TITLE:

Effect of Oligomers Derived from Biodegradable Polyesters on Eco- and Neurotoxicity

AUTHOR(S):

Yoshinaga, Naoto; Tateishi, Ayaka; Kobayashi, Yasuaki; Kubo, Tomohiro; Miyakawa, Hitoshi; Satoh, Kotaro; Numata, Keiji

CITATION:

Yoshinaga, Naoto ...[et al]. Effect of Oligomers Derived from Biodegradable Polyesters on Eco- and Neurotoxicity. Biomacromolecules 2023, 24(6): 2721-2729

ISSUE DATE:

2023-06-12

URL:

http://hdl.handle.net/2433/286258

RIGHT:

Copyright © 2023 The Authors. Published by American Chemical Society.; This publication is licensed under CC-BY-NC-ND 4.0.

京都大学学術情報リポジトリ
KURENAI 紅
Kyoto University Research Information Repository

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

Biomacromolecules

pubs.acs.org/Biomac Article

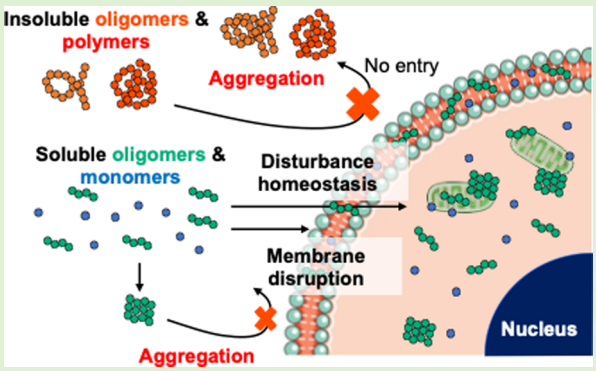
Effect of Oligomers Derived from Biodegradable Polyesters on Eco- and Neurotoxicity

Naoto Yoshinaga, Ayaka Tateishi, Yasuaki Kobayashi, Tomohiro Kubo, Hitoshi Miyakawa, Kotaro Satoh, and Keiji Numata*

Cite This: *Biomacromolecules* 2023, 24, 2721−2729 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Biodegradable polymers are eco-friendly materials and have attracted attention for use in a sustainable society because they are not accumulated in the environment. Although the characteristics of biodegradable polymers have been assessed well, the effects of their degradation products have not. Herein, we comprehensively evaluated the chemical toxicities of biodegradable polyester, polycaprolactone (PCL), and synthetic oligocaprolactones (OCLs) with different degrees of polymerization. While the PCL did not show any adverse effects on various organisms, high levels of shorter OCLs and the monomer (1 μg/mL for freshwater microorganisms and 1 mg/mL for marine algae and mammalian cells) damaged the tested organisms, including freshwater microorganisms, marine algae, and mammalian cells, which indicated the toxicities of the degradation products under unnaturally high concentrations. These results highlight the need for a further understanding of the effects of the degradation products resulting from biodegradable polyesters to ensure a genuinely sustainable society.



Downloaded via 130.54.130.250 on December 1, 2023 at 07:01:13 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

INTRODUCTION

Plastics have played an essential role in developing a convenient society due to their attractive properties, including strong mechanical properties, stability, and durability.[1,2] Despite their enormous contribution to society, the plastics remaining in natural environments may cause adverse effects.[3−5] As examples, microplastic pollutants in water and soil harm plants,[6,7] aquatic fauna,[8−10] fish,[11] zooplankton,[12] worms,[13] birds,[14] and humans.[15,16] Even biocompatible and water-soluble polymers, such as poly(ethylene glycol) (PEG), that do not show severe toxicity to humans sometimes become lethal to particular species after release into the environment.[17−19] Some biocompatible polymers have even been reported to affect the human body through frequent use, as with anti-PEG antibodies.[20] Thus, growing concerns about the toxicities of polymers in the environment have increased the international momentum for using and developing biodegradable polymers as sustainable materials.

Biodegradable polymers are defined as polymers that undergo deterioration via enzymatic cleavage of the polymer backbone and are microbially metabolized into simple natural products.[21,22] The first enzymatic degradation step depolymerizes biodegradable polymers into oligomers and monomers. Microbial take up and then metabolize these small degradative products and produce primary and secondary metabolites, followed by the mineralization of metabolites into simple products, such as carbon dioxide (CO_2), methane, and water. The biodegradability of a polymer enables us to avoid accumulation of fragmented polymers in natural environments or inside the human body. Biodegradable polymers have thus been used as eco- and human-friendly products in agriculture and as biomaterials.[23,24] The safety concerns for biodegradable products, such as agricultural mulch films, are well assessed via degradation and toxicity tests of the products and polymer pellets.[25,26] However, there has been little interest in the toxicities of the oligomers, although these biodegradable products do not rapidly degrade in the environment and may temporally remain after the initial degradation process. This insufficient understanding of oligomers might impact the health of various organisms and cause a decrement in natural populations. Very recently, degradation products from the nanoparticles released from polycaprolactone (PCL) beads were reported to be significantly toxic to marine microalgae,[27] which emphasized the importance of understanding the effects of degradable products on the ecosystem.

Received: February 15, 2023
Revised: April 8, 2023
Published: April 21, 2023

ACS Publications
© 2023 The Authors. Published by American Chemical Society

https://doi.org/10.1021/acs.biomac.3c00160
Biomacromolecules 2023, 24, 2721−2729

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

Thus, in this study, we comprehensively examined the potential eco- and neurotoxicities of polycaprolactone (PCL) and oligocaprolactones (OCLs) with different degrees of polymerization (DP). As model organisms, *Daphnia magna* (*D. magna*), *Ectocarpus siliculosus* (*E. siliculosus*), and neurons differentiated from rat adrenal gland pheochromocytoma (PC12) cells were selected to evaluate the broad effects of OCLs in different environments. Intriguingly, highly concentrated OCLs, especially short OCLs (tetramers) and monomers, demonstrated significant chemical toxicity toward *D. magna* (at 1 μg/mL), *E. siliculosus* (at 1 mg/mL), and cultured neurons (at 1 mg/mL), while the PCL showed negligible effects on these organisms. Moreover, for neurons, treatments with tetramers and monomers inhibited mitochondrial energy production and neurite growth at this concentration. These results demonstrated that the degradation products from biodegradable polyesters might be harmful to various organisms at unusually high concentrations, which requires novel and strict guidelines on the toxicities and environmental effects of the oligomers and more detailed safety assessments to develop truly eco-friendly materials.

MATERIALS AND METHODS

Materials. ε-Caprolactone (>99.0%) and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) (>98.0%) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Ethanol (EtOH, >99.5%) and acetic acid (>99.7%) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Acetic anhydride (>97%), ethyl acetate (EtOAc, >99.5%), *n*-hexane (>96.0%), chloroform (>99.0%), methanol (>99.8%), poly(ε-caprolactone) (PCL), DMSO for molecular biology, Evans blue, and sodium dodecyl sulfate (SDS) for molecular biology were purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). Dichloromethane (CH_2Cl_2, >99.5%) and tetrahydrofuran (THF, >99.5%) were purchased from KANTO Chemical Co., Inc. (Tokyo, Japan) and were dried and deoxygenized by passage through the columns of a Glass Contour Solvent System before use. ε-Caprolactone was distilled from calcium hydride before use. 6-Hydroxycaproic acid was synthesized as described in the literature.[28] EtOH was dried over 3 Å molecular sieves before use. YCT was purchased from Recenttec K.K. (Tokyo, Japan). *Chlorella* sp. was purchased from Chlorella Industry Co., Ltd. (Tokyo, Japan). Marine Art SF-1 artificial seawater was purchased from Osaka Yakken Co. Ltd. (Osaka, Japan).

