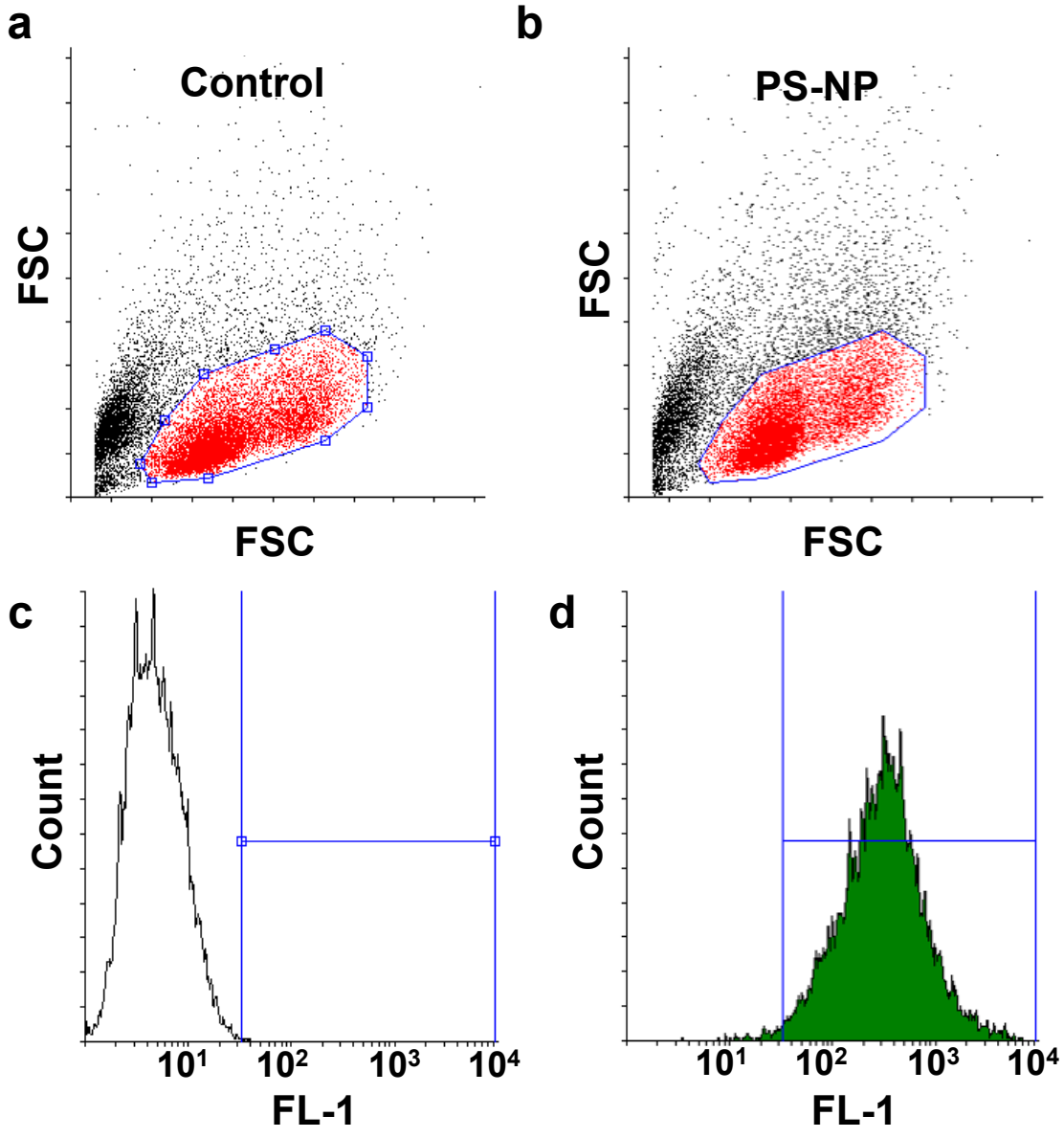
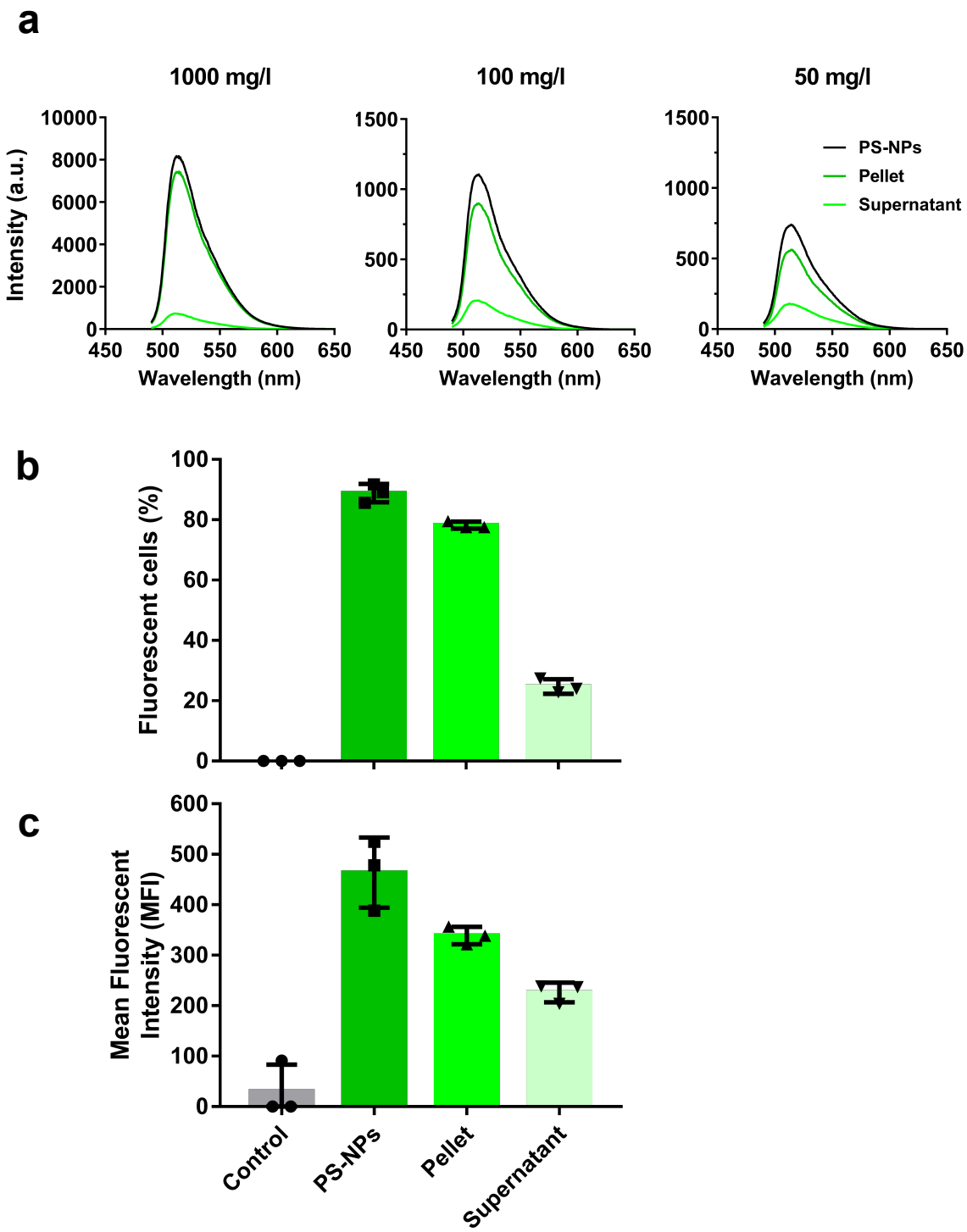
Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Figure 1



