Antibiotic chlortetracycline causes transgenerational immunosuppression via NF-κB

Qiu, Wenhui; Chen, Bei; Tang, Liang; Zheng, Chunmiao; Xu, Bentuo; Liu, Zhiyu; Magnuson, Jason T.; Zhang, Shuwen; Schlenk, Daniel; Xu, Elvis Genbo; Xing, Baoshan

Published in:
Environmental Science & Technology

DOI:
10.1021/acs.est.1c07343

Publication date:
2022

Document version:
Final published version

Environmental Science & Technology

pubs.acs.org/est

Article

Antibiotic Chlortetracycline Causes Transgenerational Immunosuppression via NF-κB

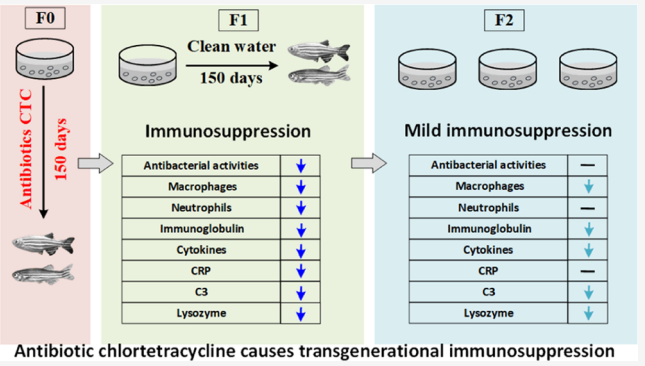
Wenhui Qiu, Bei Chen, Liang Tang, Chunmiao Zheng,* Bentuo Xu, Zhiyu Liu, Jason T. Magnuson, Shuwen Zhang, Daniel Schlenk, Elvis Genbo Xu,* and Baoshan Xing

Cite This: *Environ. Sci. Technol.* 2022, 56, 4251−4261

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The extensive and increasing global use of antibiotics results in the ubiquitous presence of antibiotics in the environment, which has made them "pseudo persistent organic contaminants." Despite numerous studies showing wide adverse effects of antibiotics on organisms, the chronic environmental risk of their exposure is unknown, and the molecular and cellular mechanisms of antibiotic toxicity remain unclear. Here, we systematically quantified transgenerational immune disturbances after chronic parental exposure to environmental levels of a common antibiotic, chlortetracycline (CTC), using zebrafish as a model. CTC strongly reduced the antibacterial activities of fish offspring by transgenerational immunosuppression. Both innate and adaptive immunities of the offspring were suppressed, showing significant perturbation of macrophages and neutrophils, expression of immune-related genes, and other immune functions. Moreover, these CTC-induced immune effects were either prevented or alleviated by the supplementation with PDTC, an antagonist of nuclear factor-κB (NF-κB), uncovering a seminal role of NF-κB in CTC immunotoxicity. Our results provide the evidence in fish that CTC at environmentally relevant concentrations can be transmitted over multiple generations and weaken the immune defense of offspring, raising concerns on the population hazards and ecological risk of antibiotics in the natural environment.



Antibiotic chlortetracycline causes transgenerational immunosuppression

KEYWORDS: *antibiotic, chlortetracycline, immunosuppression, transgenerational toxicity, NF-κB*

1. INTRODUCTION

The overuse and misuse of antibiotics is a global public health issue. Global antibiotic consumption increased by 65% between 2000 and 2015 (from 21.1 to 34.8 billion defined daily doses) and is estimated to reach 128 billion defined daily doses by 2030.[1] Their overuse results in resistance to antibiotics.[2] Over 700,000 deaths every year are attributed to antimicrobial resistance and are predicted to be 10 million deaths by 2050.[3] The estimated annual use in North America varies between 1300 and 11,200 tons,[4] whereas the total consumption in 2013 for antibiotics in China was 92,700 tons, approximately half of which was excreted by humans and animals, which eventually enters the natural environment.[5] Although the half-life of most antibiotics is short[6] (a few hours to several days), their uninterrupted and increasing emission makes them "pseudo persistent organic contaminants".[7] Antibiotics have been frequently detected in wastewater and aquatic environments at concentrations ranging from ng/L to low mg/L levels.[8,9] Among a long list of detectable environmental antibiotics, chlortetracycline (CTC) is one of the most abundant ones due to its global use.[10] High levels of CTC have been detected in surface water (a maximum level of 276.3 μg/L), groundwater (a maximum level of 126.8 μg/L), wastewater (1.8 ± 0.5 mg/L), and even bottled drinking water (a maximum level of 64 ng/L).[11−13] The ubiquity of antibiotics in the environment, especially in aquatic environments, potentially exposes them to various environmental organisms, which may threaten the whole population through the intergenerational transmission of antibiotics. Innate and adaptive immunity is the effective defense mechanism of different organisms against inherent and environmental threats; however, the immune system during early life is more susceptible and is largely influenced by parental diet, environmental contaminants, and micronutrients.[14,15] Prenatal and postnatal exposure to antibiotics may suppress the immunity over multiple generations, further affecting population structure and damaging ecological functions.[16−18]

Received: October 29, 2021
Revised: March 2, 2022
Accepted: March 3, 2022
Published: March 14, 2022

Epidemiological evidence and biological assays in mammals have demonstrated a link between the disruption of the immune response by antibiotics and autoimmune diseases.[19−21] Environmental antibiotic-induced autoimmune diseases, however, to date, have yet been demonstrated in fish.[22] Our recent nontarget transcriptomic analysis predicted the nuclear factor-κB (NF-κB) signaling pathway as one of the most important mechanisms of antibiotic toxicity.[23] NF-κB is an important molecular regulator of innate and adaptive immune responses, which can accelerate cell proliferation, inhibit apoptosis, promote cell migration and invasion, and stimulate angiogenesis and metastasis.[24] Few anti-inflammatory and immunosuppressant drugs show inhibition of the NF-κB pathway.[25−28] However, we still do not know their long-term and potential transgenerational immune effects and the exact molecular and cellular mechanisms.

In our previous study, the toxicities of 15 common antibiotics were screened in zebrafish, among which CTC was identified to exhibit the highest bioenrichment in the F0 ovary and F1 eggs and reduced the survival of F1 offspring.[29] The (eco)toxicity of antibiotics, including CTC, has also been reported in other organisms[30−34] but little is known about the chronic impacts of CTC on environmental organisms at environmentally relevant concentrations. In addition, it has been shown that relatively low levels (0.01−100 μg/L) of four antibiotics (i.e., cefotaxime, enrofloxacin, tetracycline, and sulfamonomethoxine) can induce NF-κB-mediated immune response in fish's primary macrophages.[23] Thus, we hypothesized that prolonged exposure to low environmental levels of CTC could weaken the immune defense system of animals via disrupting the NF-κB pathway during early life. To test this central hypothesis, multigenerational experiments were designed to evaluate the chronic immune effects of CTC after parental exposure to low environmental levels of CTC using the zebrafish model. By integrating chemical, toxicological, molecular, and modeling methods, we provide the comprehensive evidence that environmentally relevant concentrations of CTC itself can be transmitted over multiple generations and weaken the immune defense of offspring via NF-κB.

2. METHODS

2.1. Zebrafish. A wild-type zebrafish AB line was raised in recirculating zebrafish housing systems at the Southern University of Science and Technology (China). Tg(mpeg1:EGFP) transgenic zebrafish (labeled with the macrophage-expressed gene 1 reporter) was obtained from the China Zebrafish Resource Center (China). Animal work was done in compliance with national guidelines and approved by the Institutional Animal Care and Use Committee of Southern University of Science and Technology (SUSTC-JY2019067).

2.2. Antibodies and Reagents. Antibodies used for the western blot were: lysozyme C (LYSO, dilution 1/1000, ab229657) and anti-succinate dehydrogenase complex flavoprotein subunit A (SDHA) antibody (dilution 1/1000, ab137040) were obtained from Abcam (U.K.). Nuclear factor-κB 3 (NFKB3) antibody (dilution 1/1000, catalog no. GTX107678) was obtained from Genetex. Chlorotetracycline hydrochloride ($C_{22}H_{23}ClN_2O_8$·HCl, molecular weight 515.34, CAS No. 64-72-2) was obtained from Sigma-Aldrich. NF-κB antagonist pyrrolidine dithiocarbamate (PDTC) was obtained from Sigma-Aldrich. All other chemicals used were of analytical grade and were obtained from Sigma-Aldrich.

