Moldable plastics (polycaprolactone) can be acutely toxic to developing zebrafish and activate nuclear receptors in mammalian cells

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Abstract

Popularized on social media, hand-moldable plastics are formed by consumers into tools, trinkets, and dental prosthetics. Despite the anticipated dermal and oral contact, manufacturers share little information with consumers about these materials. Inherent to their function, moldable plastics pose a risk of dermal and oral exposure to unknown leachable substances. We analyzed 12 moldable plastics advertised for modeling and dental applications and determined them to be polycaprolactone (PCL) or thermoplastic polyurethane (TPU). The bioactivities of the most popular brands advertised for modeling applications of each type of polymer were evaluated using a zebrafish embryo bioassay. Both products were sold as microplastic-sized resin pellets. While water-borne exposure to the TPU pellets did not affect the targeted developmental endpoints at any concentration tested, the PCL pellets were acutely toxic above 1 pellet/mL. Aqueous leachates of the PCL pellets demonstrated similar toxicity. Methanolic extracts from the PCL pellets were assayed for their bioactivity using the Attagene FACTORIAL platform. Of the 69 measured endpoints, the extracts activated nuclear receptors and transcription factors for xenobiotic metabolism (pregnane X receptor, PXR), lipid metabolism (peroxisome proliferator-activated receptor γ, PPARγ), and oxidative stress (nuclear factor erythroid 2-related factor 2, NRF2). By non-targeted high-resolution comprehensive two-dimensional gas chromatography (GC×GC-HRT), we tentatively identified several compounds in the methanolic extracts, including PCL oligomers, a phenolic antioxidant, and residues of suspected anti-hydrolysis and crosslinking additives. In a follow-up zebrafish embryo bioassay, because of its stated high purity, biomedical grade PCL was tested to mitigate any confounding effects due to chemical additives in the PCL pellets; it elicited comparable acute toxicity. From these orthogonal and complementary experiments, we suggest that the toxicity was due to oligomers and nanoplastics released from the PCL rather than chemical additives. These results challenge the perceived and assumed inertness of plastics and highlight their multiple sources of toxicity.

Keywords: *nanoplastics, oligomers, polyesters, pollution, biocompatibility, biomaterials*

INTRODUCTION

Moldable plastics frequently trend on social media, showing their versatility in making artistic and practical items. These are pelletized plastics (~3 mm in diameter; microplastic-sized) with low melting temperatures (~60 ºC) advertised as durable and usable modeling materials. Consumers are instructed to melt the pellets by heating them in boiling water for several minutes and then mold the plastic by hand.

Moldable plastics are marketed as non-toxic; however, evidence shows that everyday consumer plastic products leach and expose us to bioactive compounds (e.g., phthalates).[1–6] Concerned consumers query online forums, such as Physics Forum, and product pages for answers, asking whether these materials are toxic and receive little definitive guidance from other users and vendors.[7]

Due to their white color, moldable plastics have also been patented[8] and marketed to consumers for fashioning or securing false or prosthetic teeth (e.g., Instant Smile[9]). As such, this implies short- and potentially long-term oral exposure from something purchased for a positive outcome. Dentists have urged consumers not to use moldable plastics in this way primarily because of potential choking hazards.[10,11] To our knowledge, these products are neither cleared nor listed as medical devices by the United States Food and Drug Administration (FDA).[12] It should be noted that the FDA only approves medical devices, not their materials, i.e., using a material that is part of one approved medical device does not indicate that the material is safe for use in another application.

Hence, we became curious about the potential toxicity of moldable plastic, especially because the plastics' molding process leads to dermal and oral exposure with the potential for increased

risk for additives to leach and migrate when exposed to elevated temperatures. Given that moldable plastics are marketed as non-toxic and perceived to be safe by vendors and consumers, it is prudent to determine their bioactivity (if any), as well as the potential for intentionally or unintentionally added substances to transit from the material. Herein, we purchased commercially available moldable plastic products and evaluated the toxicity of the two most popular brands using a zebrafish developmental bioassay, assessed the bioactivity of their methanolic extracts using the advanced Attagene FACTORIAL platform, and characterized the extracts by high-resolution comprehensive two-dimensional gas chromatography (GC×GC-HRT). Our results indicated that some products exhibit acute toxicity and bioactivity that originated from a mixture of degradation and residual oligomers of the plastic (and less likely chemical additives), conflicting with the presumed biological inertness of these polymers by vendors and consumers.

MATERIALS AND METHODS

Materials included in a survey of moldable plastics on the market

All moldable plastic products were purchased on Amazon.com. Products were selected by searching with combinations of "PCL", "moldable", "dental", "teeth", and "pellet". These terms yielded ~500 results, many of which were redundant. Twelve different products were chosen to have a range of customer ratings, number of reviews and ratings, and various forms (e.g., pellets, sheets, and filaments) (**Table 1**).

Moldable plastics advertised for general purpose included,

- "InstaMorph | Thermoplastic Beads, Meltable Polymorph Pellets | Lightweight Modeling Compound for DIY Crafts, Sculpting, Cosplay Accessories | Temporarily Repair | Six Ounce White" sold by Instamorph,

- “Moldable Plastic Thermoplastic Beads 8OZ, White” sold by JXE JXO,
- "Polly Plastics Heat Moldable Plastic Sheets" sold by Polly Plastics,
- "50g Thermoplastic Models Moldable Low-Melting Polycaprolactone PCL Crystalline Hydrophobic Polyester Polymers Plastic Beads Pellets" sold by PeakCargo HK and branded as Perstorp CAPA 6800 grade PCL on the packaging, and
- "uxcell 3D Pen Filament Refills,16Ft,1.75mm PCL Filament Refills, Dimensional Accuracy +/- 0.02mm, for 3D Printer, White" sold by uxcell.

Moldable plastics advertised for use as oral prosthetics included,

- "Rubie's Costume Co Teeth Pellets" sold by Rubie's,
- “Fitting Beads, 3 Pack Included, Can Be Used for Any Billy Bob Teeth OR Instant Smile Teeth!”, sold by Billy Bob,
- "Imako Cosmetic Teeth Extras (Pink and White Fitting Material)" sold by Imako, "SmileFix Basic Dental Repair Kit - Missing or Broken Tooth. Gaps, Broken Teeth Filled Space Temporary Quick & Safe. Regain Your Confidence and Beautiful Smile in Minutes at Home!" sold by Smile Fix,
- "JJ CARE Temporary Tooth Replacement Kit with Dental Tools, Moldable Thermoplastic Beads Tooth Filler for Gaps, Missing or Broken Tooth, DIY Chipped Tooth Repair Kit for up to 20 Teeth Repair" sold by JJ Care,
- "Brige Temporary Tooth Repair kit for Filling The Missing Broken Tooth and Gaps-Moldable Fake Teeth and Thermal Beads Replacement Kit" sold by Brige, and
- "Temporary Tooth Repair Kits, Dental Repair Denture Repair Beads, Tweezers, Dental Pick, Dental Tools for Temporary Fixing Filling Missing Broken Tooth Moldable Fake Teeth" sold by Waxxy and labeled as J Moldable.

Biomedical-grade polycaprolactone (PCL) (Purasorb PC17; GMP grade homopolymer) was purchased from Sigma Aldrich (Product Number: 900820, Batch Number: MKCN6057). Each plastic was stored at room temperature under ambient conditions.

Polymer identification by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

An IR spectrum of each plastic was collected using an Agilent Cary 630 FTIR with a diamond crystal ATR module, with an average of 32 scans with 2 cm^{-1} resolution. Spectra were processed in Open Specy,[13] applying a linear baseline and first-order smoothing, and assigned polymer identity (Pearson's r > 0.95) based on comparison to the Open Specy database of ~600 spectra, consisting of a range of polymers and materials. Pearson's r statistic was calculated automatically in Open Specy.