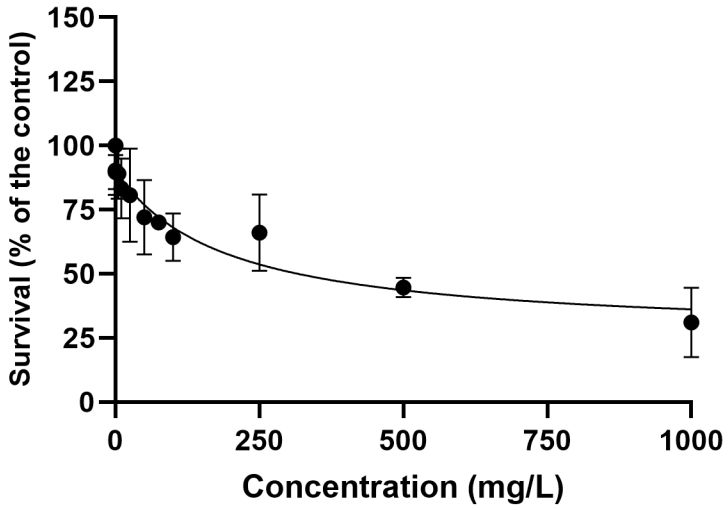
Supplementary Figure 1. ZFL cells Flow cytometry analysis strategy. Representative dot plots and the gating strategy (a, b), with red dots representing the gated population and black dots showing cellular debris and events outside of the relevant population. Histograms of fluorescence are shown (c, d). Cells were incubated as follows: unexposed control cells (a, c) and 50 mg/l PS-NPs exposed cells (b, d).