2.3. Animals and CTC Treatments. Adult zebrafish (AB) were fed live brine shrimp (*Artemia nauplii*) twice daily and maintained in flow-through aquarium systems for a 14 h light/10 h dark cycle at 28 ± 0.5 °C. Embryonic zebrafish were collected and examined to remove unfertilized and poor-quality embryos. Embryonic zebrafish (2 hpf) were randomly transferred into glass beakers that contained 500 mL of CTC solutions at environmentally relevant concentrations of 0, 0.01, 0.1, 1, 10, and 100 μg/L CTC. Each treatment consisted of three replicate beakers (n = 3), with each replicate containing 200 embryos per beaker. At 20 dpf, zebrafish larvae were transferred into 25 L glass tanks, and at 90 dpf, each treatment group was separated into males and females and separately raised in 25 L tanks. Zebrafish were continuously exposed to CTC treatments until 150 days, and the exposure medium was renewed daily. After exposure, zebrafish were allowed to mate (F0; males: females was 1:1) in clean water, and the offspring (F1) were collected for biological assays or continually raised in clean water for 150 days to mate to get the F2 generation embryos. F1 and F2 generation embryos were both tested for antibacterial ability between 0−72 hpf and 5 dpf for immunodevelopmental functions. All experiments were approved by the Institutional Animal Care and Use Committee at the Southern University of Science and Technology (SUSTC-2019-049).

2.4. Chemical Analysis. CTC levels in fish were analyzed using high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS, Agilent) with quality assurance (QA) and quality control (QC) based on our previous study[29] and described in the Supporting information (Text S1). The optimized HPLC−MS/MS parameters for CTC are shown in Supporting Information Table S1. CTC concentrations in the test solutions were measured to make sure that the exposure doses were similar to targeted nominal exposure concentrations (Supporting Information Table S2).

2.5. Developmental and Behavioral Measurements and Imaging. Body weight (g), body length (cm), intestinal weight (g), and ovary weight (g) in F0 zebrafish; egg production (number per parent) of F0; and egg death rate at birth (0 hpf, %), fertilization rate (4 hpf, %), egg death rate at 120 hpf (%), hatching rate (72 hpf, %), body length at 120 hpf (mm), swimming speed at 120 hpf (mm, 0−10 min), and swimming distance at 120 hpf (mm, 0−10 min) in F1 and F2 fish larvae were determined as previously described.[29] Images of Tg(mpeg1:EGFP) transgenic zebrafish at 5 days were acquired using a LEICA M205 FCA microscope, and macrophage numbers were counted using ImageJ software (version 1.8.0).

2.6. Antibacterial Ability. Antibacterial ability of F1 and F2 larvae against Gram-negative *Vibrio parahaemolyticus* (CGMCC 1.1615, common pathogenic bacteria in fish) was determined following modified protocols described by the previous studies.[35] Embryos were collected at 0 hpf and then inoculated with freshly prepared concentrations of bacteria (0, 10, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 cfu/mL). Bacterial solutions were renewed every 12 h. The mortality of larvae was detected after a pathogenic challenge for 72 hpf. Bacteria on the surface of F1 larvae were analyzed with a fluorescence microscope (LSM 780 NLO, ZEISS).

2.7. Whole-Mount *In Situ* Hybridization (WISH). As the lysozyme C gene is specifically expressed in fish neutrophils, WISH of the lysozyme C gene was used to define the neutrophils in F1 larvae as previously described.[36,37] The

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

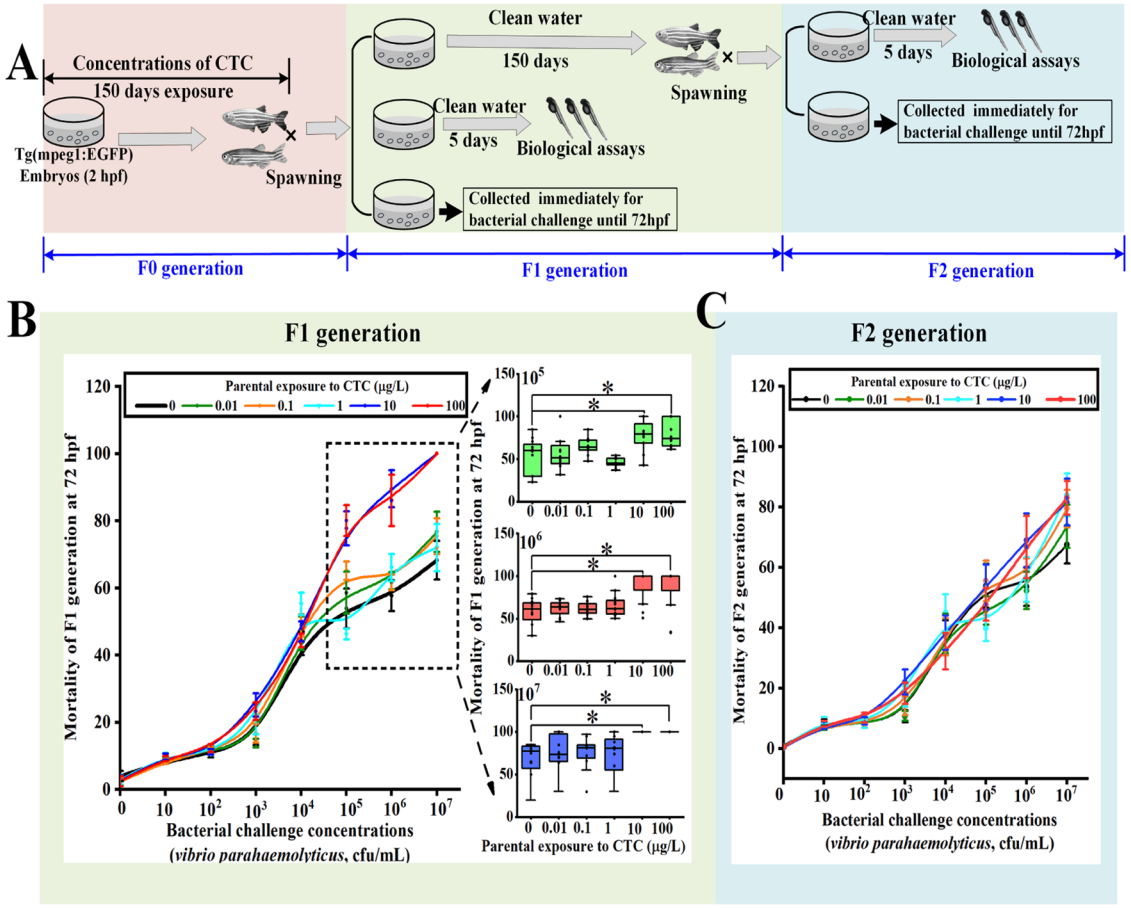


Figure 1. Antibiotic CTC exposures inhibit antibacterial activities of zebrafish offspring. (A) Experimental scheme for CTC exposures. (B) Antibacterial ability of the F1 larvae (after F0 fish exposure to CTC) against Gram-negative *Vibrio parahaemolyticus* (CGMCC 1.1615) determined by measuring the mortality of 72 hpf larvae after pathogen challenge. Concentrations of challenged bacteria were 0, 10, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 cfu/mL. Significant differences between the control group and the 10 or 100 μg/L CTC group were detected at 10^5, 10^6, and 10^7 cfu/mL challenged bacteria (n = 12; $P < 0.05$, ANOVA). (C) Antibacterial ability of the F2 larvae (after F0 fish exposure to CTC) against Gram-negative *V. parahaemolyticus*. *$P < 0.05$, by one-way ANOVA with LSD's test (n = 12). Error bars indicate the s.e.m.

antisense RNA probes were generated *in vitro* from linear plasmids using RNA polymerase T3 or Digoxigenin T7 (Promega, Madison, WI). WISH was performed as previously described.[38]

2.8. Quantitative PCR (qPCR). Total RNA was isolated using the TRIzol reagent (Invitrogen), and cDNA was generated by following the instruction of a Transcriptor First Strand cDNA Synthesis Kit (Roche). Transcripts were quantified via SYBR Green qPCR (Roche) performed using the iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad). The primers for zebrafish genes are presented in Table S3.

2.9. Immune Indicators. Concentrations of C3, CRP, IgM, and LYSO were measured using zebrafish special ELISA kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute). The intraassay and interassay coefficients of variance (CVs) were <10 and <12%, respectively. The R^2 of dilution test (specificity) ranged from 97.5 to 99.3%.

2.10. Immunoblotting Analysis. Immunoblotting was performed as previously described.[39] Antibodies for immunoblots were used before specific detection for zebrafish samples. Immunoblots were detected via standard secondary detection and chemiluminescent exposure to the film. Target proteins were normalized with the reference protein SDHA.[40] Digitally captured films were analyzed densitometrically using ImageJ software.

2.11. NF-κB Pathway Inhibition Test. An NF-κB inhibition experiment was designed separately using the exposure of embryonic Tg(mpeg1:EGFP) zebrafish (2 hpf) to 10 or 100 μg/L CTC and in the presence or absence of NF-κB antagonist pyrrolidine dithiocarbamate (PDTC, Sigma) at 1 μM. Zebrafish were exposed for 150 days and then mated to get F1 embryos. All of the procedures were in the same conditions as CTC treatments. F1 generation embryo was collected for biological assays at 5 dpf.