Bulk elemental analysis

The bulk elemental carbon, hydrogen, and nitrogen content of the moldable plastic products (samples of 15 mg or more) was measured by Midwest Microlabs (Indianapolis, IN, USA) (**Table 1**). The reported accuracy was ~0.3%, with a minimal detection limit of 0.15% for each element.[14,15]

Morphometric and colorimetric analysis

Individual moldable plastic pellets were illuminated on a tracing board and imaged using a Celestron digital microscope (Product #44308). Images were processed with the National Institutes of Health (NIH) ImageJ (1.53f51) software using the methods of James et al.[16]

previously applied to analyzing images of polyethylene pellets. Several image-based metrics were determined, including the pellet's perimeter, area, circularity, aspect ratio, hue, saturation, and brightness.

Animal husbandry

Adult wild-type AB strain zebrafish (*Danio rerio*) were housed in 10 L tanks in a fish-rearing system (Iwaki Aquatic Systems, Holliston, MA, USA). The fish were held in approximately 2:1 female to male cohorts at a density of 3-4 fish/L in buffered freshwater (475.5 mg/L Instant Ocean, 79.3 mg/L $NaHCO_3$, and 53.8 mg/L $CaSO_4$, pH 7.2.-7.5). The photoperiod was set to a 14:10 h light:dark cycle, and the water temperature was kept at 28.5 °C. The fish were fed twice daily, consisting of live brine shrimp (*Artemia salina*) in the morning and GEMMA Micro 300 micro-pellets (Skretting) in the afternoon. Freshly fertilized eggs were obtained by breeding multiple tanks. Viable embryos were collected, pooled, and maintained at 28-28.5 °C with a 14:10 light-dark cycle in egg water (60 µg/mL Instant Ocean) with a drop of methylene blue. The Woods Hole Oceanographic Institution Animal Care and Use Committee (Assurance D16-00381 from the NIH Office of Laboratory Animal Welfare) approved all experiments.

Static developmental bioassays

The toxicity of the plastics and their leachates were tested using three different configurations of a zebrafish developmental bioassay. For configuration one, embryos were continuously exposed to each plastic starting at ~4 h post-fertilization (hpf) until three days post-fertilization (dpf), unless otherwise noted, in freshly made, sterile filtered (0.2 µm pore size) 10% Hank's embryo medium[17] (10.37 mM NaCl, 0.54 mM KCl, 0.025 mM Na_2HPO_4, 0.044 mM KH_2PO_4, 0.13 mM $CaCl_2$, 0.1 mM $MgSO_4$, 0.42 mM $NaHCO_3$, pH 7.2). Treatments included Instamorph and JXE JXO pellets, as

well as biomedical-grade PCL. Embryos were evaluated daily for mortality. For configuration two, embryos were continuously exposed to 4 Instamorph pellets/mL starting at ~4, 24, and 48 hpf in freshly made, sterile filtered 10% Hank's embryo medium. After 24 h of exposure, embryos were assessed for mortality. The time points for starting exposure were selected because they correspond to different stages of zebrafish embryo development, 4 hpf being the segmentation period, 24 hpf being the pharyngula period, and 48 hpf being the hatching period. For configuration three, embryos were continuously exposed to leachates prepared from Instamorph pellets or pre-leached Instamorph pellets starting at ~4 hpf in freshly made, sterile filtered 10% Hank's embryo medium. Leachates were prepared immediately before the exposure experiment by leaching Instamorph pellets for 24 h at room temperature in freshly made, sterile filtered 10% Hanks embryo medium with 4 pellets/mL. After leaching, the pellets were collected and used as pre-leached pellets. Embryos were evaluated daily for mortality. In all configurations, viable AB strain zebrafish embryos were used, untreated embryos were used as a control treatment, and each replicate had ten embryos in 5 mL of medium maintained in 60 mm diameter combusted borosilicate glass Petri dishes at 28 ± 0.5°C.

Solvent extracts

Three different solvent extracts were prepared by incubating three sets of 10 Instamorph pellets in 5 mL analytical grade methanol (~30 mg/mL) for 24 h at room temperature in combusted borosilicate glass vials with PTFE/F217 lined caps. Methanol was chosen because it can extract polar compounds[1,2,5] without dissolving PCL. After extraction, half of the extracts (2.5 mL) were evaporated under a gentle stream of nitrogen at room temperature and reconstituted in 100 µL of molecular biology grade dimethyl sulfoxide (DMSO) for high-throughput screening bioassays. Additionally, 1.5 mL of an extract was exposed to a gentle stream of nitrogen at room temperature until dryness and reconstituted in 100 µL analytical grade dichloromethane (DCM) for non-

targeted analyses by GC×GC. An extraction blank without plastic was also prepared for the bioassays and the GC analyses. Specifics of each extract are provided in **Table S1**.

High-throughput screening bioassays

DMSO-reconstituted methanolic extracts were shipped to Attagene, Inc. (Morrisville, NC, USA) for testing by their TF-FACTORIAL (45 TF specific reporters) and NR-FACTORIAL (24 human NRs) assays (previously named cis- and trans- FACTORIAL assays, respectively).[18,19] The assays use HepG2 cells to assess the activity of endogenous transcription factors (TF) or transfected hybrid proteins consisting of a yeast GAL4 DNA binding domain and ligand-binding domain of the human nuclear receptors (NR). These multiplexed assays comprised 69 measured endpoints (**Table S2**) related to cell stress, endocrine activity, growth and differentiation, immunity, and lipid, xenobiotic, and general metabolism. Extracts were tested at a single concentration (3 µL DMSO extract/mL cell culture medium) for 24 h for the NR-FACTORIAL assay at three concentrations (1, 3, and 9 µL DMSO extract/mL cell culture medium) for 24 h for the TF-FACTORIAL assay. The TF-FACTORIAL assay was repeated twice at the midpoint concentration. Final DMSO concentrations were 0.1-0.9% (v/v), depending on the concentration of extract used in the assay. Three to six technical replicates of DMSO solvent controls matched to the DMSO concentration of the extracts were run with each sample set. Each extract was run as three technical replicates in Dulbecco's Modified Eagle Medium (DMEM) containing 1% charcoal-stripped fetal bovine serum (FBS). Reporter RNA was isolated, amplified by reverse-transcription polymerase chain reaction (RT-PCR), labeled with fluorescent markers, and quantitively assayed by capillary electrophoresis. Bioassay responses were expressed as fold-induction relative to the DMSO control by dividing the treated cells' average technical replicate expression by the average technical replicate expression of the appropriate DMSO control. Additional details of the bioassays are provided in Blackwell et al.[20]

Non-targeted comprehensive two-dimensional gas chromatography (GC×GC)

Because the three methanolic extracts were each prepared from a random composite of 10 pellets of the same material (Instamorph pellets), as a representative sample, only one DCM-reconstituted methanolic extract (sample one, **Table S1**) was analyzed by GC×GC. The extraction blank was analyzed as well. Samples were analyzed by GC×GC-FID and GC×GC-HRT using published methods[14,21–24] routine to the Organic Geochemistry Analysis Laboratory - GC×GC Facility at the Woods Hole Oceanographic Institution. Chromatographic peaks were tentatively identified based on mass spectral matches (above 80% similarity; NIST/EPA/NIH 20 Mass Spectral Library) and mass spectral interpretation.[23] See the **Supporting Information** for complete methods.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 10.1.0 (264). Data are presented as the mean ± standard deviation (n = replication). Groups were considered significantly different for a p value less than 0.05. Sample sizes and statistical tests are included in the text and figure captions where appropriate. Data evaluated by ANOVA satisfied normality and variance assumptions as determined by the D'Agostino-Pearson omnibus test for normality of the residuals and the Brown-Forsythe test for homoscedasticity.