Supplementary Figure 2



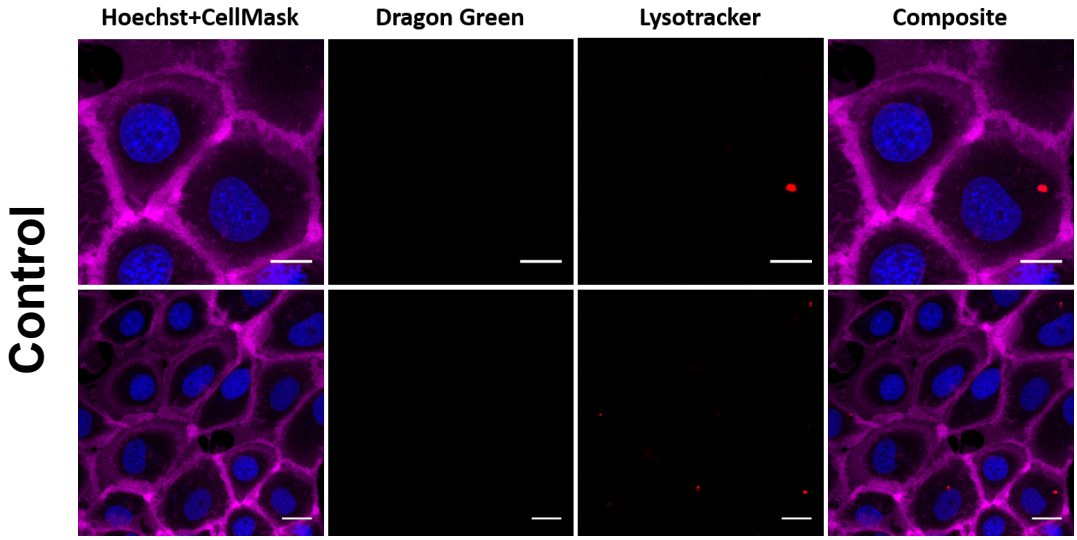
Supplementary Figure 2. Fluorophore leaking analysis from labelled PS-NPs. **(a)** Curves of fluorescence intensity of PS-NPs diluted from stock solution, without further manipulation (PS-NPs), and of obtained fractions after manipulation (Pellet, Supernatant). **(b, c)** Cytometry analysis showing the internalization of PS-NPs, Pellet and Supernatant fractions by ZFL cells (*in vitro*). **(b)** Percentage of fluorescent cells of PS-NPs (dark green), Pellet (green) and Supernatant (light green). **(c)** Mean fluorescence intensity (MFI) of PS-NPs (dark green), Pellet (green) and Supernatant (light green). Data represent the mean ± SD of three samples.