Organisms. *D. magna* (NIES strain) was obtained from the National Institute for Environmental Studies. The PC12 cell line was obtained from the RIKEN Cell Bank. *E. siliculosus* was obtained from the Kobe University Macro-Algal Culture Collection. *D. magna* was cultured in the M4 medium[29] at 20 °C under 16 h light/8 h dark conditions with an LED (16,300 lux). PC12 cells were cultured in DMEM containing 5% horse serum, 5% bovine fetal serum, and 1% penicillin/streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. Before use, the cells were differentiated into neuronal cells using the neuro growth factor (NGF; Cosmo Bio Co., Ltd., Tokyo, Japan) according to a previous report.[30] Briefly, the PC12 cells were differentiated into neurons in Opti-MEM containing 0.5% FBS, 1% penicillin/streptomycin, and 50 ng/mL NGF for 3 days. *E. siliculosus* was cultured in artificial seawater, Marine Art SF-1, with a half-strength Provasoli-enriched seawater (PES) medium at 20 °C in a 12 h light/12 h dark condition with an LED (4000 lux).

Syntheses of OCL Samples. *OCL_{4OH} Synthesis.* 6-Hydroxycaproic acid (1.65 g, 12.5 mmol) and ε-caprolactone (4.16 mL, 37.5 mmol) were dissolved in THF (169 mL) at 30 °C. TBD (75 mL, 15 mmol, 200 mM in THF) was added to the mixture. The reaction solution was stirred at 30 °C for 96 h. Polymerization was quenched by the addition of acetic acid (1.72 mL, 30.0 mmol), followed by evaporation of the solvent. The product was dissolved in acetic acid (8.6 mL, 150 mmol) again, followed by the addition of distilled water (250 mL). The resulting material was extracted with EtOAc and purified by column chromatography with hexane/EtOAc (from 80/20 to 20/80) and chloroform/EtOAc (from 100/0 to 80/20) to give OCL_{4OH} (1.42 g, $M_{n,NMR}$ = 450, $M_{n,SEC}$ = 530, M_w/M_n = 1.85). ^{1}H-NMR spectra were recorded on a JEOL ECZ-400S spectrometer operating at 400 MHz (JEOL Ltd., Tokyo, Japan). The number average molecular weights and molecular weight distributions of the OCL samples were determined by size exclusion chromatography (SEC) in THF at 40 °C on two polystyrene gel columns [Tosoh Multipore HXL-M (7.8 mm i.d. × 30 cm) × 2; flow rate 1.0 mL min^{-1}] connected to a JASCO PU-2085 precision pump and a JASCO RI-2031 detector (JASCO Co., Tokyo, Japan). The columns were calibrated with standard polystyrenes (Agilent Technologies; M_p = 580−3,152,000, M_w/M_n = 1.02−1.12).

OCL_{4Ac} Synthesis. TBD (41.8 mg, 0.300 mmol) was dissolved in DCM (24.2 mL). EtOH (0.88 mL, 15.0 mmol) and ε-caprolactone (5.0 mL, 45 mmol) were added. The reaction solution was stirred at room temperature for 2 h. The polymerization was quenched by the addition of acetic anhydride (30.0 mL, 317 mmol), and the solution was stirred for an additional 42 h. The resulting mixture was diluted with DCM and washed with saturated aqueous NH_4Cl (×3), H_2O, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting material was dissolved in hexane/chloroform (10/1) again, washed with H_2O (×6) and brine, dried over $MgSO_4$, filtered, and concentrated to give OCL_{4Ac} (3.22 g, M_n = 490, $M_{n,SEC}$ = 690, M_w/M_n = 1.26). The product was characterized by ^{1}H-NMR and SEC using the same procedures described above.

OCL_{13OH} Synthesis. TBD (418 mg, 3.00 mmol) was dissolved in THF (23.2 mL). To this was added H_2O (0.16 mL, 9.0 mmol) and ε-caprolactone (6.7 mL, 60 mmol). The reaction solution was stirred at room temperature for 1.5 h. The polymerization was quenched by the addition of benzoic acid (366 mg, 3.00 mmol). The resulting mixture was purified by precipitation from hexane and MeOH. The resulting material was dissolved in chloroform and washed with aq. 1 wt/vol% oxalic acid (×3) and H_2O, dried over $MgSO_4$, filtered, and concentrated. The resulting product was precipitated into hexane to give OCL_{13OH} (0.46 g, $M_{n,NMR}$ = 1500, $M_{n,SEC}$ = 2300, M_w/M_n = 1.16). The product was characterized by ^{1}H-NMR and SEC using the same procedures described above.

OCL_{13Ac} Synthesis. TBD (41.8 mg, 0.300 mmol) was dissolved in DCM (23.2 mL). EtOH (0.18 mL, 3.0 mmol) and ε-caprolactone (6.7 mL, 60 mmol) were added. The reaction solution was stirred at room temperature for 2.5 h. The polymerization was quenched by the addition of acetic anhydride (15.0 mL, 159 mmol), and the solution was stirred for an additional 45 h. The resulting mixture was diluted with DCM and washed with H_2O (×3), aq. sat. NH_4Cl (×3), and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting material was purified by precipitation into hexane three times to give OCL_{13Ac} (3.26 g, $M_{n,NMR}$ = 1500, $M_{n,SEC}$ = 2100, M_w/M_n = 1.13). The product was characterized by ^{1}H-NMR and SEC using the same procedures described above.

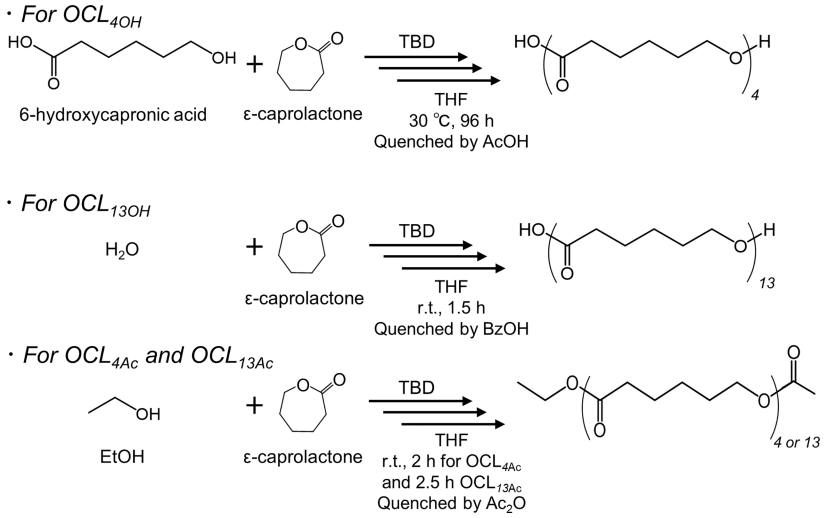
Solubility of the OCL Samples. The aqueous solubilities of the samples were measured with turbidimetric solubility assays. The sample stock solutions in DMSO (100 mg/mL) were diluted with DI water. The final DMSO concentration was 1%. The absorbance of each dilution was measured using a SpectraMax iD3 microplate reader (Molecular Devices, LLC, California) at 650 nm at room temperature.

