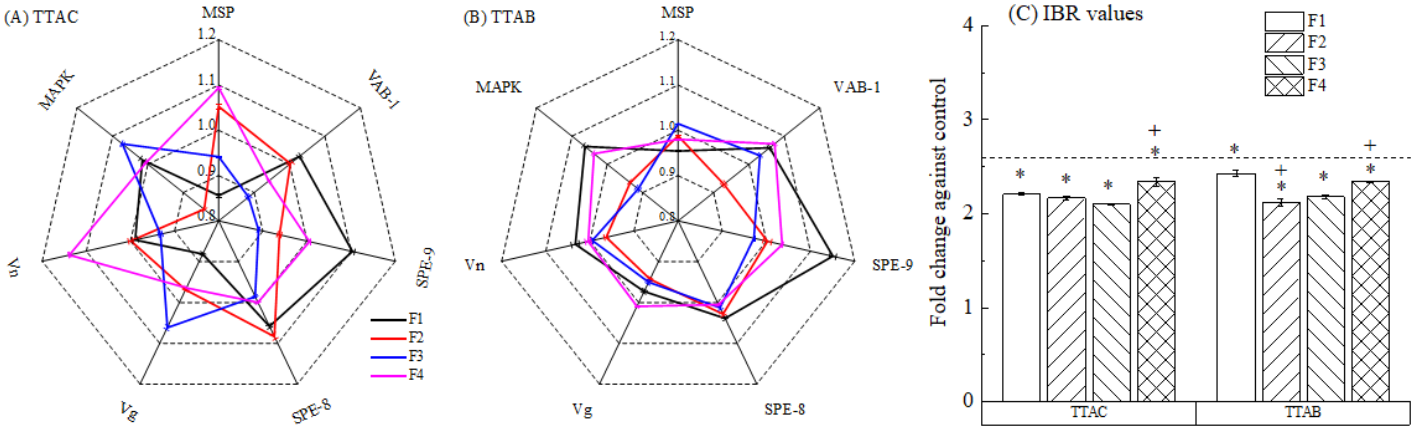

Figure 2

Multi-generational effects of TTAC and TTAB on major sperm protein (MSP), ephrin receptor protein tyrosine kinase (VAB-1), spermatocyte protein 8 (SPE8), sperm transmembrane protein 9 (SPE9), vitellogenin (Vg), vitellin (Vn) and mitogen-activated protein kinase (MAPK) of *C. elegans.* *, significantly different from control group (2.60) at 0.05 level by ANOVA; +, significantly different from the earlier generation at the same concentration 0.05 level. The data in the figure were represented as mean ± standard deviation.

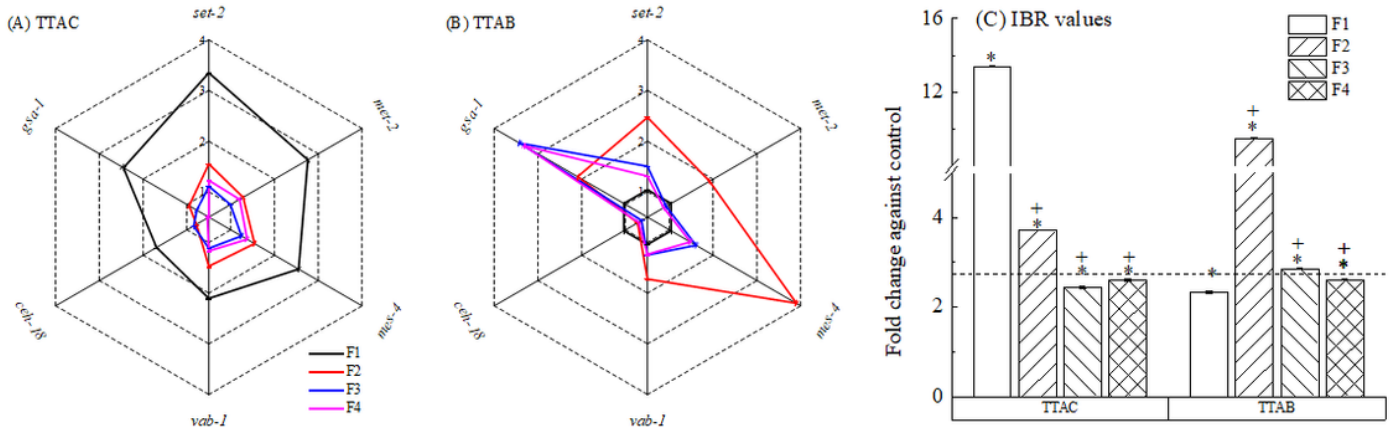

Figure 3

Multi-generational effects of TTAC and TTAB on expressions of *set-2, met-2, mes-4, vab-1, ceh-18, gsa-1* in *C. elegans*. *, significantly different from control group (2.74) at 0.05 level by ANOVA; #, significantly different between low and high concentrations in the generation at 0.05 level; +, significantly different from the earlier generation at the same concentration 0.05 level. The data in the figure were represented as mean ± standard deviation.