a

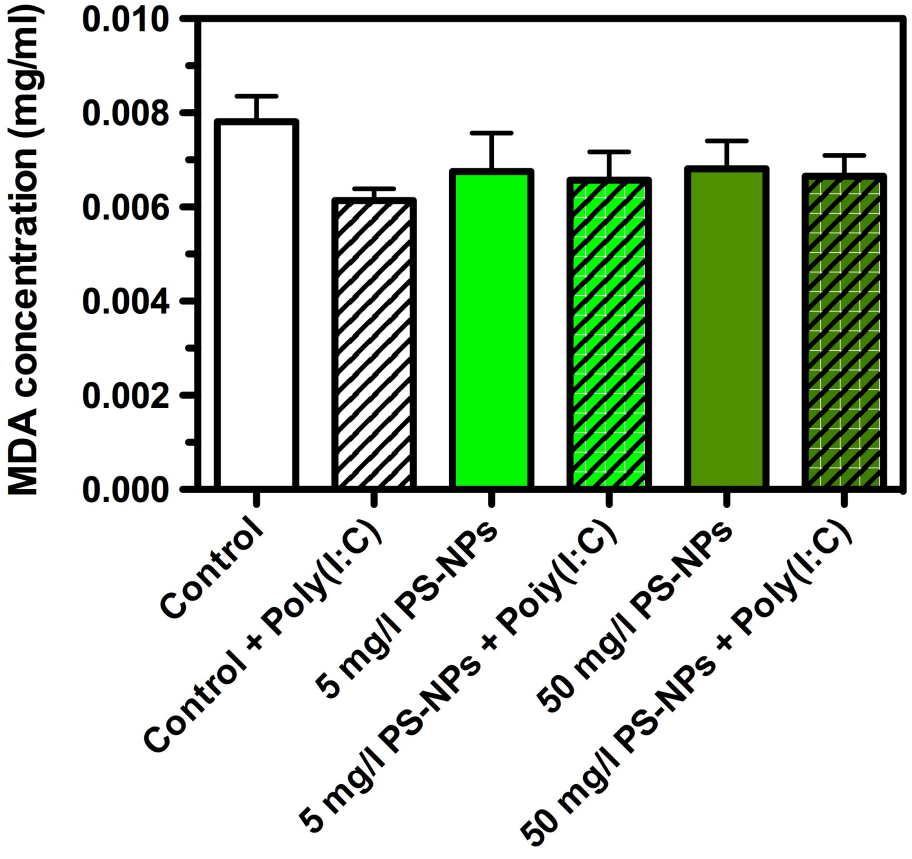


Supplementary Figure 3. Dose-response curve. ZFL cells were exposed to an increasing concentration range (from 0.05 to 1000 mg/l) of PS-NPs. Lethal doses (LD50) were estimated using a nonlinear regression fitting curve (3 P) using GraphPad Prism 8.0.1 (244). The lethal dose for 50% (LD50) of the cells were estimated at 188.8 mg/l.