Toxicity of OCLs to *D. magna*. Toxicity tests to *D. magna* were performed by referencing test guideline 211 of the Organization for Economic Cooperation and Development (OECD).[31] The OCL samples were first dissolved in DMSO at 10 mg/mL and 10 μg/mL and then diluted with M4 culture medium to a final concentration of 1 μg/mL or 1 ng/mL for OCL samples and 0.01 v/v% for DMSO. Female *D. magna* aged less than 24 h from nonfirst broods were cultured separately in 50 mL of the prepared media in glass cups (diameter: 5.0 cm and height 5.5 cm) under semistatic test conditions. The culture medium was replaced with fresh medium three times per week with a feeding of YCT (yeast, cerophyll, and trout chow) and green alga *Chlorella vulgaris* (Chlorella Industry Co. Ltd., Fukuoka, Japan). The feeding amounts of YCT were 50 μL/

https://doi.org/10.1021/acs.biomac.3c00160
Biomacromolecules 2023, 24, 2721−2729

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

Scheme 1. Syntheses of OCLs with Different Molecular Weights and/or Chain-End Structures



individual, and those of chlorella were 0.115 mg C/individual for the first 7 days and 0.384 mg C /individual from day 8 to day 21. The effects of the OCLs were validated by observing the swimming and spawning behavior, the number and sex of the offspring, and the mortality rate. The mortality rate was calculated as follows:

Mortality rate (%) = (Number of dead parental individuals)/(Number of total parental individuals).

Evans Blue Assay of *E. siliculosus* after OCL Treatment. *E. siliculosus* (1 ∼ 3 mg) was cultured in Marine Art SF-1 artificial seawater containing each sample at various concentrations. It should be noted that the final DMSO concentration was 1%. After 24 h of incubation, the tissues were stained with Evans blue (final concentration: 0.15 mg/mL) for 40 min at room temperature. After washing the samples with fresh artificial seawater twice, the samples were incubated in 150 μL of 50% methanol solution with 1% sodium dodecyl sulfate (SDS) for 30 min at room temperature to extract Evans blue from the samples. The absorbance of the extracted solution was measured at 605 nm with a SpectraMax iD3 microplate reader.

Cell Titer Assay of *E. siliculosus* after OCL Treatment. *E. siliculosus* (1−3 mg) was cultured for 24 h in Marine Art SF-1 artificial seawater containing each sample at 1 mg/mL. It should be noted that the final DMSO concentration was 1%. The tissues were washed twice with fresh artificial seawater and incubated for 2 h at 20 °C in fresh seawater containing Cell Titer 96 AQueous One solution (Promega, Madison, WI). The absorbance of the medium at 490 nm was measured with a SpectraMax iD3 microplate reader.

Cytotoxicity of OCLs to PC12-Derived Neurons. PC12 cells were seeded on poly-L-lysine-coated 96-well plates (IWAKI, AGC TECHNO GLASS Co., Ltd., Tokyo, Japan) and differentiated into neurons by NGF for 3 days. The culture medium was replaced with fresh medium containing various concentrated samples and 1% DMSO. After 24 h of incubation, the cell viability was evaluated using a Cell Counting Kit-8 (CCK-8) assay kit (Dojindo Laboratories Co., Ltd., Kumamoto, Japan) and by observing absorbance at 450 nm. Additionally, the mitochondrial membrane potential was evaluated using a Cell Meter JC-10 kit (AAT Bioquest, Inc., Sunnyvale, CA) by observing fluorescence intensities with an excitation wavelength of 485 nm and emission wavelengths of 515 nm for the green channel and 590 nm for the red channel.

Confocal Microscope Observations of the PC12-Derived Neurons. PC12 cells were seeded on 35 mm glass-bottom dishes (IWAKI) and differentiated into neurons by NGF for 3 days. The culture medium was replaced with fresh medium containing 1 mg/mL samples and 1% DMSO. After 24 h of incubation, the cells were washed with D-PBS (+) twice and then stained by using a Neurite Outgrowth Staining Kit (Thermo Fisher Scientific Inc., Waltham, MA) according to the manufacturer's protocol. The staining reagent was removed, and a solution containing a background suppression dye was added. The cells were observed using an LSM 700 confocal laser scanning microscopy system (Zeiss, Germany) equipped with a × 40 objective lens with excitation wavelengths of 488 nm for the cytosol and 543 nm for the plasma membrane.

Quantification of Neuronal Markers in PC12-Derived Neurons. PC12 cells were seeded on poly-L-lysine-coated 96-well plates (IWAKI, AGC TECHNO GLASS CO., LTD., Japan) and differentiated into neurons by NGF for 3 days. The culture medium was replaced with fresh medium containing 1 mg/mL of each sample and 1% DMSO. The cells were washed with D-PBS (+) once and lysed with 1% 2-mercaptoethanol-containing RLT buffer in an RNeasy mini kit (Qiagen, Germany). After RNA extraction with an RNeasy mini kit, the total RNA was reverse-transcribed with a ReverTra Ace qPCR RT Kit (TOYOBO, Co., Ltd., Osaka, Japan). The complementary DNA was analyzed by quantitative real-time PCR (qRT−PCR) using the following primer sets:

β3-Tubulin. ATAGACCCCAGCGGCAACTATGTG (forward), AGGCCTGAATAGGTGTCCAAAGGC (reverse).

MAP2. GACAGAGAAACAGCAGAGGAAGTG (forward), TGTTCTGATGCTGGCGATGGT (reverse).

GAP43. GATGGTGTCAAACCGGAGGAT (forward), CTTGTTATGTGTCCACGGAAGC (reverse).

GAPDH. AGTTCAACGGCACAGTCAAG (forward), TACTCAGCACCAGCATCACC (reverse).

RESULTS AND DISCUSSION

Syntheses of OCLs. We synthesized four distinct well-defined OCLs with different DPs and terminal structures via ring-opening polymerization of ε-caprolactone with 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as the catalyst (Scheme 1). TBD is an effective bicyclic guanidine-based catalyst for ring-opening polymerization of cyclic esters because of its unique ability to simultaneously activate esters and alcohols.[32,33] The synthesized samples were characterized by ^{1}H-NMR, SEC, and water solubility (Figure S1 and S2), and the data are summarized in Table 1. The DPs of the OCLs were determined from the ^{1}H-NMR spectra by using the integral ratio of the peaks attributed to the terminal and repeating units. The different compositions of the OCLs with a relatively narrow molecular weight distribution allowed us to evaluate the effects of the OCL length on various organisms precisely. Moreover, we synthesized end-capped OCLs to assess the effects of end structures on toxicity, which were capped with ethyl ester and acetyl groups at the ends. In total, four distinct

https://doi.org/10.1021/acs.biomac.3c00160
Biomacromolecules 2023, 24, 2721−2729

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

Table 1. Molecular Characteristics of the Samples

	DP	M_n	M_w/M_n	solubility in water at 25 °C
PCL	88	10,000	1.94	10 μg/mL
OCL_{13OH}	13	1400	1.16	25 μg/mL
OCL_{13Ac}	13	1500	1.13	40 μg/mL
OCL_{4OH}	3.8	400	1.85	125 μg/mL
OCL_{4Ac}	4.0	490	1.26	125 μg/mL
6-HCa	1	132	-	100 μg/mL
ε-CL	-	114	-	> 1000 μg/mL

OCLs were prepared, and these were denoted as OCL_{nX} (n indicates the DP, and X indicates the end structure (OH or Ac)). In addition to the OCLs, two monomeric compounds were used as the control groups: 6-hydroxycaproic acid (6-HCa) and ε-caprolactone (ε-CL). 6-HCa is a linear monomer and likely the shortest degradation product, and ε-CL is a cyclic monomer used for the PCL synthesis. As PCL and OCL_{13X} showed poor solubilities in aqueous solutions (Table 1 **and** Figure S3), all samples were first dissolved in DMSO and then diluted with each culturing medium in the following experiments.