2.12. Protein−Ligand Docking. Molegro virtual docker 7.0 software was used for molecular docking analysis. The chemical structure of CTC was taken from PubChem (CID: 54675777), and crystal structures of NFκB1 (PDB: 1SVC), NFκB2 (PDB: 1A3Q), NFκB3 (PDB: 2RAM), IL-6R (Interleukin-6 receptor, PDB: 1P9M), BCR (B-cell receptor, PDB: 1IGY), TCR (T-cell receptor, PDB: 1NFD), TLR2 (Toll-like receptors 2, PDB:), and TLR4 (Toll-like receptors 4, PDB: 4G8A) were obtained from Protein Data Bank. Then, all cavities of proteins were set as binding sites separately and other parameters were set as defaults. The molecular docking simulations of proteins and CTC were run based on MolDock SE algorithm and achieved binding energy minimization after docking. The number of all trial runs for calculations was 10.

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

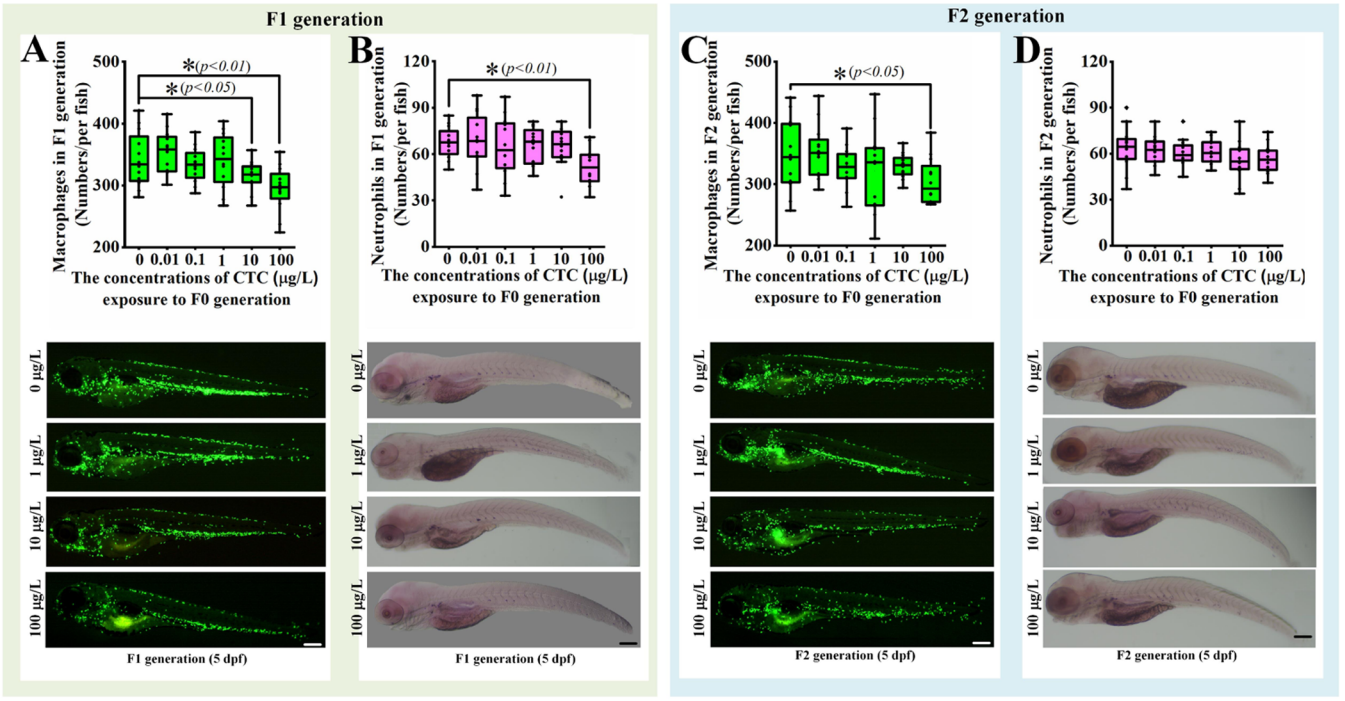


Figure 2. Antibiotic CTC exposures decrease the immune cell abundance of zebrafish offspring. (A) Number of macrophages in F1 larvae (5 dpf) after F0 exposure to CTC. The green fluorescent dots represent macrophages in Tg(mpeg1:EGFP) transgenic zebrafish, and the fluorescent images compare macrophage numbers between the control group and the 1, 10, and 100 μg/L CTC groups. Scale bar = 200 μm. (B) Number of neutrophils in F1 larvae (5 dpf). The purple points in fish represent neutrophils using whole-mount *in situ* hybridization, and the hybridization images compare the neutrophil numbers between the control group and the 1, 10, and 100 μg/L CTC groups. (C) Number of macrophages in F2 larvae (5 dpf). (D) Number of neutrophils in F2 larvae (5 dpf). *$P < 0.05$, by one-way ANOVA with LSD's test ($n = 12$). Error bars indicate the s.e.m.

Finally, the top pose based on the highest MolDock score (kcal/mol) of docking result of each protein was selected to be visualized by Discovery Visualizer version 20.

2.13. Statistical Analysis. Statistical analysis was performed with SPSS Statistics 18.0 (SPSS, Inc., Chicago, IL). All results were checked for normality and homogeneity of variance using Kolmogorov−Smirnov one-sample test and Levene's test. All statistical tests were justified as appropriate and data met the assumptions of the tests. Significant differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by LSD's test. All data are shown as the mean ± standard error. Each experiment was repeated independently with similar results. The number of animals, number of independent experiments, and methods of the statistical tests used are indicated for each experiment in the figure legends.

3. RESULTS

3.1. CTC Suppresses the Antibacterial Activities of the Offspring. It is increasingly recognized that antibiotics are related to immune interference but the potential impacts on the immune system of offspring are unclear. We phenotyped antibacterial activities in the larvae in zebrafish offspring (F1 and F2 generations) after parental exposure (F0) to the antibiotic CTC (Figure 1a). After a 10^5, 10^6, and 10^7 cfu/mL pathogen challenge, the F1 larvae at 72 hpf showed a significantly increased mortality and decreased antibacterial activity under parental exposure to 10 or 100 μg/L CTC (Figure 1b), but no significant changes following a 0, 10, 10^2, 10^3, or 10^4 cfu/mL pathogen challenge. The results indicated a significantly decreased antibacterial activity in the offspring after a high-level pathogen challenge under parental exposure to CTC. Further microscopic observation showed that the number of bacteria on the body surface of zebrafish offspring also increased after parental exposure to CTC (Figure S1). It should also be noted that after a one-generation recovery in clean water, the antibacterial activities of F2 larvae were not significantly affected (Figure 1c).

3.2. CTC Reduces Immune Cell Abundance in Offspring. To understand the cellular mechanisms of the reduced antibacterial activities of offspring by CTC exposure, we measured the number of innate immune cells, macrophages, and neutrophils in zebrafish offspring after F0 exposure to CTC. In F1 larvae (5 dpf), the number of macrophages was significantly decreased at parental exposure to the 10 or 100 μg/L CTC group, and the number of neutrophils was significantly decreased in the 100 μg/L group (Figure 2a,b). In F2 larvae (5 dpf), the inhibition of immune cell number was only observed for macrophages at F0 exposure to the 100 μg/L CTC group and the number of neutrophils was not affected (Figure 2c,d). The decreased numbers of macrophages and neutrophils suggest significant immunosuppression in zebrafish offspring in response to parental exposure to CTC, which is associated with the reduced resistance to acute bacterial challenges in CTC parental treatment (Figure 1b,c).

3.3. CTC Inhibits the Expression of Immune-Related Indicators. Immunoglobulin has a wide spectrum of antibodies to pathogens, and the main immunoglobulin classes in zebrafish include IgD, IgM, and IgZ. In 5 dpf of F1 larvae, the expressions of immunoglobulin genes *igd*, *igm*, and *igz* were significantly inhibited after parental exposure to CTC (Figure 3a). The gene expressions of immune indicators of *c3*, *crp*, and