Supplementary Figure 4

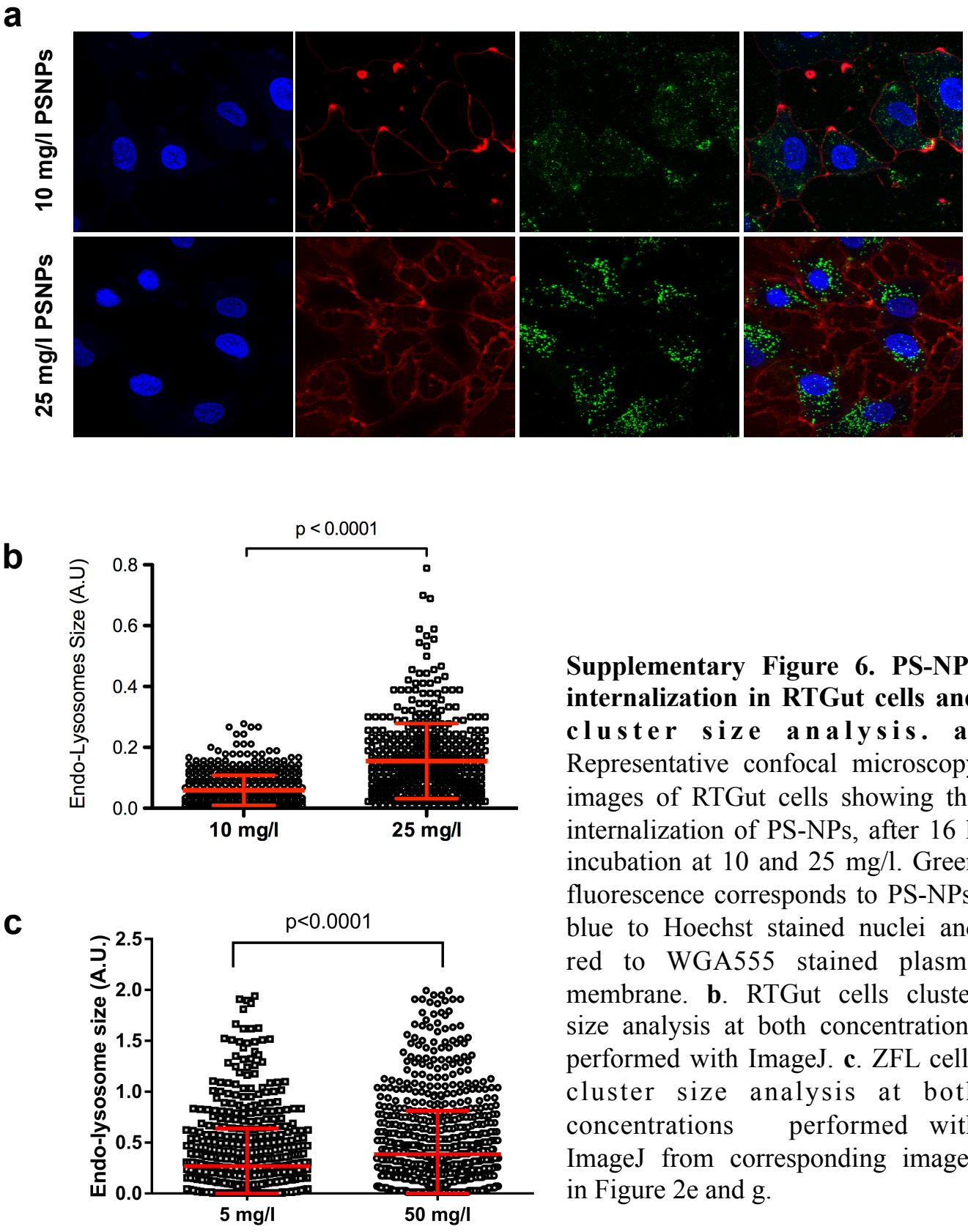


Supplementary Figure 4. Confocal images of control ZFL cells. Blue fluorescence corresponds to Hoechst stained nuclei, magenta to Cell Mask stained plasma membrane and red to Lysotracker stained lysosomes.



Supplementary Figure 5. Lipid peroxidation in ZFL cells exposed to PS-NPs. Lipid peroxidation assessed through the presence of malondialdehyde (MDA) in ZFL cells after exposure to PS-NPs followed by Poly(I:C) stimulus for 16 h. Cells were incubated as follows: unexposed control cells (white), 5 mg/l PS-NPs (light green) and 50 mg/l PS-NPs (dark green).

Supplementary Figure 6



Supplementary Figure 6. PS-NPs internalization in RTGut cells and cluster size analysis. a. Representative confocal microscopy images of RTGut cells showing the internalization of PS-NPs, after 16 h incubation at 10 and 25 mg/l. Green fluorescence corresponds to PS-NPs, blue to Hoechst stained nuclei and red to WGA555 stained plasma membrane. **b**. RTGut cells cluster size analysis at both concentrations performed with ImageJ. **c**. ZFL cells cluster size analysis at both concentrations performed with ImageJ from corresponding images in Figure 2e and g.

Supplementary Table 1

Table 1. Real Time primers.

Gene	Primer sequence (5´-3´)	Product size (bp)	Accession number
ef1-α	FW_CTTCTCAGGCTGACTGTGC RV_ACGATCAGCTGTTTCACTCCC	133	AY422992
mx	FW_ACATCTTGGATCGTTCAGGGGA RV_AACGCAGGTTCCTCCAACAG	163	NM_182942.4
vig-1	FW_CTTATAGGTCGAGCACAGGGC RV_ACGTACTGGATTGAGAGCGGTG	165	NM_001025556.1
gig2	FW_AGGGTACGACACTGCCTGGT RV_ AGGGTCACCAAAGCCACAAT	148	NM_001245989.1

Table 2. Zebrafish larvae mortality (%) after continuous exposure to different concentrations of PS-NPs.

ZEBRAFISH LARVAE MORTALITY (%)

Exposure Group	60 h	96 h
0 mg /l	6,7%	6,7%
50 mg/l	7,1%	35,7%
100 mg/l	71.4%	100%