Toxicities of the OCLs to *D. magna*. The ecotoxicities of the synthesized OCLs were validated with *D. magna*, an indicator species in freshwater due to its sensitivity to environmental pollution.[34] We conducted a long-term chronic reproduction test after referencing the OECD guidelines for testing soluble chemicals (OECD 211). In this test, a female *D. magna* was cultured in each cup so that it developed parthenogenetic eggs and had only female hatchlings. In these experiments, we evaluated both the chronic toxicities and hormonal effects by counting the total offspring and by checking the sex of the hatchlings. To avoid turbidity stress, which influences *D. magna* behavior and mortality rate,[35] the sample concentrations were set to relatively low values (1 μg/mL and 1 ng/mL). It should be noted that under these less concentrated conditions, none of the samples exhibited acute toxicity within 48 h. The total offspring and mortality rates of the DMSO control group were more than 60 and less than 20%, respectively, which meant that the experiments met the validity criteria of the guideline. While PCL did not show considerable adverse effects at the tested condition, culturing *D. magna* in the presence of OCLs and monomers except 1 μg/mL OCL_{13OH} significantly decreased the total offspring compared with the DMSO control group, even at a relatively low concentration (1 ng/mL) (Figure 1). Especially, OCL_{4OH} demonstrated more reduction of the total offspring of *D. magna* than other OCLs. Some *D. magna* cultured with the OCLs stopped laying eggs after approximately 10 days of incubation (Figure S4), which was also not observed in the DMSO- and PCL-treated groups. These results indicated the potential toxicities of the OCLs to *D. magna*.

The decreases in the total offspring were not dependent on the concentration of the OCLs and monomers. The details were unclear, but the threshold concentrations for toxicity of the OCLs and monomer to *D. magna* might be much lower than 1 ng/mL, and the toxic effects plateaued above the threshold. Interestingly, there was no significant difference between OCL_{nOH} and OCL_{nAc} regarding toxicity to *D. magna*, which suggested that the DPs of the oligomers, not the end structures, were crucial in determining the toxicity. The mortality rates among the samples were random, and no male hatchlings were observed in any of the tested samples (Table 2), so the degradation products did not cause severe acute toxicity. As PCL is widely used as a multifilm and as bags for composting in agricultural fields and can flow into rivers after fragmentation, the degradation products may chemically disturb the ecosystem via toxicity to *D. magna*, which plays an important role in freshwater ecosystems as a predator and prey.

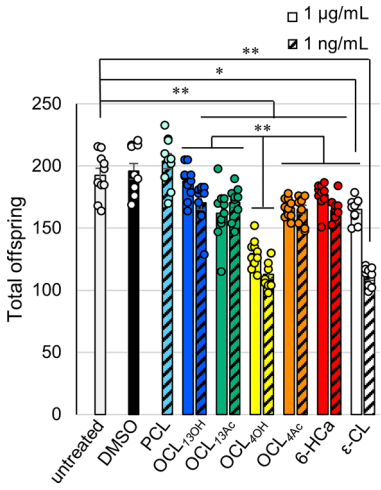


Figure 1. Ecotoxicities of OCLs to *D. magna*. The total offspring after 21 days of incubation with each sample at 1 μg/mL (closed bar) or 1 ng/mL (hatched bar). Data represent the mean ± SEM. Each dot represents biological replicates. n = 10. *p < 0.05 and **p < 0.01 (one-way ANOVA followed by Tukey's post hoc test).

Toxicities of OCLs to Marine Organisms. We then examined the ecotoxicity effects of the OCLs toward marine organisms. Brown algae are multicellular marine organisms and play an essential role as primary producers in the ecosystems of coastal areas. Assessing toxicity to brown algae is important because the effect on primary producers may spread to other organisms. The toxicity to *E. siliculosus*, a widely used model brown alga, was thus evaluated through the Evans blue assay. Since Evans blue can only pass through the damaged plasma membrane, the toxicity can be determined by measuring the absorbance of Evans blue in the cell.[36] After 24 h of incubation with each sample, the absorbances for Evans blue in *E. siliculosus* treated with the OCL_{4X} and monomers were significantly higher than that in the DMSO control group (Figure 2a), and PCL and OCL_{13X} showed no effect. In *E. siliculosus*, the end structures of the OCLs were not crucial for toxicity, as in the results obtained with *D. magna*. These results indicated that the amphiphilic OCL_{4X} and monomers accessed the plasma membrane, leading to tissue damage in *E. siliculosus*. The effect of the oligomers on energy production by *E. siliculosus* was investigated to gather detailed toxicity data. The NADH level in *E. siliculosus* was measured using a Cell Titer 96 Aqueous One solution, which is highly soluble in aqueous solutions, including seawater. After incubation in the presence of 1 mg/mL samples, the OCL_{4X} and monomers showed significant decreases in absorbance compared with the DMSO-treated control group (Figure 2b), indicating less energy production by the *E. siluculosus* treated with OCL_{4X}- and the monomer. The amphiphilic OCL_{4X} and monomers may have physically destabilized the plasma membrane, as shown by the Evans blue assay, and biologically disturbed the cellular energy metabolism, as demonstrated by the Cell Titer assay. The sea is the final destination for many plastics, including degradation

https://doi.org/10.1021/acs.biomac.3c00160
Biomacromolecules 2023, 24, 2721−2729

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

Table 2. Mortality Rate and Number of Male Hatchlings after the Sample Treatment (N = 10)

	concentration (ng/mL)														
	PCL		OCL_{13OH}		OCL_{13Ac}		OCL_{4OH}		OCL_{4Ac}		6-HCa		ε-CL		DMSO
	1 μg/mL	1 ng/mL	1 μg/mL	1 ng/mL	1 μg/mL	1 ng/mL	1 μg/mL	1 ng/mL	1 μg/mL	1 ng/mL	1 μg/mL	1 ng/mL	1 μg/mL	1 ng/mL	-
mortality rate (%)	0	0	20	30	10	20	0	30	10	10	20	0	30	10	10
male hatchlings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