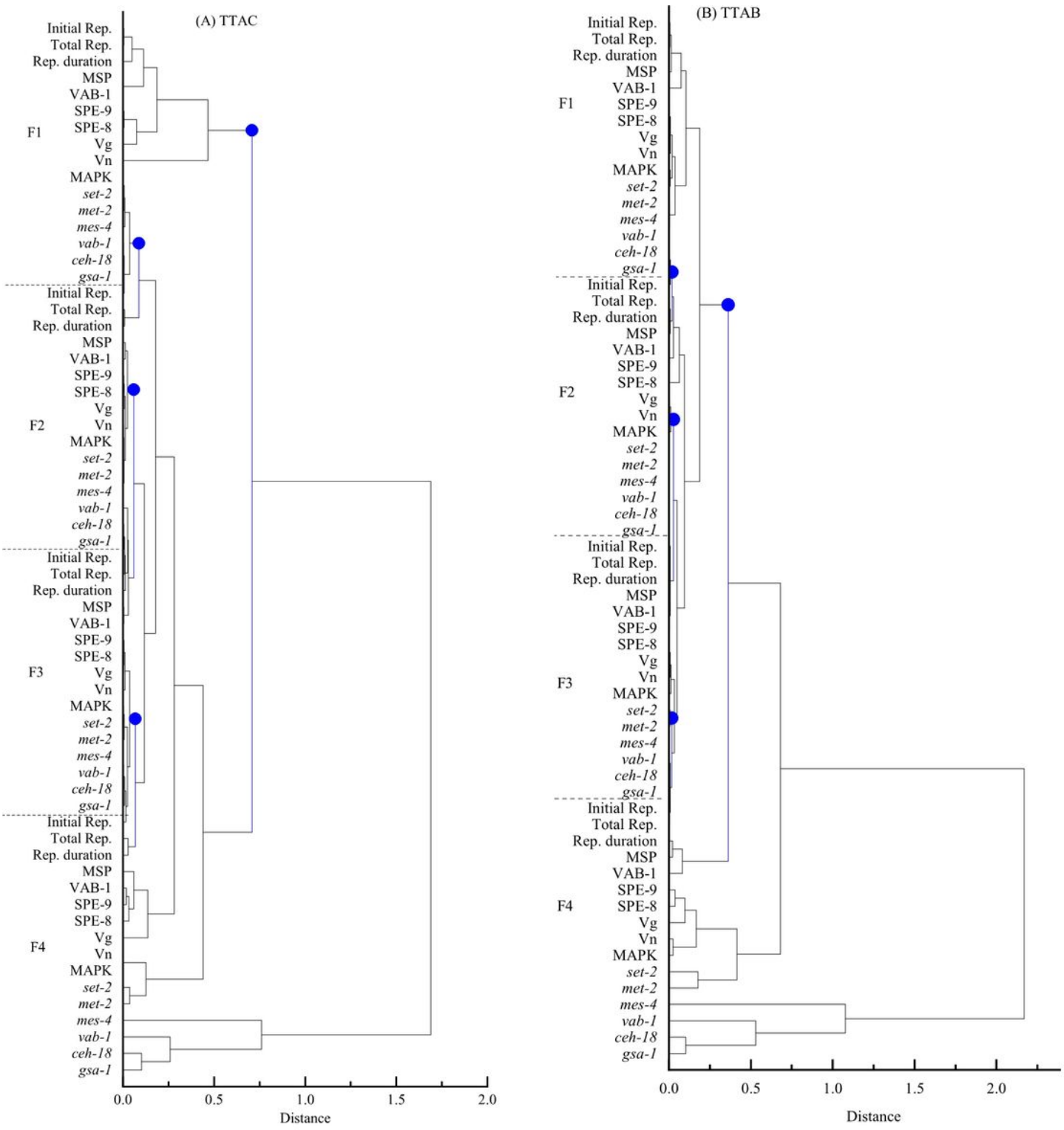

Figure 4

Hierarchical cluster analysis on the multi-generational effects of TTAC and TTAB on initial reproduction (Initial Rep.), total reproduction (Total Rep.), reproduction duration (Rep. duration), essential reproduction proteins including spermatocyte protein 8 (SPE8), sperm transmembrane protein 9 (SPE9), major sperm protein (MSP), ephrin receptor protein tyrosine kinase (VAB-1), vitellogenin (Vg), vitellin (Vn), and mitogen-

activated protein kinase (MPK-1) and gene expressions of *set-2*, *met-2*, *mes-4*, *vab-1*, *ceh-18*, *gsa-1* in *C. elegans*.

Supplementary Files

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