RESULTS

Details on moldable plastics were scant and non-specific

We purchased 12 moldable plastics from Amazon.com that were advertised for modeling and dental applications (**Table 1**). We reviewed each plastic's product page on Amazon.com and the vendor website (if applicable) for details about the plastic. Descriptions and information on the polymers were limited. Many of the plastics were described in vague and generic terms such as "polyester", "white beads", "thermoplastic polymer", "shapeable resin", and "thermoplastic beads". Only a few products had readily available safety data sheets (SDS) that were accessible to download on the product page or vendor's website. None of the plastics advertised for dental applications had SDSs. According to the few available SDSs, the materials were PCL.[25–28] Additionally, PCL is listed as the preferred embodiment material in the patent describing the use of moldable plastics to fashion dental prosthetics.[8] Therefore, we initially assumed that all moldable plastics on the market were PCL. According to reviews, customers also believed that these materials were PCL. However, they noted differences between products. For instance, one reviewer stated that, when melted, JXE JXO plastic was "stickier" than Instamorph plastic,[29] suggesting that it might be a different polymer.

The moldable plastics ranged in shape and color. The majority of moldable plastic products were ellipsoid resin pellets. Visually, the pellets were indistinguishable from product to product (**Figure S1**). One product was a thick sheet (Polly Plastic), and another was a filament (uxcell). All the products were opaque and white. However, upon detailed quantitative inspection by optical microscopy, morphometric and colorimetric differences were detected amongst the pellets in their projected perimeter and area, circularity and aspect ratio, and hue, saturation, and brightness (**Figures S2-S8**).

Consumer-grade moldable plastics were polycaprolactone (PCL) or thermoplastic polyurethane (TPU)

The polymer type for each moldable plastic was determined by IR spectroscopy. Five plastics were identified as PCL based on spectral matching to reference spectra (Pearson's r >0.96) (**Figures S9-S13**). The remaining seven plastics had IR spectra that were inconsistent with PCL (**Figure S14**) and instead matched reference spectra of thermoplastic polyurethane (TPU) (Pearson's r >0.95) (**Figures S15-S21**). For example, the IR spectra of these samples had a weak νNH stretching vibration at ~3350 cm^{-1}, a shouldering amide I band at ~1685 cm^{-1}, an amide II band at ~1530 cm^{-1}, and νC–O and νC–O–C vibrations at ~1310 cm^{-1} and ~1260 cm^{-1}, respectively, peaks characteristic of polyurethanes.[30] The carbon, hydrogen, and nitrogen content of selected plastics provided additional support to the TPU identification. Those identified as TPU by IR spectroscopy contained bulk nitrogen and had ratios of H/C less than expected for PCL (**Table 1**).

Reanalyzing the morphometrics and colorimetrics with respect to polymer type instead of product identified features that distinguished PCL and TPU moldable plastic pellets from one another (**Figures S22-S28**). Notably, the combination of a pellet's aspect ratio and brightness robustly discriminated whether the pellet was PCL or TPU (**Figure S29**). Presumably, the subtle differences in these features result from the properties and processing of the two types of plastic.

Three modeling plastics accurately reported their polymer type (**Table 1**). Only one of the plastics advertised for oral prosthetics specified their polymer type (**Table 1**). Two plastics, including one sold for dental applications, were incorrectly specified as PCL by the manufacturer; these

misreported plastics were determined to be TPU (**Figures S16**, **S18**). We suspect that the products identified as TPU are polycaprolactone-based TPUs in which a polycaprolactone polyol was used as the chain extender or soft segment in the TPU. This may explain, to some extent, the misreporting and incomplete reporting of the materials underlying these products. Regardless, without detailed chemical analysis, distinguishing whether a product is TPU or PCL is infeasible, leaving consumers largely uninformed about the materials they are buying and potentially putting in their bodies.

Table 1. Survey of several moldable plastics on the market.

Product[a]	Use	Form	# of Ratings[b]	SDS Readily Available	Bulk Elemental Analysis %C	%H	%N	H/C[c]	IR Polymer Assignment[d]	Polymer Specified by Vendor
Instamorph	Consumer	Pellet	12250	Yes	63.96	8.93	0.00	1.66	PCL (S9)	PCL
JXE JXO	Consumer	Pellet	4638	No	60.06	7.83	0.70	1.55	TPU (S15)	Unspecified
uxcell	Consumer	Filament	5	No	61.16	8.06	1.16	1.57	TPU (S16)	PCL
Polly Plastic	Consumer	Sheet	3359	Yes	Not measured				PCL (S11)	PCL
Perstorp	Consumer	Pellet	2	Yes	Not measured				PCL (S10)	PCL
Rubies	Dental	Pellet	562	No	63.77	9.02	0.55	1.69	PCL (S13)	Unspecified
InstantSmile	Dental	Pellet	9977	No	61.20	8.08	1.05	1.57	TPU (S17)	Unspecified
Imako	Dental	Pellet	563	No	64.06	9.00	0.49	1.67	PCL (S12)	Unspecified
SmileFix	Dental	Pellet	699	No	61.31	8.10	1.39	1.57	TPU (S18)	PCL
JJ Care	Dental	Pellet	34	No	61.23	8.08	2.07	1.57	TPU (S19)	Unspecified
Brige	Dental	Pellet	2414	No	Not measured				TPU (S20)	Unspecified
J Moldable	Dental	Pellet	115	No	Not measured				TPU (S21)	Unspecified

[a]Those in bold were tested for toxicity and bioactivity.
[b]On Amazon.com as of 6/29/2023
[c]The theoretical value of H/C for PCL is 1.67; Purasorb PC17 (biomedical-grade PCL) was used as a PCL standard and had a value for H/C of 1.68. Pure PCL is expected to be devoid of N.
[d]Text in parentheses indicates the figure number of the product's IR spectrum.

Consumer-grade PCL can be acutely toxic to developing zebrafish

We evaluated the potential toxicity of two moldable plastic products sold on Amazon.com (JXE JXO and Instamorph pellets) by directly exposing zebrafish embryos to them. These products were selected because they were the most popular consumer moldable plastics included in our survey, and consumers mentioned using them for dental applications in their reviews. For clarity in the subsequent sections, the Instamorph and JXE JXO pellets will be referred to as consumer-grade PCL and TPU, respectively.

No mortality was observed for embryos exposed to ~60 mg/mL (4 pellets/mL) of consumer-grade TPU. Conversely, ~60% of embryos perished within 24 h of continuous exposure to the same concentration of consumer-grade PCL (**Figure 1A**). No changes in mortality for the consumer-grade TPU-treated embryos were observed for the remainder of the exposure experiment (through 72 hpf). Given the significant acute toxicity caused by the consumer-grade PCL and the lack of acute toxicity caused by the consumer-grade TPU, we focused our investigation on the consumer-grade PCL. No further experiments were conducted with the consumer-grade TPU. Presumably, the other TPU-based moldable plastics will not cause acute toxicity to zebrafish embryos based on the results for JXE JXO moldable plastics. Nonetheless, this possibility does not dismiss their potential to elicit bioactivity and cause sublethal effects, as polyurethanes have been shown to leach bioactive compounds.[1–3] Further evaluation of TPU-based moldable plastics, particularly those sold for dental applications, is warranted.

We completed several additional exposure experiments using the consumer-grade PCL to determine its toxicity to developing zebrafish in more detail. Embryo mortality followed a sigmoidal-like concentration dependence with an LC_{50} of ~30 mg/mL (2 pellets/mL) (**Figure 1B**). There was no statistical difference in embryo susceptibility to the consumer-grade PCL when

exposure to ~60 mg/mL (4 pellets/mL) began at 4, 24, or 48 hpf (**Figure 1C**). A replicated independent experiment at the LC_{50} concentration affirmed the observed acute toxicity for the consumer-grade PCL (**Figure S30**).

Despite the LC_{50} for consumer-grade PCL being well above the concentration of plastic found in natural waters (~10s ng/L to ~100s mg/L; <100 particles/L),[31–35] the plastic's acute toxicity raises concern. Mortality of zebrafish embryos from exposure to relatively large pieces of plastic compared to the size of an embryo is rare. For instance, toxicity studies using zebrafish embryos are often conducted in polystyrene well plates because of the material's apparent inertness. Similarly, in an experiment complementary to those presented here, we observed no acute toxicity to zebrafish embryos upon exposure to polyethylene pellets (data unpublished).