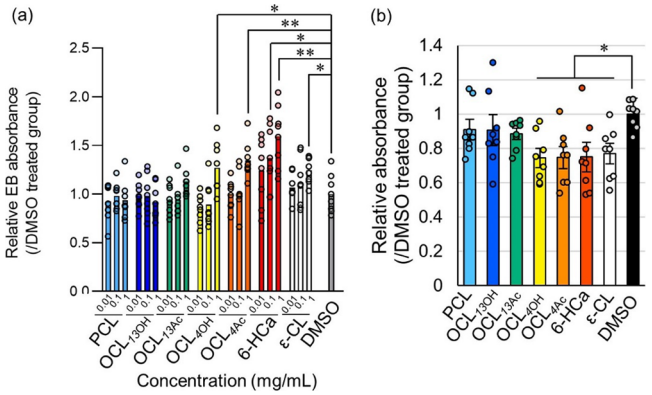


Figure 2. Ecotoxicities of OCLs to *E. siliculosus*. (a) Cell damage evaluated by Evans blue assays after 24 h incubations with each sample at various concentrations. (b) Energy production efficiency of *E. siliculosus* 24 h after the sample treatments at 1 mg/mL. Data represent the mean ± SEM. Each dot in (b) represents biological replicates. n = 8. *p < 0.05 and **p < 0.01 (one-way ANOVA followed by Dunnett's post hoc test compared with the DMSO-treated group).

products, released into the environment, even if the plastics are biodegradable. The biodegradable plastics accumulated by the neighboring brown algae in the sea may cause damage to the brown algae as a result of the degradation products.

Toxicities of the OCLs to PC12-Derived Neurons. The acute toxicities of OCLs to neurons were evaluated by measuring the cell viabilities, mitochondrial conditions, and neuronal markers. The PC12 cell line, which is widely used to assess the neuronal function of various substances after differentiation into neuron-like cells,[37,38] was selected as a model neuron. After incubating the cells with each sample, the cytotoxicities were determined with a CCK-8 kit, which measures the level of the electron carrier (NADH) correlated to energy production. PCL and OCL_{13X} did not influence cell viability, but OCL_{4X} and the monomers showed significant reductions after 24 h of incubation, even though there was no information in the safety data sheet of the monomer indicating cytotoxicity (Figure 3a). In contrast, there was no difference in cytotoxicity between OCL_{4OH} and OCL_{4Ac}, which was consistent with the results from the *D. magna* and *E. siliculosus* studies (Figure 1, 2a). These results indicated that the small oligomers and monomers were toxic regardless of the end structures. As the reduction in NADH levels raised concerns about impaired mitochondrial function, the mitochondrial membrane potential was therefore measured with a JC-10 kit, which shows red fluorescence in the healthy mitochondria and green fluorescence in the damaged mitochondria. Compared with the DMSO-treated control group, OCL_{4X} and 6-HCa significantly increased the green/red fluorescence ratio (Figure 3b), indicating that the OCL_{4X} and monomers damaged the mitochondrial membrane, as seen with the energy production test (Figure 3a). The effect of the OCLs on the mitochondria may be a serious issue as the usage of biodegradable products is expanded. Further analysis is required to elucidate the detailed mechanism through which the degradation products affect the energy production pathways.

Treatments with the OCL_{4X} and monomer also changed the cell morphology. The confocal images showed that the plasma membrane was stained red, and the inside of the cell was stained green, which revealed that the neurons had lost their dendritic structures and became spherical after treatment with the OCL_{4X} and monomer (Figure 3c). The OCL_{4X} and monomer may have prevented neuronal homeostasis. To gain further insight into the morphological changes occurring in neurons, the messenger RNAs encoding the neuronal markers were evaluated by quantitative real-time PCR after incubation with each sample. Neuron-specific class III β-tubulin (Tuj1), microtube-associated protein 2 (MAP2), and growth-associated protein 43 (GAP43) were selected as the neuronal markers. Tuj1 is associated with neurogenesis, MAP2 stimulates the dendritic growth of neurons, and GAP43 works on axonal growth.[39] Interestingly, a decrease in *GAP43* expression was observed after the OCL_{4X} and 6-HCa treatments, while *Tuj1* and *MAP2* expression was not significantly affected in any of the samples (Figure 3d). ε-CL tended to decrease *GAP43* expression, but not significantly. These results confirmed that the OCL_{4X} and monomer inhibited axonal growth and led to morphological changes in the PC12-derived neurons, as shown in Figure 3c.

Mechanism for the Toxicity of the Short OCLs. PCL did not demonstrate unfavorable effects on the cultured neurons, as reported elsewhere,[40] or toward *D. magna* and *E. siliculosus*. The longer OCL_{13X} analogs were also safe for *E. siliculosus* and the neurons, probably because OCL_{13X} still behaved as a hydrophobic polymer and aggregated in the culture medium, which prevented cellular uptake. For *D. magna*, the OCL_{13X} showed toxicity, and *D. magna* was sensitive to the OCLs, even for the low concentrations at which the OCL_{13X} dissolved. On the other hand, the OCL_{4X} and the monomer demonstrated adverse effects on all tested organisms. Presumably, the amphiphilicity of OCL_{4X} and the monomer induced these adverse effects because the amphiphilic molecules likely entered the plasma membrane and were taken into the cells (Figure 4), similar to surfactants, which can destabilize cell membrane with disturbing cellular functions.[41−44] This is related to the observation that the soluble degradation products and assemblies showed neurotoxicity in the case of amyloid and fibrous proteins.[37,45,46] Access of the OCL_{4X} and monomer to the plasma membrane decreased the membrane integrity and might have caused toxic effects (Figures 1, 2a, and 3a). Entry of the OCL_{4X} and the monomer into the cells disturbed cellular homeostasis, especially energy production in the mitochondria (Figures 2b