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

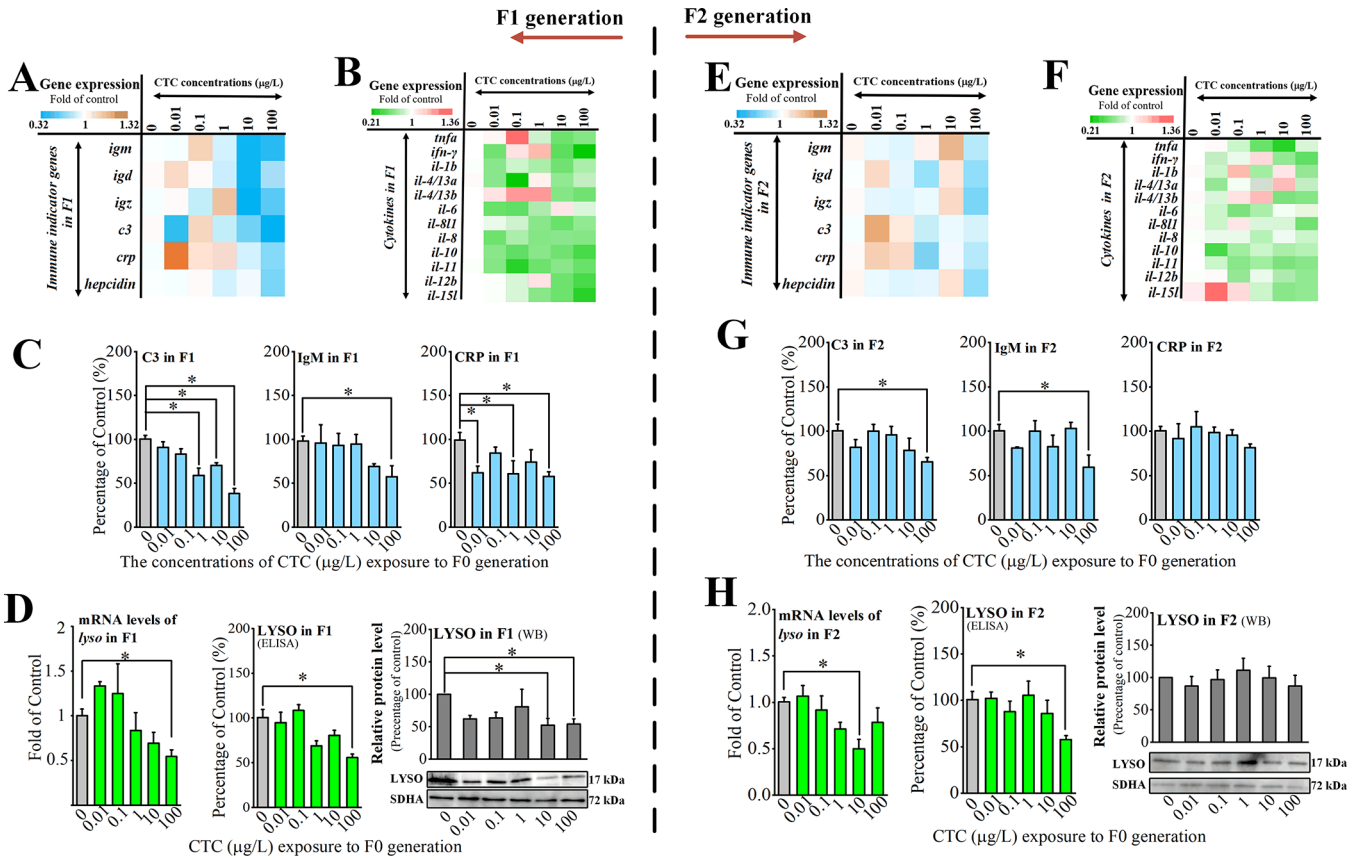


Figure 3. Antibiotic CTC exposures inhibit the expression of immune-related indicators in zebrafish offspring (5 dpf). (A) Inhibited expression of immune indicator genes in F1 larvae after F0 exposure to CTC. (B) Inhibited expression of cytokine genes in F1 larvae. (C) Inhibition of immune indicator (C3, IgM, CRP) levels in F1 larvae by ELISA assay. ELISA analysis, the intraassay and interassay coefficients of variance (CVs) were <10 and <12%, respectively. (D) Inhibition of lysozyme gene expression and protein levels in F1 larvae. (E) Inhibited expression of immune indicator genes in F2 larvae. (F) Inhibited expression of cytokine genes in F2 larvae. (G) Inhibition of immune indicator (C3, IgM, CRP) levels in F2 larvae by ELISA assay. (H) Inhibition of lysozyme gene expression and protein levels in F2 larvae. *$P < 0.05$, by one-way ANOVA with LSD's test ($n = 3$ or 4). Error bars indicate the s.e.m.

antimicrobial peptide *hepcidin* were also significantly downregulated. The mRNA levels of cytokines including *tnfa, ifn-γ, il-1b, il-4/13a, il-4/13b, il-6, il-8l1, il-8, il-10, il-11, il-12b*, and *il-15l* were also significantly downregulated in response to CTC parental treatment (Figure 3b). Consistently, the results of ELISA showed a significant decrease of immune indicators including C3, IgM, and CRP in the 0.01, 1, 10, or 100 μg/L CTC treatment groups (Figure 3c). Low expression of lysozyme gene *lyso* and low protein levels of LYSO were also found in F1 larvae in the 100 μg/L CTC treatment group (Figure 3d). Compared with F1, similar trends were also found in F2 larvae (Figure 3e−h). In summary, the low expression of immunoglobulin and cytokines, as well as low LYSO content in both F1 and F2 larvae evidenced a persistent immunocompromisation across two generations after CTC parental exposure. In addition, developmental and behavioral effects of CTC exposure, including survival rate, sex differentiation, body weight, body length, and tissue weight, were recorded in F0, as well as in F1 and F2 generations (egg production, egg death, fertilization rate, body length, swimming behavior; Table S4).

3.4. CTC Activates NF-κB Pathway. Based on our earlier nontarget transcriptomic results,[43] we hypothesized that NF-κB signaling the key pathway is involved in CTC immunotoxicity. The results of the molecular binding modeling suggested that CTC can bind to the groove in the RING domain of NF-κB1, NF-κB2, and NF-κB3 (Figure 4a). The MolDock score of highest activities on CTC binding to NF-κB1, NF-κB2, and NF-κB3 were −104.394, −104.434, and −93.0463 kcal/mol, respectively (Supporting Information Table S5). The binding between the ligand and NF-κB was predicted to be stabilized as the formation of conventional hydrogen bonds and Pi-Alkyl (Figure 4a and Supporting Information Figure S2). Moreover, the MolDock score of highest activities on CTC binding to NF-κB1, NF-κB2, and NF-κB3 were the three lowest values among the classical immune pathway including B-cell receptor, T-cell receptor, toll-like receptor, and IL-6 receptor, suggesting the most stabilized binding of CTC to NF-κB (Supporting Information Table S5). Also, the transcriptional levels of nfκb1, nfκb 2, and nfκb3 (Figure 4b), the protein level of nfκb3 (Figure 4c), and their related genes (Figure 4d) were all significantly increased in F1 and F2 larvae in response to parental exposure to CTC. Interestingly, CTC was still detectable in F1 and F2 embryos (Figure S3; Supporting Information Table S6), suggesting that residual CTC in offspring may bind to NF-κB molecules and activate NF-κB pathway, interfering with the immune system.

3.5. NF-κB Antagonist Alleviates Immunosuppression by CTC. To confirm the activation of NF-κB by CTC, the immunosuppression of CTC was investigated by introducing an NF-κB antagonist, PDTC. PDTC could inhibit the activation of NF-κB by suppressing both NF-κB DNA binding