Only in a few instances have plastic items been acutely toxic to zebrafish embryos, and in these cases, toxicity was attributed to the release of residual acrylate monomer or surfactant.[36,37] We hypothesized that the consumer-grade PCL was releasing some toxicant(s). To test this, we leached 4 pellets/mL of the consumer-grade PCL for 24 h at room temperature in zebrafish embryo medium and exposed embryos to the resulting leachate. The leachate was slightly more toxic than direct exposure to the pellets and displayed less variability (**Figure 1D**). We also exposed embryos to the pellets used to prepare the leachate ("pre-leached"). Mortality was delayed somewhat for embryos exposed to these pellets, i.e., pre-leaching the pellets appeared to reduce the toxicity of the consumer-grade PCL, at least initially.

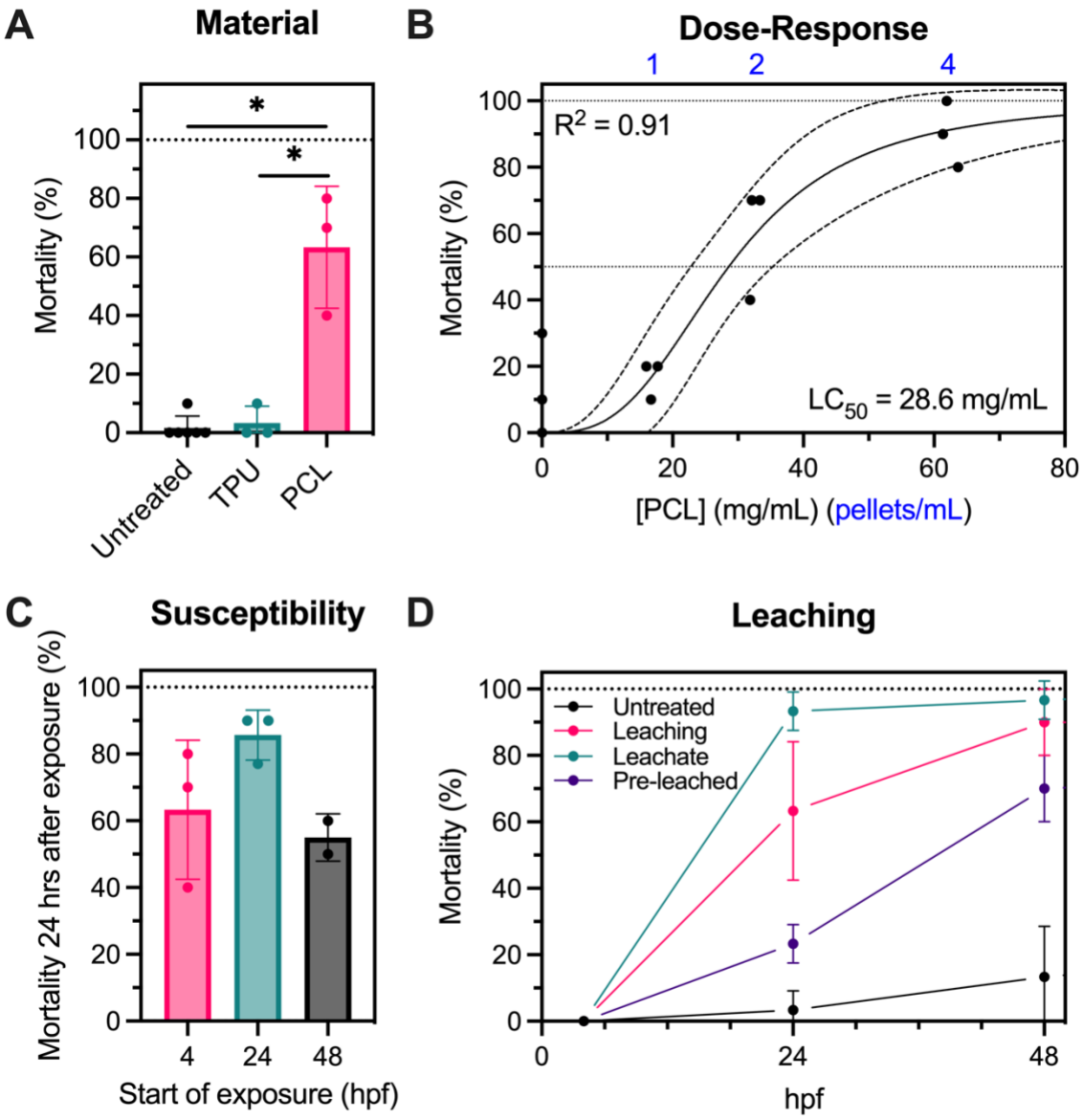

Figure 1. (**A**) Mortality of zebrafish embryos at 24 hpf after a 20 h exposure to 4 pellets/mL of consumer-grade PCL or TPU. Exposures of each material were conducted independently with their own untreated controls. In the figure, the untreated condition presents data combined from both exposures. Statistical differences were determined by Welch's t-test. * corresponds to a *p* value <0.05. (**B**) Dose-response relationship for mortality of zebrafish embryos continuously exposed to consumer-grade PCL from 4-48 hpf. Data were fit to a two-parameter normalized Hill equation, $(\text{Mortality }(\%) = \frac{100}{1+\left(\frac{EC_{50}}{[PCL]}\right)^{n}})$. Residuals were normally distributed and homoscedastic. Dashed lines indicate 95% confidence intervals. (**C**) Mortality of zebrafish embryos after 24 h of exposure to 4 pellets/mL of consumer-grade PCL starting at different stages of development. Treatments were not statistically different as determined by an ordinary one-way ANOVA with Tukey's test for multiple comparisons. (**D**) Mortality of zebrafish embryos exposed to consumer-grade PCL leachate, 4 pellets/mL (leaching), or 4 pre-leached pellets/mL. Each treatment was assessed using three biological replicates unless otherwise noted.

Bioactivity and characterization of consumer-grade PCL-associated chemicals

In parallel to the zebrafish embryo bioassays, we prepared methanolic extracts of the consumer-grade PCL to screen the bioactivity and composition of plastic-associated chemicals using high-throughput *in vitro* bioassays and non-targeted GC×GC analyses, respectively. Methanolic extracts from consumer plastics have proved instructive for assessing the toxic potential of leachable plastic-associated chemicals.[1,3] Additionally, analyses of solvent extractable material are routine parts of food-contact and medical device regulatory frameworks.[38] Blank-corrected methanolic extractable mass for the consumer-grade PCL was 6.59 ± 4.16 mg/g PCL (n=3). The variability in extractable content (coefficient of variation = ~63%) provides a possible explanation for some of the variability observed in the zebrafish bioassays. Due to pellet variability, replicates with lower mortality could have been exposed to pellets with less leachable content and vice-versa.

High-throughput in vitro bioassays.

Unlike previous studies of consumer plastics, which used single-target reporter assays,[1–3,5] we took an unbiased approach to identify potential sources of toxicity. We used TF-FACTORIAL and NR-FACTORIAL *in vitro* bioassays, which measure the activation of 45 human transcription factor response elements and 24 nuclear receptors, respectively.[19] With these assays, specific biological responses yield unique bioassay profiles that can be used to identify potential modes of action.[39] Of the 69 endpoints measured in the bioassays, only five were activated within the range of concentrations tested. The activities of all other response elements and receptors were well below an operationally defined 1.5 fold-induction cut-off. Results were consistent across three extracts prepared from three independent sets of plastic. All activities of the extraction blank were below the induction cut-off (**Figure S31**).

The five endpoints that were activated by the extracts included, the TF and NR endpoints for the pregnane X receptor (PXR/PXRE), the TF and NR endpoints for the peroxisome proliferator activated receptor γ (PPARγ/PPRE), and the only endpoint for the nuclear factor erythroid 2-related factor 2 (NRF2) (**Figure 2**, **Tables S3-S4**). The dose-response relationship of PXRE appeared to follow a bell shape, being more stimulatory at lower concentrations than at higher concentrations of the extract (**Figure 2B**, **Table S4**). The dose response of PPRE and NRF2 appeared sigmoidal within the range of concentrations tested (**Figure 2C-D**, **Table S4**). The elevated activity of PXR/PXRE and PPARγ/PPRE in both TF and NR assays suggested that active components of the extracts acted as direct ligands of PXR and PPARγ.