https://doi.org/10.1021/acs.biomac.3c00160
Biomacromolecules 2023, 24, 2721−2729

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

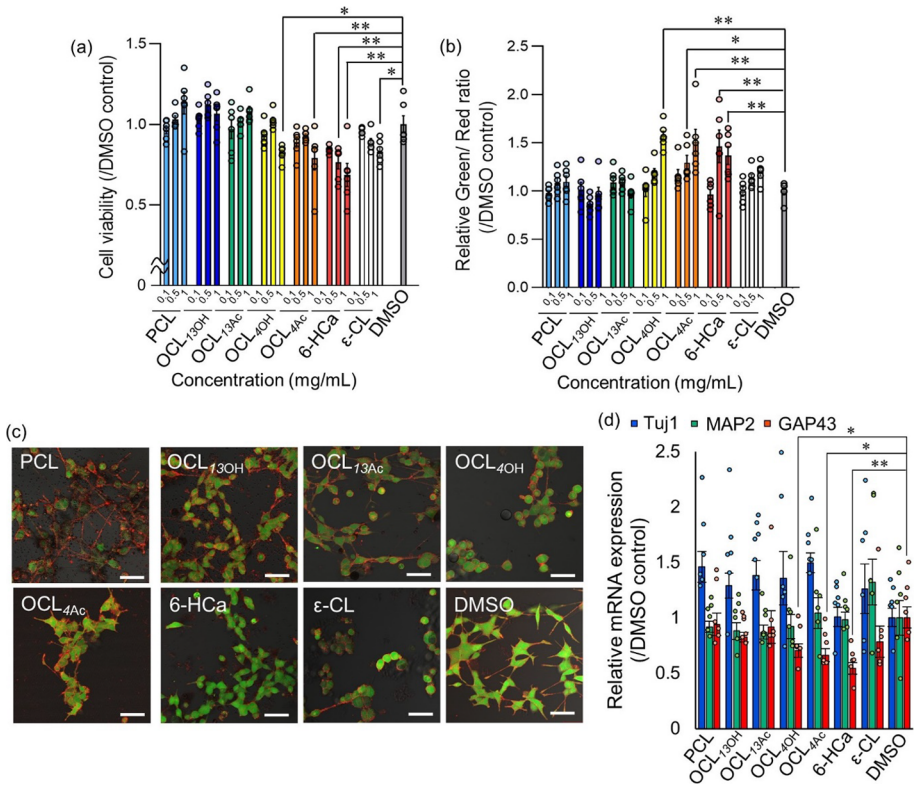


Figure 3. Neurotoxicities of the OCLs. (a) Cell viability of PC12-derived neurons, (b) mitochondrial membrane potential, (c) confocal images with staining the plasma membrane red and inside the cells green, and (d) neuron markers after 24 h of incubation with various concentrated samples. The sample concentration in (c) and (d) was 1 mg/mL. Scale bar in (c): 50 μm. Data represent the mean ± SEM. Each dot in (d) represents biological replicates. $n = 6$. *$p < 0.05$ and **$p < 0.01$ (one-way ANOVA followed by Dunnett's post hoc test compared with the DMSO-treated group).

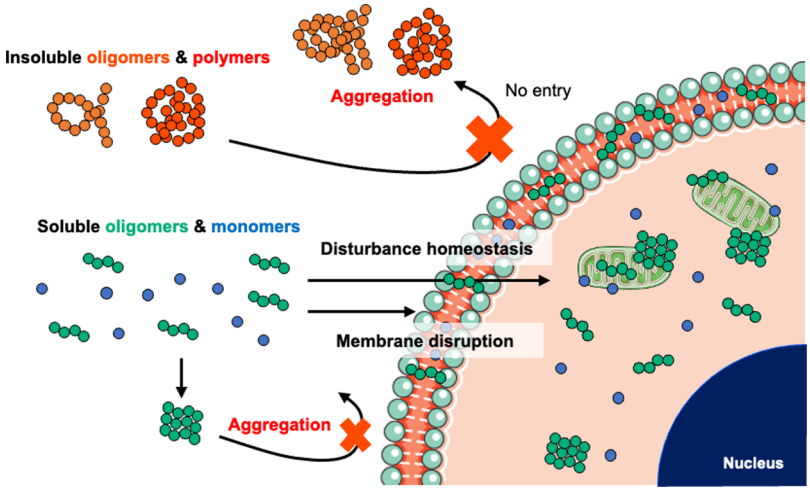


Figure 4. Schematic illustration of the cytotoxicity mechanism for the OCLs and monomers.

and 3b). The OCL_{4X} and its monomer might accumulate in the mitochondria and disrupt the membrane as they did with the plasma membrane, although the detailed mechanism is unclear. Furthermore, OCL_{4OH} exhibited significantly higher toxicity to *D. magna* compared with other OCLs. As the difference between end structures in OCL_{13X} was not observed, the end structure might not be dominant to explain the enhanced toxicity of OCL_{4OH}. Instead, the molecular weight distribution of OCL_{4OH}, which was relatively wider than other OCLs, could affect the toxicity to *D. magna* because of the small components in OCL_{4OH}. Notably, these experiments were conducted at a high sample concentration, which cannot naturally occur in the environment. However, the harmful properties of the degradation products toward several organisms should warn us of overconfidence in the biodegradable polymeric materials because they can be taken up by our bodies as microplastics.[47]

CONCLUSIONS

In this study, we synthesized OCLs as model degradation products from PCL by controlling the degrees of polymer-

https://doi.org/10.1021/acs.biomac.3c00160
Biomacromolecules 2023, 24, 2721−2729

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

ization and the end structures, and we comprehensively evaluated their toxicities to various organisms. Our results indicated that the oligomers, especially the shorter OCL_{4X}, and monomers damaged the cells and tissues for multiple species. In particularly adverse cases, the OCL treatments resulted in a decrease in the total offspring of *D. magna* and reduced the levels of mitochondrial energy production in *E. siliculosus* and cultured neurons. As PCL demonstrated no cytotoxicity toward the tested organisms, the molecular weights and solubilities of the degradation products could be critical factors in the chemical toxicity. The amphiphilic OCL_{4X} and monomer probably entered the cells by diffusion, consequently leading to toxic effects. Studying the fates of OCLs and the monomer in cells after cellular uptake is necessary to reveal the mechanism for toxicity. Our study demonstrated that biodegradable polyesters are not always safe for various species due to their degradation products, even though the polyester itself is quite harmless. The potential toxicity warns us of the risks of the degradation products that have been missed, probably due to the positive impression of "biodegradability" regarding the environment. Biodegradable materials can ultimately be decomposed to water and CO_2, but the findings in this study imply that the products produced during the degradation process must be addressed to ensure that the materials are sustainable and safe. The potential toxicity may also provide the design principle of biodegradable polymers; a quite rapid degradation, which has been attracted to avoid the accumulation of plastics in the environment,[48] might be harmful due to the dramatical increase of degradation products in the local environment. Therefore, in addition to product safety and degradation rate, the toxicities of the intermediate products formed by the initial degradation process should be integrated into the life-cycle assessments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biomac.3c00160.

^{1}H-NMR; SEC; images of the solubility tests; and time-lapse data for the total offspring of *D. magna* (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Keiji Numata − *Biomacromolecule Research Team, RIKEN Center for Sustainable Resource Science, Wako-shi, Saitama 351-0198, Japan; Institute for Advanced Biosciences, Keio University, Tsuruoka-shi, Yamagata 997-0017, Japan; Department of Material Chemistry, Kyoto University, Kyoto-shi, Kyoto 615-8510, Japan;* orcid.org/0000-0003-2199-7420; Email: keiji.numata@riken.jp

Authors

Naoto Yoshinaga − *Biomacromolecule Research Team, RIKEN Center for Sustainable Resource Science, Wako-shi, Saitama 351-0198, Japan; Institute for Advanced Biosciences, Keio University, Tsuruoka-shi, Yamagata 997-0017, Japan;* orcid.org/0000-0003-4399-0289

Ayaka Tateishi − *Biomacromolecule Research Team, RIKEN Center for Sustainable Resource Science, Wako-shi, Saitama 351-0198, Japan*

Yasuaki Kobayashi − *School of Materials and Chemical Technology, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8550, Japan*

Tomohiro Kubo − *School of Materials and Chemical Technology, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8550, Japan;* orcid.org/0000-0003-3913-5845

Hitoshi Miyakawa − *Center for Bioscience Research and Education, Utsunomiya University, Utsunomiya, Tochigi 321-8505, Japan*

Kotaro Satoh − *School of Materials and Chemical Technology, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8550, Japan;* orcid.org/0000-0002-3105-4592

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.biomac.3c00160

Author Contributions

N.Y., K.S., and K.N. wrote the manuscript. K.N. conceived the project. N.Y., A.T., Y.K., and T.K. conducted the experiments. All authors have given approval to the final version of the manuscript.

Funding

This work was financially supported by Japan Science and Technology Agency Exploratory Research for Advanced Technology (JPMJER1602 to K.N.), by MEXT Program: Data Creation and Utilization-Type Material Research and Development Project (JPMXP1122714694 to K.N.), Environment Research and Technology Development Fund (1RF-2202), Ministry of the Environment (to H.M.), and by Japan Society for the Promotion of Science Grant-in-Aid for Transformative Research Areas (B) (20H05732 and 20H05735 to K.N. and 20H05734 to K.S.)