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

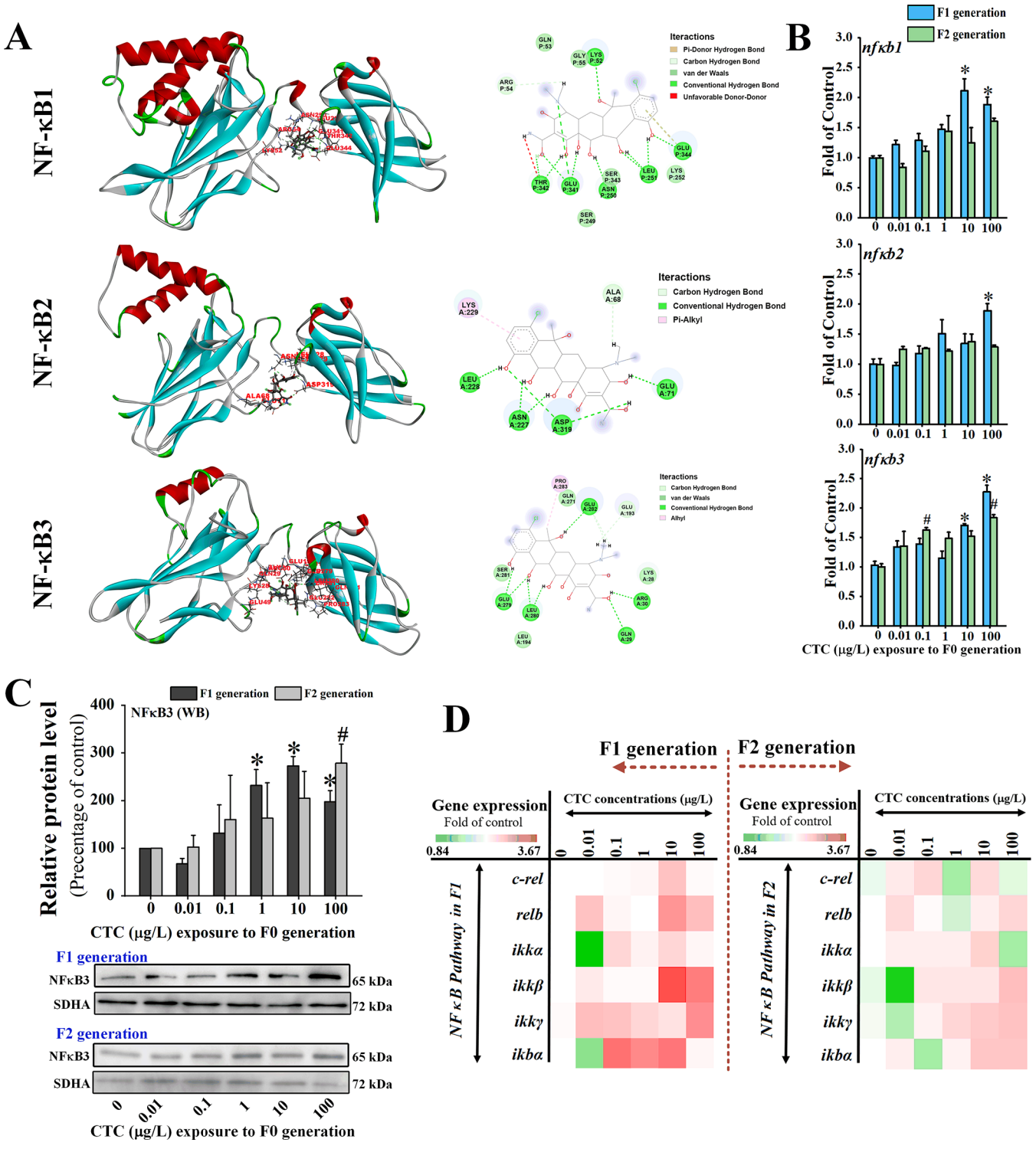


Figure 4. CTC binds to NF-κB and actives the nuclear factor-κB (NFκB) pathway in 5 dpf zebrafish offspring. (A) Molecular modeling indicates CTC binding to NFκB1, NFκB2, and NFκB3 by Molegro Virtual Docker software X7. The mRNA expression levels of *nfκb1*, *nfκb 2*, and *nfκb3* (B) and related genes (C). (D) Protein level of nfκb3 by western blot was significantly induced in F1 larvae in response to parental exposure to CTC. (E) CTC levels (ng/g) in F0 female ovary, F0 male testis, F1 embryos, and F2 embryos after F0 exposure to CTC. *$P < 0.05$, by one-way ANOVA with LSD's test. Error bars indicate the s.e.m. B, $n = 4$; C, $n = 4$; D, $n = 3$; and E, $n = 8$.

and NF-κB-dependent transcriptional activity.[41] If the inhibitor blocked the immunosuppression of CTC, it would suggest that the NF-κB pathway mediates the immunotoxicity of CTC. The effects were monitored in F1 larvae after F0 coexposure to PDTC alone, CTC alone, and CTC (10 or 100 μg/L) + PDTC (Figure 5a). PDTC significantly inhibited the NF-κB pathway in F1 larvae (Supporting Information Figure S4). Moreover, in F1 larvae, PDTC significantly attenuated the inhibitory effects of CTC on macrophage numbers, neutrophil numbers, and LYSO levels at 100 μg/L, and IgM levels at 10 and 100 μg/L (Figure 5b−e). Also, developmental and behavioral interferences of F1 larvae, including egg production,

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

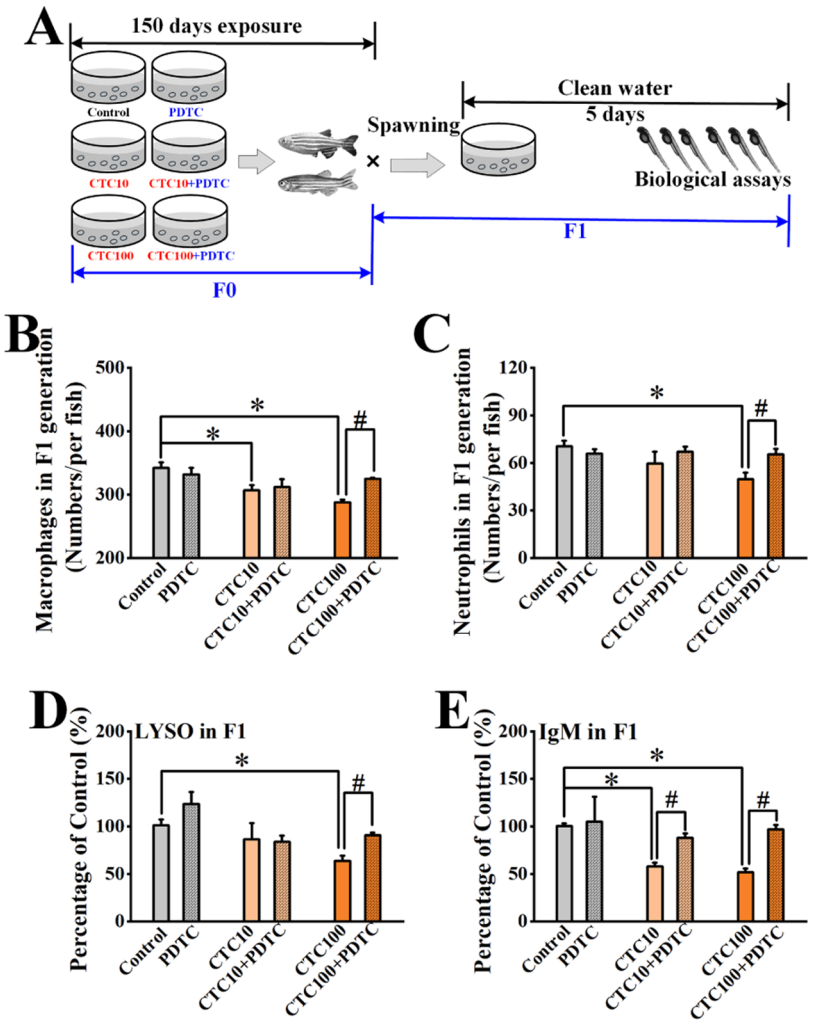


Figure 5. NF-κB antagonist PDTC rescues the immunosuppression of CTC in zebrafish offspring (5 dpf) mediated via NF-κB. (A) Experimental scheme for the NF-κB pathway inhibition test. CTC10, 10 μg/L CTC; CTC100, 100 μg/L CTC; CTC10 + PDTC, coexposure to 10 μg/L CTC with 1 μM PDTC; and CTC100 + PDTC, coexposure to 100 μg/L CTC with 1 μM PDTC. (B) Macrophage numbers in F1 larvae were decreased in 10 and 100 μg/L CTC parental exposure but increased by parental coexposure to 100 μg/L CTC with PDTC. (C) Parental coexposure to 100 μg/L CTC with PDTC increased neutrophil numbers in F1 larvae. (D) Parental coexposure to 100 μg/L CTC with PDTC increased LYSO levels in F1 larvae. (E) Parental coexposure to 10 or 100 μg/L CTC with PDTC increased IgM levels in F1 larvae. *$P < 0.05$, by one-way ANOVA with LSD's test. Error bars indicate the s.e.m. B, $n = 12$; C, $n = 12$; D, $n = 4$; and E, $n = 4$.

egg death, fertilization rate, body length, and swimming by CTC treatment, were significantly attenuated after parental coexposure to PDTC (Supporting Information Table S7). These results provided strong evidence that the transgenerational immunosuppression of CTC was mediated via NF-κB.

4. DISCUSSION

The prolonged use of antibiotics increases the susceptibility and severity of secondary infections,[42] which is closely related to the development of antibiotic resistance.[43,44] Antibiotics may interfere with the host's immune system,[45,46] but the drivers of such immune interference remain nebulous. Our previous studies found that antibiotics can disrupt the normal immune responses of the host: inflammation, cytokines, and host-dependent disease tolerance in primary macrophages *in vitro* and trigger immune and inflammatory response of the healthy host in zebrafish larvae *in vivo*.[23,47] Combined with our findings on the intergenerational transmission effects of antibiotics,[29] we suggested and focused for the first time on the transgenerational immunomodulatory effects of antibiotics. In the present study, we uncover that antibiotic CTC transgenerationally reduces macrophage and neutrophil numbers, immune indicators, as well as the host's antibacterial ability, indicating the immunosuppressive effects of CTC over two generations. Our findings suggest that immunosuppressive effects of antibiotics increase the susceptibility to secondary infections, highlighting the need to consider the hosts' immunosuppression of antibiotics besides antibiotic-resistant pathogens.