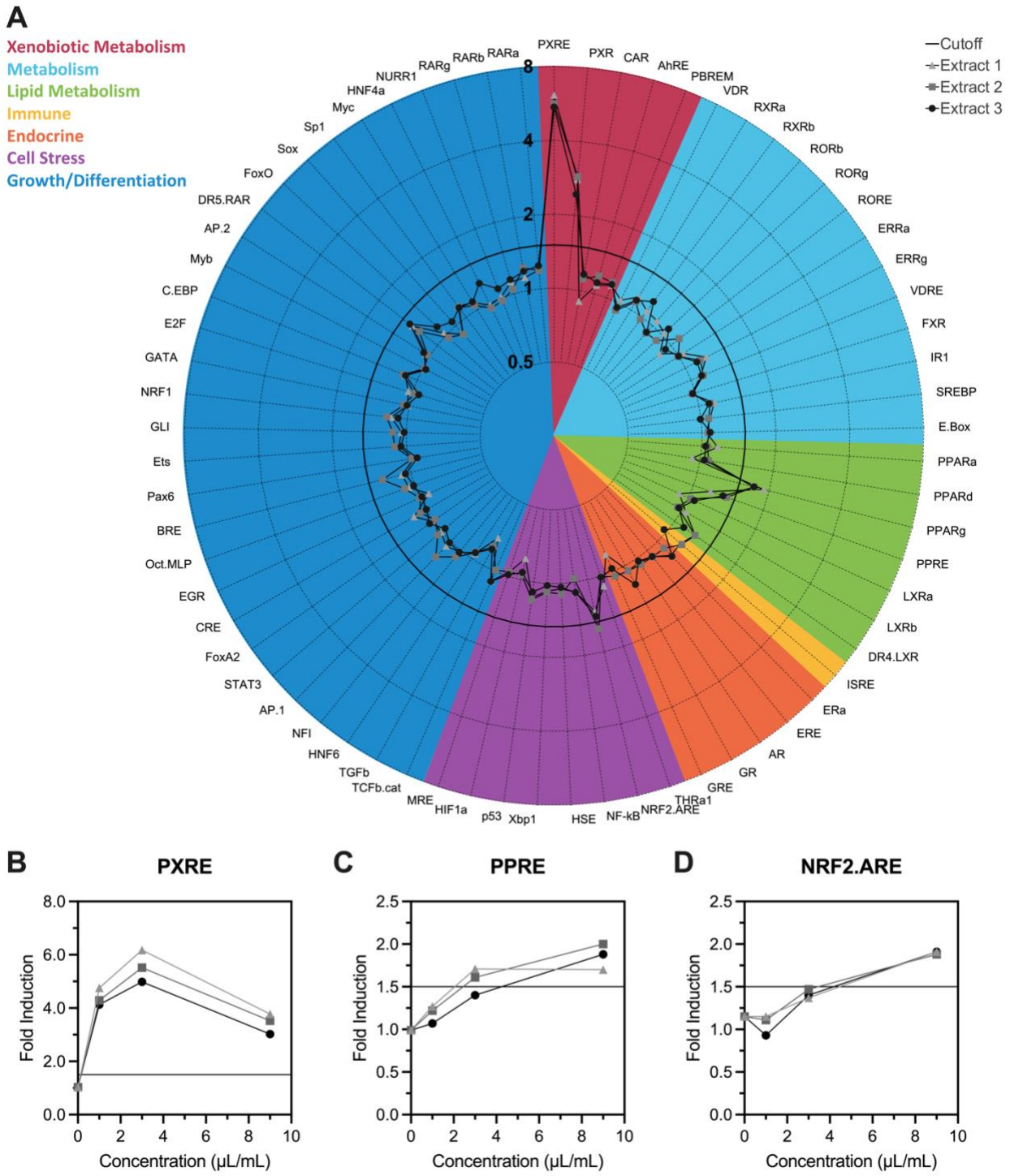

Figure 2. TF-FACTORIAL and NR-FACTORIAL endpoints for consumer-grade PCL methanolic extracts assayed at 3 µL DMSO reconstituted extract/mL cell culture medium (A). Fold induction of all 45 human transcription factor response elements and 24 nuclear receptors tested for activity in the bioassays are included in **Table S3**. Endpoints were grouped and color-coded by biological role.[20] Dose-response relationships of the three extracts for PXRE (B), PPRE (C), and NRF2.ARE (D) at concentrations of 1-9 µL DMSO reconstituted extract/mL cell culture medium. The values at zero concentration were those of the extraction blank (n=1).

Non-targeted GC×GC analyses.

GC×GC-HRT was used to gauge the relative abundance and tentatively identify molecules in the methanol extract.[23] This approach chromatographically separates components relative to their vapor pressure and polarity, yielding ordered two-dimensional chromatograms with a high-resolution mass spectrum for each peak.[22] The GC×GC-HRT chromatogram of the methanol extract contained 11 peaks that can broadly be binned into two elution windows. Peaks 1 through 6 were grouped in a narrow band with limited retention in both dimensions. Peaks 7 through 11 eluted along a wide range of retention times.

Peaks 1 and 2 were tentatively identified as 2,6-diisopropylphenyl isocyanate (Peak 1; **Figure 3**, **Figure S32**; CAS# 28178-42-9) and 2,6-diisopropylaniline (Peak 2; **Figure 3**, **Figure S33**; CAS# 24544-04-5). Peaks 5 and 6 shared spectral features with Peaks 1 and 2 (e.g., fragments indicative of a 2,6-diisopropylphenyl unit) and shared fragments indicative of amide bonding (**Figures S36-S37**). The tentative occurrence of isocyanates, anilines, and amides on a 2,6-diisopropylphenyl structural unit likely indicates that these relate to bis(2,6-diisopropylphenyl)carbodiimide (CAS# 2162-74-5),[40] an anti-hydrolysis additive used for polyester stabilization.[41,42] Carbodiimides react with carboxylic acids to form *N*-acylureas that can fragment into amides and isocyanates at elevated temperatures (such as during melt processing).[40] In particular, the absence of bis(2,6-diisopropylphenyl)carbodiimide and the presence of 2,6-diisopropylphenyl isocyanate and 2,6-diisopropylaniline agrees with previous reports on the presence of the compound in plastic leachates.[43] Peak 3 was tentatively identified as methyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (**Figure 3**, **Figures S34**; CAS# 41088-52-2). Cycloaliphatic epoxides are commonly used as hardeners/crosslinkers with PCL.[44–46] Peak 9 was tentatively identified as 4,4′-butylidenebis(3-methyl-6-*t*-butylphenol) (**Figure 3**, **Figure S40**; CAS# 85-60-9), a phenolic antioxidant used to prevent thermal degradation during melt processing of

polymers (tradename Santowhite[47]). The remaining five peaks (peaks 4, 7, 8, 10, and 11; **Figure 3**, **Figures S35, S38-S39, S41-S42**) were tentatively identified as PCL oligomers owing to their base ion of m/z 115.071 ($C_6H_{11}O_2^+$) and regular addition of m/z 114 with later eluting peaks.[48] Additionally, these peaks formed a "fairway" in the GC×GC chromatogram, a typical chromatographic feature for compounds of the same class with increasing molecular weight.[22] These results reinforce that plastics are not exclusively single compounds but are diverse, complex mixtures of many known and unknown compounds.[4,49]

Previous work has shown that extractables from commercially produced PCL can include ε-caprolactone (CAS# 502-44-3), 6-hydroxyhexanoic acid (CAS# 1191-25-9), and phthalates (unspecified).[50,51] One of the most conventional synthesis routes of PCL uses stannous 2-ethylhexanoate (CAS# 301-10-0) as a catalyst for the ring-opening polymerization of ε-caprolactone.[52] Residual ε-caprolactone, 6-hydroxyhexanoic acid, 2-ethylhexanoic acid (CAS# 149-57-5), and phthalates were not detected.