Notes

The authors declare no competing financial interest.

K.S. and T.K. thank Dr. Kazuyuki Osaka for his help with the sample preparations.

■ REFERENCES

(1) Balakrishnan, P.; Sreekala, M. S. In *Recycling of Polymers: Methods, Characterization and Applications*; Francis, R., Eds.;Wiley-VCH: Weinheim, Germany, 2016; p 251.

(2) De Sousa, F. D. B. The role of plastic concerning the sustainable development goals: The literature point of view. *Cleaner and Responsible Consumption* **2021**, *3*, No. 100020.

(3) Zhang, S.; Wang, J.; Liu, X.; Qu, F.; Wang, X.; Wang, X.; Li, Y.; Sun, Y. Microplastics in the environment: A review of analytical methods, distribution, and biological effects. *TrAC Trends Anal. Chem.* **2019**, *111*, 62−72.

(4) Shen, M.; Huang, W.; Chen, M.; Song, B.; Zeng, G.; Zhang, Y. (Micro)plastic crisis: Un-ignorable contribution to global greenhouse gas emissions and climate change. *J. Cleaner Prod.* **2020**, *254*, No. 120138.

(5) Tarazona, N. A.; Machatschek, R.; Balcucho, J.; Castro-Mayorga, J. L.; Saldarriaga, J. F.; Lendlein, A. Opportunities and challenges for integrating the development of sustainable polymer materials within an international circular (bio)economy concept. *MRS Energy Sustain.* **2022**, *9*, 28−34.

(6) Kalčíková, G.; Žgajnar Gotvajn, A.; Kladnik, A.; Jemec, A. Impact of polyethylene microbeads on the floating freshwater plant duckweed *Lemna minor*. *Environ. Pollut.* **2017**, *230*, 1108−1115.

(7) De Souza Machado, A. A.; Kloas, W.; Zarfl, C.; Hempel, S.; Rillig, M. C. Microplastics as an emerging threat to terrestrial ecosystems. *Glob. Change Biol.* **2018**, *24*, 1405−1416.