Newborns are particularly vulnerable to infections,[48,49] and the ontogeny of immunity during early life is of high importance as it shapes the immune system for the entire course of life.[50,51] Embryonic and transgenerational toxicity can affect the whole population of a species.[52] **Our results provide the evidence in fish that antibiotic CTC can be transmitted over two generations and weaken the antibacterial activities of offspring after parental exposure to CTC.** This implies that F1 fish with impaired immunity by CTC parental exposure in the environment may be more suspectable to infections in the environment. Antibiotic

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

exposures in parental mammals have also been reported to be associated with both short-term (e.g., congenital abnormalities, low birth weight) and long-term effects (e.g., atopic dermatitis, changes in the gut microbiome, and asthma in the newborn[53]). Abuse and widespread antibiotics expose more and more species in the environment,[54,55] which appeals for increasing attention and a better understanding of the unknown long-term impacts of antibiotics on environmental health.

We revealed that NF-κB is the molecular target of the immunosuppressive activity of CTC, which resulted in the activation of downstream genes and the downregulation of cytokines and immune responses. NF-κB is considered a key player in inflammatory processes and autoimmune diseases.[56−58] Some anti-inflammatory drugs and immunosuppressants have been confirmed in the disturbance of NF-κB pathways.[25,26,28,59] For example, fluoroquinolone antibiotics of levofloxacin and ciprofloxacin can attenuate microglia inflammatory response via TLR4/NF-κB pathway.[60] Betalactam antibiotic amoxicillin inhibits the endocytosis and allostimulatory capacity, depending on hyperactivated MAPK/NF-κB systems, in monocytes of allergic patients.[61] Tetracycline antibiotic doxycycline suppresses proinflammatory cytokines via the modulation of MAPK/NF-κB pathways.[62] In primary microglia cells, minocycline was showed to induce neuroinflammation via inhibiting NF-κB signaling pathways.[62,63] These findings suggest that some antibiotics can modulate the NF-κB signaling pathways, but their molecular targets and the detailed cellular and molecular mechanisms remain to be elucidated. NF-κB activation involves IκB-α phosphorylation and the subsequent nuclear translocation of NF-κB p65 component to promote the transcription of responsive genes.[64] Here, we found significantly increased levels of nfκB1/nfκB2/nfκB3, hyperactivated expression of c-rel, relb, ikkα, ikkβ, ikkγ, and iκBα, as well as inhibition of cytokines in zebrafish larvae after parental exposure to CTC. The molecular docking data showed that CTC can bind to NF-κB1/NF-κB2/NF-κB3 via stabilized conventional hydrogen bonds and Pi-Alkyl. Moreover, the NF-κB inhibitor, PDTC, was shown to significantly attenuate the inhibition actions of CTC on macrophage numbers, neutrophil numbers, and LYSO levels, further confirming the central role of NF-κB. However, the immunosuppressive effects of CTC cannot be completely rescued. Thus, besides NF-κB, other pathways might also be involved in the observed immunosuppression. For example, moxifloxacin, doxycycline, and erythromycin were shown to possess the strongest immunomodulatory effects through modulation of toll-like receptors (TLR).[65] Scott et al. showed that antibiotics perturbed mucosal macrophages, key cells for mounting immune responses via dysregulation of intestinal T-cell immunity.[66] Moreover, NF-κB signaling can crosstalk with signaling pathways that involve toll-like receptors (TLR), STAT3, MAPK, and T-cell receptor.[67−69] Thus, our results confirmed the activation of NF-κB-dependent signaling as an important molecular mechanism contributing to the transgenerational immune effects of CTC in zebrafish, but other potential mechanisms or interactions with other pathways should not be excluded,[60] which requires future investigations.

Exposure to CTC directly led to significant immune interferences of F1 and F2 fish. It should be noted that parental exposure to an NF-κB inhibitor mitigated the inhibitory effects of CTC in offspring that were not directly exposed to CTC. This implies that the immune interferences of offspring might be generated in F0 during the gravid period and transmitted to offspring through reproduction, which may combine with the chemical transmission of CTC to F1, explaining the transgenerational toxicity and immunosuppression. Since CTC was also detected in offspring, the offspring were at a dual risk of toxicity transmission and chemical exposure, which may result in the inhibition of immune defense function at the population level. Particularly, the ubiquity and variety of antibiotics in the environment could lead to synergistic or cross-acting effects on wild organisms;[23] therefore, the ecological risk of antibiotics might be underestimated.

In summary, antibiotics are extensively administered but can affect more than just the infection for which they are prescribed. The present study provides the comprehensive evidence that antibiotic CTC can strongly inhibit the antibacterial activities of fish offspring by transgenerational immunosuppression. Parental exposure to CTC transgenerationally perturbs macrophages, neutrophils, expression of immune-related genes, and NF-κB-dependent signaling, resulting in immune dysfunction of multiple generations. We highlight the depleted immune resistance of multiple generations after CTC exposure in fish, at low environmental concentrations. The potential environmental impacts of the broad-spectrum antibiotic mixture on long-term adaptive immunity and susceptibility to infections and inflammation should be further understood.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c07343.

Additional experimental details, methods, and results, including quality assurance (QA) and quality control (QC) for chemical analysis; optimized HPLC−MS/MS parameters for CTC; CTC concentrations in the test solutions and fish; primers of zebrafish genes; developmental and behavioral measurements; MolDock score and molecular modeling; pathogen challenge figures; and NF-κB pathway gene expression under NF-κB antagonist exposure (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Chunmiao Zheng − *Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China;* orcid.org/0000-0001-5839-1305; Email: zhengcm@sustech.edu.cn

Elvis Genbo Xu − *Department of Biology, University of Southern Denmark, Odense 5230, Denmark;* orcid.org/0000-0002-4414-1978; Email: elvis@biology.sdu.dk

Authors

Wenhui Qiu − *School of Public Health and Emergency Management, Southern University of Science and Technology, Shenzhen 518055, China; Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China;* orcid.org/0000-0002-9961-3928

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

Bei Chen − *Fisheries Research Institute of Fujian, Xiamen 361013, China*
Liang Tang − *School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China*
Bentuo Xu − *School of Life and Environmental Science, Wenzhou University, Wenzhou 325035, China*
Zhiyu Liu − *Fisheries Research Institute of Fujian, Xiamen 361013, China*
Jason T. Magnuson − *Department of Environmental Sciences, University of California, Riverside, California 92521, United States;* orcid.org/0000-0001-6841-8014
Shuwen Zhang − *Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China*
Daniel Schlenk − *Department of Environmental Sciences, University of California, Riverside, California 92521, United States*
Baoshan Xing − *Stockbridge School of Agriculture, University of Massachusetts, Amherst, Massachusetts 01003, United States;* orcid.org/0000-0003-2028-1295

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.1c07343

Author Contributions

W.Q., C.Z., and E.G.X. designed and performed fish experiments; W.Q. and E.G.X. wrote the manuscript; B.X. and L.T. contributed to the fish work and chemical analysis; B.C., Z.L., and S.Z. contributed to the antibacterial ability, immunoblotting, RNA-seq, ELISA, WISH, and so on; J.T.M., D.S., and B.X. contributed to the scientific discussion; W.Q. and C.Z. supervised the overall project; and J.T.M., D.S., B.X., and C.Z. revised the manuscript.

Notes

The authors declare no competing financial interest.
All data generated or analyzed during this study are available from the corresponding author on reasonable request.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 42077223), the National Key Research and Development Program of China (Grant No. 2021YFA1202500), the Shenzhen Science and Technology Innovation Committee (JCYJ20190809164201686, KQTD2016022619584022, KCXFZ20201221173410029), the State Environmental Protection Key Laboratory of Integrated Surface Water-Groundwater Pollution Control, the Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control (No. 2017B030301012), and the Leading Talents of Guangdong Province Program (Chunmiao Zheng). E.G.X. acknowledges the support of the Department of Biology, University of Southern Denmark and Danmarks Frie Forskningsfond.