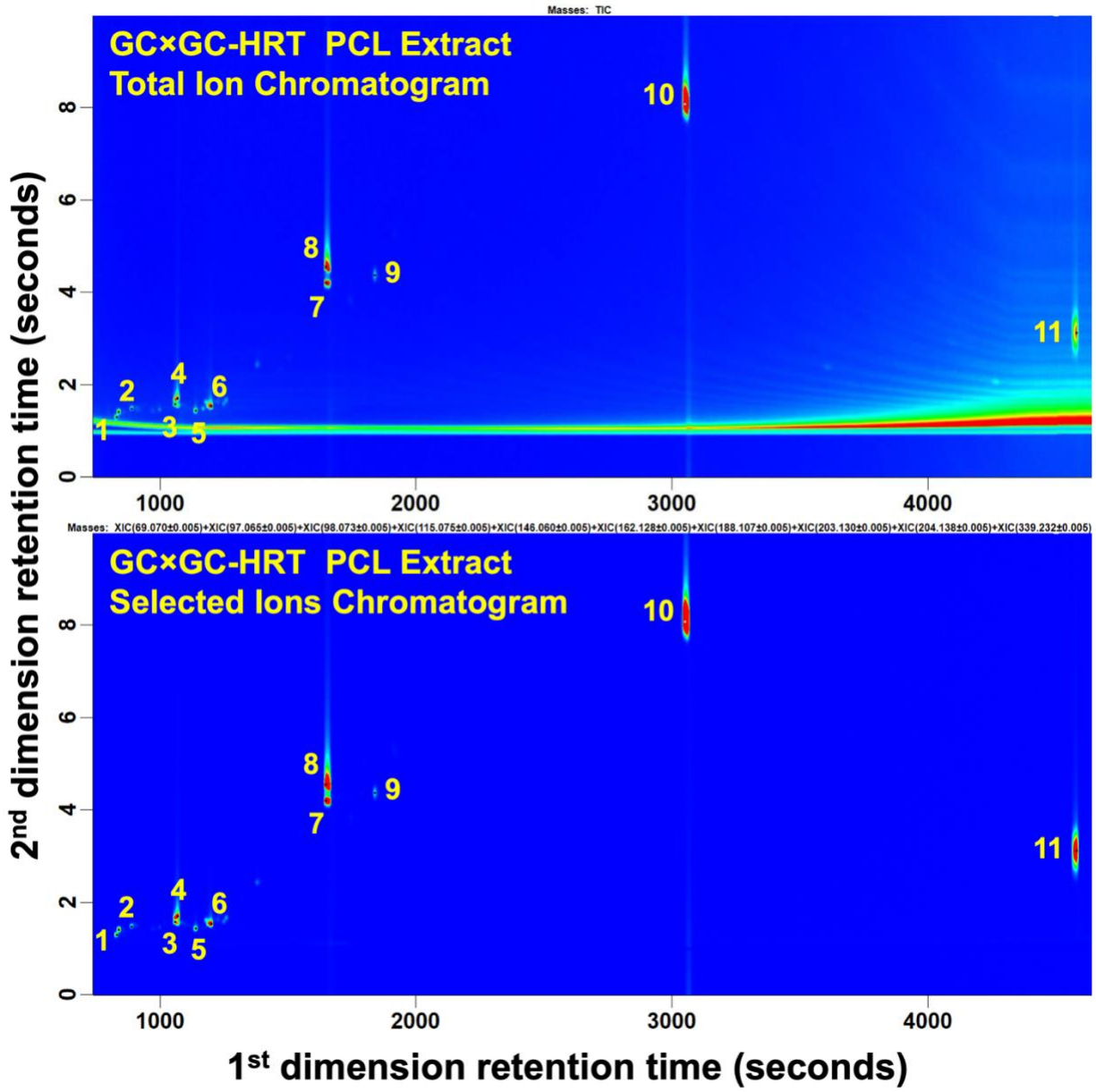

Figure 3. GC×GC-HRT total ion (top) and selected ion (bottom) chromatograms. Selected ions included m/z 97.065, 98.073, 115.075, 146.060, 162.128, 188.107, 203.130, 204.138, and 339.232. A high resolution mass spectrum for each peak are included in the **Supporting Information**.

High purity biomedical-grade PCL was acutely toxic to zebrafish embryos.

Given the presence of several concerning compounds and those unidentified in the consumer-grade PCL extracts, we hypothesized that the acute toxicity of the consumer-grade PCL to developing zebrafish embryos could be due to these impurities and additives. In a follow-up experiment, we tested biomedical grade PCL for its toxicity to zebrafish embryos in an effort to reduce any confounding effects from leachable chemical additives. Because residual tin catalysts can reduce the biocompatibility of PCL-based biomedical implants,[53] biomedical-grade PCL is purified to reduce residual tin below 50 ppm.[53] Purification presumably also removes residual catalysts and other non-intentionally added substances. If the acute toxicity persisted for this material, it would suggest that components intrinsic to the polymer, i.e., nanoplastics and oligomers, were more likely the cause of toxicity than chemical additives. To test this, we evaluated the toxicity of a commercially available biomedical-grade PCL certified to have residual tin content of 18 ppm, residual monomer content ≤0.5%, and other elemental impurities ≤10 ppm by the United States Pharmacopeial method 232. Dosed at the greatest plastic concentration for consumer-grade PCL tested (~60 mg/mL), we observed an ~80% mortality of zebrafish embryos within 3 dpf when directly exposed to biomedical-grade PCL (**Figure 4**). These results indicated that the observed acute toxicity for PCL was unlikely to be from a chemical additive, residual catalyst, or non-intentionally added substance and suggest that polymer breakdown products (i.e., nanoplastics and oligomers) were the source of the toxicity.

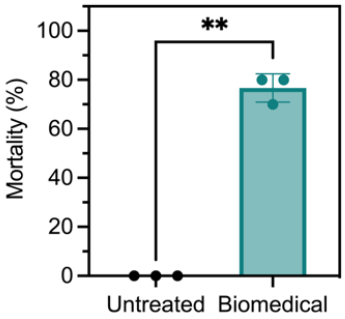

Figure 4. Mortality of zebrafish embryos at 72 hpf after ~3 days of exposure to biomedical-grade PCL (~60 mg/mL). Statistical significance was determined by an unpaired Welch's t test. ** corresponds to a *p* value <0.01.

DISCUSSION

Potential explanations for the acute toxicity of consumer-grade PCL to early developing zebrafish

Additives.

Acute toxicity from plastic items is often attributed to the leaching of toxic additives, which include non-intentionally added substances such as reaction by-products and breakdown products.[49] One value of the TF-FACTORIAL and NR-FACTORIAL platforms is that they are part of the U.S. Environmental Protection Agency (EPA) ToxCast program[54] and have been used to screen >3500 compounds, of which a significant portion are also part of the multi-agency Tox21 program.[55] This extensive database presumably enables these platforms to help narrow the number of compounds potentially responsible for toxicity in a complex mixture. As a first pass, we compared the results of the bioassays to those available on the U.S EPA CompTox dashboard[56] for compounds reported to occur in PCL formulations and those tentatively identified by GC×GC in the consumer-grade PCL.

The dashboard indicated that several compounds reported to occur in PCL formulations i.e., ε-caprolactone,[57] 2-ethylhexanoic acid,[58] and common phthalates[59–62] were not PXR activators, or their bioassay profiles did not match those of the consumer-grade PCL extracts. Nonetheless, common phthalates can activate PPAR,[63] suggesting that they may contribute to the PPARγ activity. Several common phthalates (e.g., benzyl butyl phthalate (CAS# 85-68-7)[64] and di(2-ethylhexyl) phthalate (CAS# 117-81-7)[60]) also elicit estrogen or androgen nuclear receptor activity, which was not observed for the extracts (**Figure 2**). These phthalates tend to be orders of magnitude more acutely toxic (benzyl butyl phthalate, EC_{50} = ~50 ng/mL[65]) to developing zebrafish embryos than those without sex hormone activity (e.g., di-*n*-octyl phthalate (CAS# 117-84-0)[66], EC_{50} = ~150 µg/mL[65]), suggesting that any phthalates associated with the PCL are those that are less acutely toxic. Additionally, phthalates minimally leach from PCL in aqueous media, further limiting their potential as the source of harm, and phthalates were not detected by the GC analyses.[50] Collectively, these arguments suggest that it is unlikely that phthalates were the source of acute toxicity for the zebrafish embryos following exposure to consumer-grade PCL.