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

(8) Martínez-Gómez, C.; León, V. M.; Calles, S.; Gomáriz-Olcina, M.; Vethaak, A. D. The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins. *Mar. Environ. Res.* **2017**, *130*, 69−76.
(9) Guilhermino, L.; Vieira, L. R.; Ribeiro, D.; Tavares, A. S.; Cardoso, V.; Alves, A.; Almeida, J. M. Uptake and effects of the antimicrobial florfenicol, microplastics and their mixtures on freshwater exotic invasive bivalve *Corbicula fluminea*. *Sci. Total Environ.* **2018**, *622-623*, 1131−1142.
(10) Reichert, J.; Schellenberg, J.; Schubert, P.; Wilke, T. Responses of reef building corals to microplastic exposure. *Environ. Pollut.* **2018**, *237*, 955−960.
(11) Barboza, L. G. A.; Vieira, L. R.; Guilhermino, L. Single and combined effects of microplastics and mercury on juveniles of the European seabass (*Dicentrarchus labrax*): Changes in behavioural responses and reduction of swimming velocity and resistance time. *Environ. Pollut.* **2018**, *236*, 1014−1019.
(12) Rehse, S.; Kloas, W.; Zarfl, C. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of *Daphnia magna*. *Chemosphere* **2016**, *153*, 91−99.
(13) Huerta Lwanga, E.; Gertsen, H.; Gooren, H.; Peters, P.; Salánki, T.; van der Ploeg, M.; Besseling, E.; Koelmans, A. A.; Geissen, V. Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*. *Environ. Pollut.* **2017**, *220*, 523−531.
(14) Teuten, E. L.; Saquing, J. M.; Knappe, D. R. U.; Barlaz, M. A.; Jonsson, S.; Björn, A.; Rowland, S. J.; Thompson, R. C.; Galloway, T. S.; Yamashita, R.; et al. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc., B* **2009**, *364*, 2027−2045.
(15) Carbery, M.; O'Connor, W.; Palanisami, T. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. *Environ. Int.* **2018**, *115*, 400−409.
(16) Campanale, C.; Massarelli, C.; Savino, I.; Locaputo, V.; Uricchio, V. F. A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1212.
(17) Nascimento, Í. F.; Guimarães, A. T. B.; Ribeiro, F.; Rodrigues, A. S. d. L.; Estrela, F. N.; Luz, T. M. d.; Malafaia, G. Polyethylene glycol acute and sub-lethal toxicity in neotropical *Physalaemus cuvieri* tadpoles (Anura, Leptodactylidae). *Environ. Pollut.* **2021**, *283*, No. 117054.
(18) Rozman, U.; Kalčíková, G. The first comprehensive study evaluating the ecotoxicity and biodegradability of water-soluble polymers used in personal care products and cosmetics. *Ecotoxicol. Environ. Saf.* **2021**, *228*, No. 113016.
(19) Hering, I.; Eilebrecht, E.; Parnham, M. J.; Günday-Türeli, N.; Türeli, A. E.; Weiler, M.; Schäfers, C.; Fenske, M.; Wacker, M. G. Evaluation of potential environmental toxicity of polymeric nanomaterials and surfactants. *Environ. Toxicol. Pharmacol.* **2020**, *76*, No. 103353.
(20) Kozma, G. T.; Shimizu, T.; Ishida, T.; Szebeni, J. Anti-PEG antibodies: Properties, formation, testing and role in adverse immune reactions to PEGylated nano-biopharmaceuticals. *Adv. Drug Delivery Rev.* **2020**, *154-155*, 163−175.
(21) Haider, T. P.; Völker, C.; Kramm, J.; Landfester, K.; Wurm, F. R. Plastics of the Future? The Impact of Biodegradable Polymers on the Environment and on Society. *Angew. Chem., Int. Ed.* **2019**, *58*, 50−62.
(22) Joseph, E.; Tohidifar, P.; Sarver, C. T.; Mackie, R. I.; Rao, C. V. Fundamentals of Polymer Biodegradation Mechanisms. In *Biodegradable Polymers in the Circular Plastics Economy;* Dusselier, M.; Lange, J.-P., Eds.; Wiley-VCH: Weinheim, Germany, 2022; p 17.
(23) Sikder, A.; Pearce, A. K.; Parkinson, S. J.; Napier, R.; O'Reilly, R. K. Recent Trends in Advanced Polymer Materials in Agriculture Related Applications. *ACS Appl. Polym. Mater.* **2021**, *3*, 1203−1217.
(24) Song, R.; Murphy, M.; Li, C.; Ting, K.; Soo, C.; Zheng, Z. Current development of biodegradable polymeric materials for biomedical applications. *Drug Des. Devel. Ther.* **2018**, *Volume 12*, 3117−3145. 10.2147/DDDT.s165440 PubMed
(25) Ghosh, K.; Jones, B. H. Roadmap to Biodegradable Plastics—Current State and Research Needs. *ACS Sustainable Chem. Eng.* **2021**, *9*, 6170−6187.
(26) Ahmed, T.; Shahid, M.; Azeem, F.; Rasul, I.; Shah, A. A.; Noman, M.; Hameed, A.; Manzoor, N.; Manzoor, I.; Muhammad, S. Biodegradation of plastics: current scenario and future prospects for environmental safety. *Environ. Sci. Pollut. Res.* **2018**, *25*, 7287−7298.
(27) Tamayo-Belda, M.; Pulido-Reyes, G.; González-Pleiter, M.; Martín-Betancor, K.; Leganés, F.; Rosal, R.; Fernández-Piñas, F. Identification and toxicity towards aquatic primary producers of the smallest fractions released from hydrolytic degradation of polycaprolactone microplastics. *Chemosphere* **2022**, *303*, No. 134966.
(28) Li, M.; Ling, L.; Xia, Q.; Li, X. A reduction-responsive drug delivery with improved stability: disulfide crosslinked micelles of small amiphiphilic molecules. *RSC Adv.* **2021**, *11*, 12757−12770.
(29) Elendt, B. P.; Bias, W. R. Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of *D. magna*. *Water Res.* **1990**, *24*, 1157−1167.
(30) Hu, R.; Cao, Q.; Sun, Z.; Chen, J.; Zheng, Q.; Xiao, F. A novel method of neural differentiation of PC12 cells by using Opti-MEM as a basic induction medium. *Int. J. Mol. Med.* **2017**, *41*, 195−201.
(31) OECD. *Test No. 211: Daphnia magna Reproduction Test*; OECD Guidelines for the Testing of Chemicals, Section 2; OECD Publishing: Paris, 2012; p 25, DOI: 10.1787/9789264185203-en.
(32) Pratt, R. C.; Lohmeijer, B. G. G.; Long, D. A.; Waymouth, R. M.; Hedrick, J. L. Triazabicyclodecene: A Simple Bifunctional Organocatalyst for Acyl Transfer and Ring-Opening Polymerization of Cyclic Esters. *J. Am. Chem. Soc.* **2006**, *128*, 4556−4557.
(33) Chuma, A.; Horn, H. W.; Swope, W. C.; Pratt, R. C.; Zhang, L.; Lohmeijer, B. G. G.; Wade, C. G.; Waymouth, R. M.; Hedrick, J. L.; Rice, J. E. The Reaction Mechanism for the Organocatalytic Ring-Opening Polymerization of l-Lactide Using a Guanidine-Based Catalyst: Hydrogen-Bonded or Covalently Bound? *J. Am. Chem. Soc.* **2008**, *130*, 6749−6754.
(34) Nasser, F.; Lynch, I. Updating traditional regulatory tests for use with novel materials: Nanomaterial toxicity testing with *Daphnia magna*. *Saf. Sci.* **2019**, *118*, 497−504.
(35) Chen, L.; Fu, X. e.; Zhang, G.; Zeng, Y.; Ren, Z. Influences of Temperature, pH and Turbidity on the Behavioral Responses of *Daphnia magna* and Japanese Medaka (*Oryzias latipes*) in the Biomonitor. *Procedia. Environ. Sci.* **2012**, *13*, 80−86.
(36) Miyamoto, T.; Tsuchiya, K.; Numata, K. Endosome-escaping micelle complexes dually equipped with cell-penetrating and endosome-disrupting peptides for efficient DNA delivery into intact plants. *Nanoscale* **2021**, *13*, 5679−5692.
(37) Numata, K.; Kaplan, D. L. Differences in Cytotoxicity of *β*-Sheet Peptides Originated from Silk and Amyloid *β*. *Macromol. Biosci.* **2011**, *11*, 60−64.
(38) Chua, P.; Lim, W. K. Optimisation of a PC12 cell-based in vitro stroke model for screening neuroprotective agents. *Sci. Rep.* **2021**, *11*, 8096.
(39) Adak, A.; Das, G.; Khan, J.; Mukherjee, N.; Gupta, V.; Mallesh, R.; Ghosh, S. Extracellular Matrix (ECM)-Mimicking Neuroprotective Injectable Sulfo-Functionalized Peptide Hydrogel for Repairing Brain Injury. *ACS Biomater. Sci. Eng.* **2020**, *6*, 2287−2296.
(40) Koch, F.; Möller, A.-M.; Frenz, M.; Pieles, U.; Kuehni-Boghenbor, K.; Mevissen, M. An in vitro toxicity evaluation of gold-, PLLA- and PCL-coated silica nanoparticles in neuronal cells for nanoparticle-assisted laser-tissue soldering. *Toxicol. In Vitro* **2014**, *28*, 990−998.
(41) Manaargadoo-Catin, M.; Ali-Cherif, A.; Pougnas, J.-L.; Perrin, C. Hemolysis by surfactants — A review. *Adv. Colloid Interface Sci.* **2016**, *228*, 1−16.
(42) Yuan, S.; Ji, X.; Ma, M.; Ding, F.; Rao, K.; Wang, Z.; Yang, R.; Liu, Y. Comparative toxicity study of a novel non-ionic surfactant, vanillin ethoxylates, and nonylphenol ethoxylates in Chinese hamster ovary cells in vitro. *J. Environ. Sci.* **2019**, *82*, 70−81.

京都大学 KYOTO UNIVERSITY
A Self-archived copy in
Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
京都大学学術情報リポジトリ KURENAI 紅 Kyoto University Research Information Repository

(43) Inácio, Â. S.; Costa, G. N.; Domingues, N. S.; Santos, M. S.; Moreno, A. J. M.; Vaz, W. L. C.; Vieira, O. V. Mitochondrial Dysfunction Is the Focus of Quaternary Ammonium Surfactant Toxicity to Mammalian Epithelial Cells. *Antimicrob. Agents Chemother.* **2013**, *57*, 2631−2639.
(44) Inácio, Â. S.; Mesquita, K. A.; Baptista, M.; Ramalho-Santos, J.; Vaz, W. L. C.; Vieira, O. V. In Vitro Surfactant Structure-Toxicity Relationships: Implications for Surfactant Use in Sexually Transmitted Infection Prophylaxis and Contraception. *PLoS One* **2011**, *6*, No. e19850.
(45) Chimon, S.; Shaibat, M. A.; Jones, C. R.; Calero, D. C.; Aizezi, B.; Ishii, Y. Evidence of fibril-like beta-sheet structures in a neurotoxic amyloid intermediate of Alzheimer's beta-amyloid. *Nat. Struct. Mol. Biol.* **2007**, *14*, 1157−1164.
(46) Numata, K.; Kaplan, D. L. Mechanisms of enzymatic degradation of amyloid Beta microfibrils generating nanofilaments and nanospheres related to cytotoxicity. *Biochemistry* **2010**, *49*, 3254−3260.
(47) Jaiswal, K. K.; Dutta, S.; Banerjee, I.; Pohrmen, C. B.; Singh, R. K.; Das, H. T.; Dubey, S.; Kumar, V. Impact of aquatic microplastics and nanoplastics pollution on ecological systems and sustainable remediation strategies of biodegradation and photodegradation. *Sci. Total Environ.* **2022**, *806*, No. 151358.
(48) Bher, A.; Cho, Y.; Auras, R. Boosting Degradation of Biodegradable Polymers. *Macromol. Rapid Commun.* **2023**, *44*, No. 2200769.

https://doi.org/10.1021/acs.biomac.3c00160
Biomacromolecules 2023, 24, 2721−2729