■ REFERENCES

(1) Klein, E. Y.; Van Boeckel, T. P.; Martinez, E. M.; Pant, S.; Gandra, S.; Levin, S. A.; Goossens, H.; Laxminarayan, R. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci.* **2018**, *115*, No. 1717295115.
(2) Jansen, K. U.; Knirsch, C.; Anderson, A. S. The role of vaccines in preventing bacterial antimicrobial resistance. *Nat. Med.* **2018**, *24*, 10−19.
(3) Humphreys, G.; Fleck, F. United Nations meeting on antimicrobial resistance. *Bull. W.H.O.* **2016**, *94*, 638−639.
(4) Sarmah, A. K.; Meyer, M. T.; Boxall, A. B. A. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* **2006**, *65*, 725−759.
(5) Zhang, Q.-Q.; Ying, G.-G.; Pan, C.-G.; Liu, Y.-S.; Zhao, J.-L. Comprehensive Evaluation of Antibiotics Emission and Fate in the River Basins of China: Source Analysis, Multimedia Modeling, and Linkage to Bacterial Resistance. *Environ. Sci. Technol.* **2015**, *49*, 6772−6782.
(6) Ji, K.; Kho, Y.; Park, C.; Paek, D.; Ryu, P.; Paek, D.; Kim, M.; Kim, P.; Choi, K. Influence of water and food consumption on inadvertent antibiotics intake among general population. *Environ. Res.* **2010**, *110*, 641−649.
(7) Hamscher, G.; Sczesny, S.; Höper, H.; Nau, H. Determination of Persistent Tetracycline Residues in Soil Fertilized with Liquid Manure by High-Performance Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry. *Anal. Chem.* **2002**, *74*, 1509−1518.
(8) Le Page, G.; Gunnarsson, L.; Snape, J.; Tyler, C. R. Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. *Environ. Int.* **2017**, *109*, 155−169.
(9) Qiu, W. H.; Sun, J.; Fang, M. J.; Luo, S. S.; Tian, Y. Q.; Dong, P. Y.; Xu, B. T.; Zheng, C. M. Occurrence of antibiotics in the main rivers of Shenzhen, China: Association with antibiotic resistance genes and microbial community. *Sci. Total Environ.* **2019**, *653*, 334−341.
(10) Mellon, M.; Benbrook, C.; Benbrook, K. L. J. R. R. *Hogging it: Estimaton of Antimicrobial Abuse in Livestock*, 2001; Vol. 2.
(11) Hou, J.; Wang, C.; Mao, D. Q.; Luo, Y. The occurrence and fate of tetracyclines in two pharmaceutical wastewater treatment plants of Northern China. *Environ. Sci. Pollut. Res.* **2016**, *23*, 1722−1731.
(12) Tong, L.; Huang, S. B.; Wang, Y. X.; Liu, H.; Li, M. J. Occurrence of antibiotics in the aquatic environment of Jianghan Plain, central China. *Sci. Total Environ.* **2014**, *497*, 180−187.
(13) Ben, Y. J.; Hu, M.; Zhang, X. Y.; Wu, S. M.; Wong, M. H.; Wang, M. Y.; Andrews, C. B.; Zheng, C. M. Efficient detection and assessment of human exposure to trace antibiotic residues in drinking water. *Water Res.* **2020**, *175*, No. 115699.
(14) Olin, A.; Henckel, E.; Chen, Y.; Lakshmikanth, T.; Pou, C.; Mikes, J.; Gustafsson, A.; Bernhardsson, A. K.; Zhang, C.; Bohlin, K.; Brodin, P. Stereotypic Immune System Development in Newborn Children. *Cell* **2018**, *174*, 1277−1292.e14.
(15) Ghazal, P.; Dickinson, P.; Smith, C. L. Early life response to infection. *Curr. Opin. Infect. Dis.* **2013**, *26*, 213−218.
(16) Neuman, H.; Forsythe, P.; Uzan, A.; Avni, O.; Koren, O. Antibiotics in early life: dysbiosis and the damage done. *FEMS Microbiol. Rev.* **2018**, *42*, 489−499.
(17) Ledger, W. J.; Blaser, M. J. Are we using too many antibiotics during pregnancy? *Bjog-an Int. J. Obstet. Gynaecol.* **2013**, *120*, 1450−1452.
(18) Du, L. F.; Liu, W. K. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agron. Sustainable Dev.* **2012**, *32*, 309−327.
(19) Wickens, K.; Pearce, N.; Crane, J.; Beasley, R. Antibiotic use in early childhood and the development of asthma. *Clin. Exp. Allergy* **1999**, *29*, 766−771.
(20) McKeever, T. M.; Lewis, S. A.; Smith, C.; Collins, J.; Heatlie, H.; Frischer, M.; Hubbard, R. Early exposure to infections and antibiotics and the incidence of allergic disease: A birth cohort study with the West Midlands General Practice Research Database. *J. Allergy Clin. Immun.* **2002**, *109*, 43−50.
(21) Altenburg, J.; de Graaff, C. S.; van der Werf, T. S.; Boersma, W. G. Immunomodulatory Effects of Macrolide Antibiotics - Part 1: Biological Mechanisms. *Respiration* **2011**, *81*, 67−74.
(22) Rehberger, K.; Werner, I.; Hitzfeld, B.; Segner, H.; Baumann, L. 20 Years of fish immunotoxicology - what we know and where we are. *Crit. Rev. Toxicol.* **2017**, *47*, 516−542.