There was no bioactivity data in the dashboard for 6-hydroxyhexanoic acid, the end hydrolysis product of PCL and an endogenous metabolite.[67,68] Given that PCL continuously releases 6-hydroxyhexanoic acid as it degrades, the contribution of this compound to the observed toxicity cannot be ruled out (Microtox assay EC_{50} = 120 µM).[69,70] However, degradation into oligomers precedes monomers, likely limiting any significant formation of 6-hydroxyhexanoic acid within the timescale of our experiments. This point is supported by the chemical analyses, which did not detect it.

According to the dashboard, 2,6-diisopropylaniline can elicit activity of PXR and RXRβ.[71] Notably, 2,6-diisopropylaniline has been shown to cause adverse bioactivity.[72] As for 2,6-diisopropylphenyl isocyanate, methyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate, 4,4'-butane-1,1-diylbis(2-*t*-butyl-5-methylphenol), and the other tentatively identified compounds, the dashboard indicated these compounds had not been analyzed by the FACTORIAL bioassays.[73–76] 4,4'-butane-1,1-diylbis(2-*t*-butyl-5-methylphenol) had been screened as part of the Tox21 program, which provided evidence for the activity of several pathways not activated in the FACTORIAL bioassays, discounting its contribution to the observed toxicity as well.[73]

Toxicity data on 2,6-diisopropylphenyl isocyanate, methyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate, and bis(2,6-diisopropylphenyl)carbodiimide were severely limited.[75–77] For instance, there were no bioactivity records on PubChem and the U.S. EPA CompTox dashboard for methyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate.[75] Some carbodiimides used in PCL have been shown to be toxic to *Daphnia magna* at concentrations of ~4-8 µM; though, bis(2,6-diisopropylphenyl)carbodiimide was not tested.[78] Additionally, this compound has been approved as an additive for plastics used in food-contact applications, indicating to an extent its perceived level of hazardousness.[43]

Searching other chemical databases for the potential compounds associated with PCL yielded some additional insights. Comparison to the PlastChem database revealed that the majority of compounds known to occur in PCL formulations and tentatively identified compounds in the extract are on the database's red list of chemicals that are not regulated internationally and considered hazardous by at least one criteria.[79] Further comparison of the FACTORIAL bioassay results to the Attagene database of 6000+ compounds yielded no hits with similarity scores greater than 0.75 (**Table S5**), suggesting the bioactivity was derived from compound(s) not in that

database or from a mixture of compounds that produced a unique response pattern in the FACTORIAL bioassays.

Nanoplastics and oligomers.

It is well recognized that PCL undergoes hydrolytic degradation and consequently releases breakdown products,[80–83] implying that breakdown products could be the dominating source of the observed toxicity and bioactivity. As with nanoplastics, oligomers are increasingly being recognized as chemicals of concern for human health and the environment.[84] Tamayo-Belda et al.[85] demonstrated that consumer-grade PCL pellets shed appreciable quantities of nanoplastics (1.7±0.1 mg/g pellet; $2 \cdot 10^9$ particles/g pellet; mean diameter 67±15 nm), small microplastics (0.7±0.2 mg/g pellet; diameter 100-1000 nm), and linear and cyclic oligomers (0.3±0.1 mg/g pellet) within 1 day of incubation in 1 mM potassium phosphate buffer at pH 7 and ~28 ºC. Hydrolytic degradation of the amorphous phase of PCL was proposed to cause the fragmentation and release of nanoplastics and oligomers from the macroscopic material. Yoshinaga et al.[86] showed that short (degree of polymerization ~4) PCL oligomers and 6-hydroxyhexanoic acid can exhibit adverse effects on freshwater microorganisms (1 µg/mL), marine algae (1 mg/mL), and mammalian cells (1 mg/mL). In contrast, longer oligomers and bulk PCL had no effect at the same concentrations, which were concentrations lower than those used in our study. Reisman et al. reported a TD_{50} for 6-hydroxyhexanoic acid of ~23 mg/mL for immortalized mammalian fibroblasts.[87] Similarly, Tamayo-Belda et al.[54] showed that PCL degradation products from PCL pellets adversely affected two freshwater cyanobacteria. These studies and our tentative identification of PCL oligomers reinforce the idea that these compounds and other degradation products contributed to the observed toxicity.

Luis et al.[88] showed that synthesized PCL nanoparticles (mean diameter 329 nm) were acutely toxic to zebrafish embryos with LC_{50} of 168.9 µg/mL at 96 hpf.[57] Based on these data in the literature, assuming the consumer-grade PCL pellets used in our study released comparable quantities of material, the estimated concentrations of potentially shed nanoplastics, small microplastics, and oligomers at the LC_{50} for consumer-grade PCL were ~50 µg/mL (~$6{\cdot}10^7$ particles/mL), ~20 µg/mL, and ~10 µg/mL, respectively. The value of 50 µg/mL (~$6{\cdot}10^7$ particles/mL) is comparable to the LC_{50} for PCL nanoparticles established by Luis et al. and is likely an underestimate because our exposure conditions were saltier and slightly more basic than those of Tamayo-Belda et al., which can increase PCL degradation.[89] Additionally, nanoparticle biological activity generally increases with decreasing particle size,[90–92] suggesting that the LC_{50} for shed nanoplastics may be lower than the LC_{50} for the synthesized PCL nanoparticles prepared by Luis et al.

PXR is touted as a master xenobiotic receptor that is activated by a wide variety of structurally diverse compounds, so its activation in the FACTORIAL bioassays was not surprising. Its measured activity further supports the idea that the toxicity to zebrafish embryos was caused by shed nanoplastics and oligomers. While PXR activity can be challenging to interpret, owing to the receptor's ligand binding promiscuity,[93] predictive models of PXR ligands have found that ester groups can be potent activators of human PXR.[94] Because PCL is a polyester, this suggests that PCL breakdown products (i.e., nanoplastics and oligomers) could be the source of bioactivity in the *in vitro* bioassays. The tentative identification of PCL oligomers in the methanolic extracts supports this idea. Because ligands for human and zebrafish PXR are not wholly identical,[95] the activation of zebrafish PXR by PCL and its degradation products will require further investigation. Nonetheless, PXR activity in rats and zebrafish has been reported upon nanoparticle exposure,[96–98] hinting that PXR may participate in nanoplastic toxicity. The co-activation of PPARγ suggests

that the PCL oligomers might perturb lipid metabolism. The activation of NRF2 suggests that the PCL extracts contained products capable of causing oxidative stress. The oxidative stress response is highly conserved in vertebrates.[99] Embryonic development involves precisely regulated changes in cellular redox balance, and thus, developing embryos are susceptible to chemicals that disrupt redox homeostasis.[100] Numerous studies report evidence of oxidative stress from exposure to plastic particles,[101,102] although the exact components triggering this response are not well understood. From our results and these arguments, the likely source for the observed acute toxicity of developing zebrafish embryos caused by passive, water-borne exposure to macroscopic PCL was its potentially rapid release of nanoplastics and oligomers within 24 h and thereafter.

Implications

Biomedical

Our findings show that PCL can be acutely toxic to early-developing zebrafish, potentially because of shed particles and oligomers, raising concerns about the widely recognized use of PCL in biomedical research and devices.[103] First, the differences in toxic outcomes between our study and previous *in vivo* studies of PCL biomaterials likely stem from differences in local concentration, material properties, exposure route, matrix composition, and the model organism used and its developmental stage. Our study passively exposed early-developing fish embryos to a relatively high concentration of macroscopic unmodified PCL. In contrast, *in vivo* biomedical studies have implanted or injected engineered PCL biomaterials into developed (adult) mammals.[81,104–111] Additionally, few studies have investigated the zebrafish response to PCL,[88,112–114] and those that have primarily focused on small particles, not macroscopic items, as in our study. One study previously investigated the toxicity of finely ground PCL particles to

developing zebrafish embryos and found no observable effect on mortality; however, this water-borne exposure was conducted at a concentration of 0.2 mg/mL, well below the concentration that acute toxicity was observed by us (150 times less than the LC_{50}).[114] Comparatively, in the same study, *in vitro* cytotoxicity at 10 mg/mL cell culture medium was observed.[114] Recent work has suggested that PCL oligomers can improve the biocompatibility of PCL materials.[115] However, these conclusions were based solely on two non-specific *in vitro* assays; thus, extrapolating these effects *in vivo* should be approached cautiously. Above all, the conditions used to test the biocompatibility of PCL medical devices are not analogous to those used in our study. So, the mechanisms of toxicity relevant to our study could have been missed or gone unrecognized previously.

Regardless, unmodified PCL implants elicit a conventional foreign body response and shed particles as the polymer hydrolytically degrades in the body.[80–82] Complete degradation of PCL implants can take years.[81,116] Particles shed *in vivo* during degradation can be phagocytosed and have been observed in cellular structures.[117] As concern for microplastics and nanoplastics in the body increases, the shedding of particles and oligomers from degradable and non-degradable[118] polymeric implants and their impact on local and systemic biocompatibility requires greater scrutiny. A recent report by the FDA determined that the systemic effects of PCL-based biomedical implants used clinically are poorly understood.[103] Much of the previous work investigating PCL biocompatibility focused on the polymer's cytocompatibility as measured by routine viability assays (e.g., Microtox, MTT, LDH, and live/dead staining),[70,87,115,119,120] the dynamics of the foreign body response to PCL,[106–111] and the excretion routes of PCL degradation products.[81,105] Few have investigated this polymer's broader bioactivity.[86,121,122] Recent work on another common biomedical polymer, polylactic acid (PLA), has shown that ingested PLA microplastics can shed nanoplastics and oligomers in the gut, leading to acute inflammation and the translocation of particles from the gut to other tissues.[123] Polyethylene wear particles have

similarly been shown to migrate from orthopedic implants to lymphatic tissues and contribute to device failure via small particle disease.[124] In particular, the acute toxicity of PCL on early developing fish embryos at least justifies considering the potential impacts of implants capable of shedding nanoparticles and oligomers and using nanocarriers in the context of fetal health. Recent work has shown the presence of nanoplastics and microplastics within the human placenta, and nanoplastics have been observed to cause damage to developing mammalian fetuses.[125–128]

Our findings can instruct the design of more biocompatible PCL and other biomaterials, in general, by applying new approach methodologies and non-targeted bioassays to assess biocompatibility.[129–131] Zebrafish have been demonstrated as effective platforms for studying the foreign body response to biomaterials and developing engineered tissues because of their optically transparent transgenic lines and rapid development.[112,113,132–135] Non-targeted bioassays can screen for dysregulation of biological processes resulting from biomaterial exposure to identify formulations that minimize adverse effects. In particular, TF-FACTORIAL and NR-FACTORIAL bioassays can reveal when treatments (pharmaceutical or otherwise) deviate from their desired activity.[39]

Environmental

PCL is often derivatized, modified, or blended with other polymers (e.g., PLA and polyvinyl chloride) to tailor a plastic's degradability and mechanical properties.[87,136–139] Additionally, PCL satisfies many degradability standards,[137] has received FDA food-contact approval when blended with PLA,[140] and is used in many biomedical devices.[103] For these reasons, as industry moves toward more degradable, ecocompatible[141] plastics, the use of PCL is likely to increase.[142,143]

PCL has not evaded marine plastic surveys despite its current, more specialized uses.[144,145] Of concern is that alkali hydrolysis of PCL has been shown to result in more bioactive PCL nanoparticles.[122] Because seawater is alkaline (pH ~8.1), this implies potentially more adverse impacts from PCL degrading in marine environments than others. Moreover, large-scale resin pellet spills have become more common,[14] which is superimposed on a well-recognized baseline of endemic resin pellet pollution from industrial practices.[146] As we have shown for one species of fish and others for primary producers,[85] PCL pellets can potentially harm a range of aquatic organisms. In light of our findings, a spill of PCL pellets in or near a waterbody is worrisome, suggesting the need for legislation and accountability to prevent resin pellet spillage.

Consumers

PCL and TPU based materials have been tried and patented for dental applications.[147–151] Most notably, a PCL-based root canal filler marketed under the tradename Resilon was introduced in 2004. However, it was pulled from the market years after following anecdotal reports of poor device performance.[150,152,153] Later clinical and retrospective studies found that Resilon-filled root canals had higher degradation rates and were 5-fold more likely to present with lesions than the conventional root canal filler material (gutta-percha).[152,153] It was suggested that, at least in part, device failure was due to the degradability of PCL and the material succumbing to microbial attack in the mouth.[150,154] In response to social media posts by consumers using moldable plastics to create temporary teeth,[11] dental professionals have warned of choking hazards, inflammation, and the potential for more serious tooth decay from trapped food.[10]

Moldable plastics sold for dental applications can be considered as either "temporary crown and bridge resin" or "tooth shade resin material",[155,156] making them class II medical devices. Thus, they are regulated by the U.S. FDA via the 510(k) pathway, requiring premarket notification to

"clear" the product before commercial distribution. None of the analyzed moldable plastics were listed on the U.S. FDA 510(k) premarket notification database (**Tables S6-S7**) nor any other FDA database as approved, cleared, or authorized medical devices.[157,158] In fact, many included a legal disclaimer on their Amazon.com webpage stating their lack of U.S. FDA evaluation. Based on the number of reviews of these products and their content, many users have enjoyed having a low-cost, over-the-counter solution for their dental challenges, particularly when practiced dentistry may be out of reach. A market for these products is unsurprising as it is estimated that ~52% of Americans are missing at least one tooth.[159] Regardless, consumers deserve transparency about the products they purchase to make an informed decision, particularly regarding their health and choice of treatment.

CONCLUSIONS

For consumers and vendors of PCL, our findings conflict with PCL's presumed biological inertness. In recent years, numerous accounts have quantified the release of nanoplastics (or been challenged as having released cyclic oligomers instead[160–163]) from plastic consumer goods, including disposable coffee cups,[164] tea bags,[165,166] baby bottles,[167] rubber teats,[168] and polyester textiles.[169,170] As listed on an SDS of a PCL-based moldable plastic,[25,27,28] "The polymer is not bioavailable because of its molecular size." This statement and view of plastic require revision in the context of released nanoplastics, oligomers, and additives from plastic items and the mounting evidence supporting the environmental and human health impacts of plastics.[4]

Supporting Information Available

The following files are available free of charge,

Extended materials and methods; image of moldable plastic pellets (Figure S1); morphometrics and colorimetrics of moldable plastic pellets (Figures S2 – S8); ATR-FTIR spectra of moldable plastics (Figures S9 – S21); morphometrics and colorimetrics of PCL and TPU-based moldable plastics (Figures S22 – S29); confirmatory zebrafish study of consumer-grade PCL mortality (Figure S30); TF- and NR- FACTORIAL assay of extraction black (Figure S31); GC×GC-HRT mass spectra of chromatographic peaks (Figures S32 – S42) (PDF).

Solvent extract data (Table S1); TF- and NR- FACTORIAL assay endpoint definitions (Table S2); TF- and NR- FACTORIAL assay endpoint data (Table S3-S4); Top library hits for FACTORIAL assay results (Table S5); List of products and companies that have 510(k) pre-market notification for Product Code EBG, "Temporary crown and bridge resin." (Table S6); List of products and companies that have 510(k) pre-market notification for Product Code EBF, "Tooth shade resin material." (Table S7) (XLSX).

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Notes

The authors declare the following competing financial interest(s): A.V.M. and S.S.M. have competing financial interests as Attagene shareholders. The authors declare no other competing interests.

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