(23) Qiu, W. H.; Hu, J. Q.; Magnuson, J. T.; Greer, J.; Yang, M.; Chen, Q. Q.; Fang, M. J.; Zheng, C. M.; Schlenk, D. Evidence linking exposure of fish primary macrophages to antibiotics activates the NF-kB pathway. *Environ. Int.* **2020**, *138*, No. 105624.
(24) Karin, M.; Greten, F. R. NF-κB: linking inflammation and immunity to cancer development and progression. *Nat. Rev. Immunol.* **2005**, *5*, 749−759.
(25) Scott, M. G.; Hancock, R. E. W. Cationic antimicrobial peptides and their multifunctional role in the immune system. *Crit. Rev. Immunol.* **2000**, *20*, No. 24.
(26) Sun, X. J.; Zeng, H. Y.; Wang, Q. S.; Yu, Q. W.; Wu, J. X.; Feng, Y.; Deng, P.; Zhang, H. X. Glycyrrhizin ameliorates inflammatory pain by inhibiting microglial activation-mediated inflammatory response via blockage of the HMGB1-TLR4-NF-kappa B pathway. *Exp. Cell Res.* **2018**, *369*, 112−119.
(27) Hunto, S. T.; Kim, H. G.; Baek, K. S.; Jeong, D.; Kim, E.; Kim, J. H.; Cho, J. Y. Loratadine, an antihistamine drug, exhibits anti-inflammatory activity through suppression of the NF-B-k pathway. *Biochem. Pharmacol.* **2020**, *177*, No. 113949.
(28) Chen, S.; Bonifati, S.; Qin, Z.; St Gelais, C.; Kodigepalli, K. M.; Barrett, B. S.; Kim, S. H.; Antonucci, J. M.; Ladner, K. J.; Buzovetsky, O.; Knecht, K. M.; Xiong, Y.; Yount, J. S.; Guttridge, D. C.; Santiago, M. L.; Wu, L. SAMHD1 suppresses innate immune responses to viral infections and inflammatory stimuli by inhibiting the NF-κB and interferon pathways. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, E3798−E3807.
(29) Qiu, W. H.; Fang, M. J.; Magnuson, J. T.; Greer, J. B.; Chen, Q. Q.; Zheng, Y.; Xiong, Y.; Luo, S. S.; Zheng, C. M.; Schlenk, D. Maternal exposure to environmental antibiotic mixture during gravid period predicts gastrointestinal effects in zebrafish offspring. *J. Hazard. Mater.* **2020**, *399*, No. 123009.
(30) Ji, K.; Kim, S.; Han, S.; Seo, J.; Lee, S.; Park, Y.; Choi, K.; Kho, Y. L.; Kim, P. G.; Park, J.; Choi, K. Risk assessment of chlortetracycline, oxytetracycline, sulfamethazine, sulfathiazole, and erythromycin in aquatic environment: are the current environmental concentrations safe? *Ecotoxicology* **2012**, *21*, 2031−2050.
(31) Kummerer, K. The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges. *J. Environ. Manage.* **2009**, *90*, 2354−2366.
(32) Park, S.; Choi, K. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. *Ecotoxicology* **2008**, *17*, 526−538.
(33) Ji, K.; Choi, K.; Lee, S.; Park, S.; Khim, J. S.; Jo, E. H.; Choi, K.; Zhang, X. W.; Giesy, J. P. Effects of sulfathiazole, oxytetracycline and chlortetracycline on steroidogenesis in the human adrenocarcinoma (H295R) cell line and freshwater fish Oryzias latipes. *J. Hazard. Mater.* **2010**, *182*, 494−502.
(34) Bojarski, B.; Kot, B.; Witeska, M. Antibacterials in Aquatic Environment and Their Toxicity to Fish. *Pharmaceuticals* **2020**, *13*, No. 189.
(35) Drevets, D. A.; Canono, B. P.; Campbell, P. A. Measurement of bacterial ingestion and killing by macrophages. *Curr. Protoc. Immunol.* **2015**, *109*, 14.6.1−14.6.17.
(36) Liu, F.; Wen, Z. L. Cloning and expression pattern of the lysozyme C gene in zebrafish. *Mech. Dev.* **2002**, *113*, 69−72.
(37) Qiu, W. H.; Chen, B.; Greer, J. B.; Magnuson, J. T.; Xiong, Y.; Zhong, H. B.; Andrzejczyk, N. E.; Zheng, C. M.; Schlenk, D. Transcriptomic Responses of Bisphenol S Predict Involvement of Immune Function in the Cardiotoxicity of Early Life-Stage Zebrafish (Danio rerio). *Environ. Sci. Technol.* **2020**, *54*, 2869−2877.
(38) Dente, L.; Gestri, G.; Tsang, M.; Kudoh, T.; Wilson, S. W.; Dawid, I. B.; Andreazzoli, M. Cloning and developmental expression of zebrafish pdzrn3. *Int. J. Dev. Biol.* **2011**, *55*, 989−993.
(39) Gallagher, S.; Winston, S. E.; Fuller, S. A.; Hurrell, J. G. R. Immunoblotting and Immunodetection. In *Current Protocols in Molecular Biology*, **2008**.
(40) Prokopec, S. D.; Watson, J. D.; Pohjanvirta, R.; Boutros, P. C. Identification of Reference Proteins for Western Blot Analyses in Mouse Model Systems of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) Toxicity. *PLoS One* **2014**, *9*, No. e110730.
(41) Németh, Z. H.; Deitch, E. A.; Szabo, C.; Hasko, G. Pyrrolidinedithiocarbamate inhibits NF-kappa B activation and IL-8 production in intestinal epithelial cells. *Immunol. Lett.* **2003**, *85*, 41−46.
(42) Wendy, A. The Antibiotic Alternative. The Natural Guide to Fighting Infection and Maintaining a Healthy Immune System. *Econ. Bot.* **2002**, *56*, 211.
(43) Fischbach, M. A.; Walsh, C. T. Antibiotics for Emerging Pathogens. *Science* **2009**, *325*, 1089−1093.
(44) Nathan, C. Antibiotics at the crossroads. *Nature* **2004**, *431*, 899−902.
(45) Wlodarska, M.; Finlay, B. B. Host immune response to antibiotic perturbation of the microbiota. *Mucosal Immunol.* **2010**, *3*, 100−103.
(46) Wlodarska, M.; Willing, B.; Keeney, K. M.; Menendez, A.; Bergstrom, K. S.; Gill, N.; Russell, S. L.; Vallance, B. A.; Finlay, B. B. Antibiotic Treatment Alters the Colonic Mucus Layer and Predisposes the Host to Exacerbated Citrobacter rodentium-Induced Colitis. *Infect. Immun.* **2011**, *79*, 1536−1545.
(47) Liu, J. Y.; Wei, T. Z.; Wu, X.; Zhong, H. B.; Qiu, W. H.; Zheng, Y. Early exposure to environmental levels of sulfamethoxazole triggers immune and inflammatory response of healthy zebrafish larvae. *Sci. Total Environ.* **2020**, *703*, No. 134724.
(48) Levy, O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat. Rev. Immunol.* **2007**, *7*, 379−390.
(49) Strunk, T.; Currie, A.; Richmond, P.; Simmer, K.; Burgner, D. Innate immunity in human newborn infants: prematurity means more than immaturity. *Journal of Maternal-Fetal & Neonatal Medicine* **2011**, *24*, 25−31.
(50) Kumar, S. K. M.; Bhat, B. V. Distinct mechanisms of the newborn innate immunity. *Immunol. Lett.* **2016**, *173*, 42−54.
(51) Bookstaver, P. B.; Bland, C. M.; Griffin, B.; Stover, K. R.; Eiland, L. S.; McLaughlin, M. A Review of Antibiotic Use in Pregnancy. *Pharmacotherapy* **2015**, *35*, 1052−1062.
(52) Zhang, Z. B.; Hu, J. Y.; Zhen, H. J.; Wu, X. Q.; Huang, C. Reproductive Inhibition and Transgenerational Toxicity of Triphenyltin on Medaka (Oryzias latipes) at Environmentally Relevant tip Levels. *Environ. Sci. Technol.* **2008**, *42*, 8133−8139.
(53) Vidal, A. C.; Murphy, S. K.; Murtha, A. P.; Schildkraut, J. M.; Soubry, A.; Huang, Z.; Neelon, S. E. B.; Fuemmeler, B.; Iversen, E.; Wang, F.; Kurtzberg, J.; Jirtle, R. L.; Hoyo, C. Associations between antibiotic exposure during pregnancy, birth weight and aberrant methylation at imprinted genes among offspring. *Int. J. Obes.* **2013**, *37*, 907−913.
(54) Gelbrand, H.; Miller-Petrie, M.; Pant, S.; Gandra, S.; Levinson, J., The State of the World's Antibiotics 2015, 2015.
(55) Kennedy, D. Time to deal with antibiotics. *Science* **2013**, *342*, 777.
(56) Taniguchi, K.; Karin, M. NF-κB, inflammation, immunity and cancer: coming of age. *Nat. Rev. Immunol.* **2018**, *18*, 309−324.
(57) Grinberg-Bleyer, Y.; Oh, H.; Desrichard, A.; Bhatt, D. M.; Caron, R.; Chan, T. A.; Schmid, R. M.; Klein, U.; Hayden, M. S.; Ghosh, S. NF-kappa B c-Rel Is Crucial for the Regulatory T Cell Immune Checkpoint in Cancer. *Cell* **2017**, *170*, 1096−1108.
(58) Sun, S. C. The non-canonical NF-kappa B pathway in immunity and inflammation. *Nat. Rev. Immunol.* **2017**, *17*, 545−558.
(59) Hunto, S. T.; Kim, H. G.; Baek, K.-S.; Jeong, D.; Kim, E.; Kim, J. H.; Cho, J. Y. Loratadine, an antihistamine drug, exhibits anti-inflammatory activity through suppression of the NF-kB pathway. *Biochem. Pharmacol.* **2020**, *177*, No. 113949.
(60) Zusso, M.; Lunardi, V.; Franceschini, D.; Pagetta, A.; Lo, R.; Stifani, S.; Frigo, A. C.; Giusti, P.; Moro, S. Ciprofloxacin and levofloxacin attenuate microglia inflammatory response via TLR4/NF-kB pathway. *J. Neuroinflamm.* **2019**, *16*, 12.
(61) Lopez, S.; Gomez, E.; Torres, M. J.; Pozo, D.; Fernandez, T. D.; Ariza, A.; Sanz, M. L.; Blanca, M.; Mayorga, C. Betalactam antibiotics affect human dendritic cells maturation through MAPK/NF-kB

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261

systems. Role in allergic reactions to drugs. *Toxicol. Appl. Pharm.* **2015**, *288*, 289−299.
(62) Santa-Cecilia, F. V.; Socias, B.; Ouidja, M. O.; Sepulveda-Diaz, J. E.; Acuna, L.; Silva, R. L.; Michel, P. P.; Del-Bel, E.; Cunha, T. M.; Raisman-Vozari, R. Doxycycline Suppresses Microglial Activation by Inhibiting the p38 MAPK and NF-kB Signaling Pathways. *Neurotoxicity Res.* **2016**, *29*, 447−459.
(63) Nikodemova, M.; Duncan, I. D.; Watters, J. J. Minocycline exerts inhibitory effects on multiple mitogen-activated protein kinases and I kappa B alpha degradation in a stimulus-specific manner in microglia. *J. Neurochem.* **2006**, *96*, 314−323.
(64) Kang, S.-M.; More, S. V.; Park, J.-Y.; Kim, B.-W.; In, P. J.; Yoon, S.-H.; Choi, D.-K. A novel synthetic HTB derivative, BECT inhibits lipopolysaccharide-mediated inflammatory response by suppressing the p38 MAPK/JNK and NF-kappa B activation pathways. *Pharmacol. Rep.* **2014**, *66*, 471−479.
(65) Bode, C.; Diedrich, B.; Muenster, S.; Hentschel, V.; Weisheit, C.; Rommelsheim, K.; Hoeft, A.; Meyer, R.; Boehm, O.; Knuefermann, P.; Baumgarten, G. Antibiotics regulate the immune response in both presence and absence of lipopolysaccharide through modulation of Toll-like receptors, cytokine production and phagocytosis in vitro. *Int. Immunopharmacol.* **2014**, *18*, 27−34.
(66) Scott, N. A.; Andrusaite, A.; Andersen, P.; Lawson, M.; Alcon-Giner, C.; Leclaire, C.; Caim, S.; Le Gall, G.; Shaw, T.; Connolly, J. P. R.; Roe, A. J.; Wessel, H.; Bravo-Blas, A.; Thomson, C. A.; Kastele, V.; Wang, P.; Peterson, D. A.; Bancroft, A.; Li, X. H.; Grencis, R.; Mowat, A. M.; Hall, L. J.; Travis, M. A.; Milling, S. W. F.; Mann, E. R. Antibiotics induce sustained dysregulation of intestinal T cell immunity by perturbing macrophage homeostasis. *Sci. Transl. Med.* **2018**, *10*, No. eaao4755.
(67) Grivennikov, S. I.; Karin, M. Dangerous liaisons: STAT3 and NF-κB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev.* **2010**, *21*, 11−19.
(68) Oeckinghaus, A.; Hayden, M. S.; Ghosh, S. Crosstalk in NF-κB signaling pathways. *Nat. Immunol.* **2011**, *12*, 695−708.
(69) Taniguchi, K.; Karin, M. NF-κB, inflammation, immunity and cancer: coming of age. *Nat. Rev. Immunol.* **2018**, *18*, 309−324.

https://doi.org/10.1021/acs.est.1c07343
Environ. Sci. Technol. 2022, 56, 4251−4261