**Supplementary information**

**Supplementary methods**

Quantification of amino groups

The number of amino groups on the surface of the PS / PMMA NPs was determined using the fluorescamine assay as described previously (Hsiao *et al.*, 2019). Briefly, fluorescamine (Sigma-Aldrich, Taufkirchen, Germany) reacts with primary amines and forms highly fluorescent products. First, 100 µL NP suspension (10 mg/mL) was added to 800 µL PBS followed by adding 100 µL fluorescamine (1 mg/mL acetone). After mixing, the suspension was incubated at room temperature for 10 min. The fluorescence intensity was measured at 485 nm with an excitation of 392 nm. Details for calculating the concentration of amino groups in µmol/g and the number of amino groups per surface area are given in Hsiao *et al.*, 2019.

Stereomicroscopic analysis of zebrafish embryos

Zebrafish embryos were anesthetized in fish water containing 0.02% (w/v) (Westerfield, 1995) MS-222 (Sigma-Aldrich, Taufkirchen, Germany) and transferred into a 6-cm petri dish. As upon exposure to ZnO NPs (1 µg/mL) embryos did not hatch, chorions were manually removed with forceps. Embryos were positioned laterally and immobilized by 0.5% (w/v) low-melting agarose (Carl Roth, Karlsruhe, Germany) prepared in fish water. Images were acquired by MZ16F stereomicroscope equipped with DFC 300FX camera (Leica Microsystems, Wetzlar, Germany).

**Table S1** Physico-chemical characteristics of the MNMs in zebrafish water (Holtfreter's medium). MNM suspensions of 125 µg/mL were prepared in zebrafish water as described in Materials and Methods. The sample was split, with one part immediately analysed by DLS (0 h) and the other part analysed after 24 h. TEM analyses were done by drying each of the as-synthesised particle suspensions (in water) onto a TEM-grid.

| MNM | coating/ functionalisation | TEM | DLS | | | |
|---|---|---|---|---|---|---|
| | | | 0h | | 24h | |
| | | [nm] | z-average [nm] | PDI | z-average [nm] | PDI |
| PS-$NH_2$[1] | $NH_2$ | 46.4 ± 7.6 | 57.1 | 0.06 | 59.9 | 0.085 |
| PS-$NH_2$[2] | $NH_2$ | 35.3 ± 7.4 | 50.5 | 0.04 | 50.8 | 0.05 |
| PS-COOH | COOH | 52 ± 5.9 | 58.6 | 0.10 | 92.3 | 0.17 |
| PMMA-$NH_2$ | $NH_2$ | 43.3 ± 11 | 47.9 | 0.09 | 48.5 | 0.14 |
| PMMA-COOH | COOH | 35 ± 4 | 41.8 | 0.06 | 42.8 | 0.08 |
| $SiO_2$-plain | COOH | 14.2 - 21.0[d] | 24 | 0.060 | 24 | 0.146 |
| $SiO_2$-$NH_2$ | $NH_2$ | 19.1 - 28.1[d] | 1069 | 0.258 | 2461 | 0.278 |
| $SiO_2$-COOH | COOH | 16.5 - 24.3[d] | 29 | 0.162 | 30 | 0.218 |
| $TiO_2$-plain | - [c] | 8.0 - 11.9[d] | 1703 | 0.332 | 2025 | 0.350 |
| $TiO_2$-PVP | PVP [c] | 9.0 - 11.9[d] | 1733 | 0.364 | 1515 | 0.315 |
| $TiO_2$-F127 | Pluronic® F127 [c] | 5.1 - 13.4[d] | 1518 | 0.281 | 1712 | 0.474 |
| $TiO_2$-AA4040 | Dispex® AA4040 [c] | 5.1 - 13.9[d] | 1555 | 0.261 | 1828 | 0.246 |
| $TiO_2$ (NM-103) | $Al_2O_3$, Dimethicone*[a,b] | 25.4 - 40.7[d] | 937 | 0.361 | 965 | 0.400 |
| $TiO_2$ (NM-104) | $Al_2O_3$ [b] | 23.2 - 42.5[d] | 866 | 0.294 | 1495 | 0.328 |
| $CeO_2$-plain | - | 17.1 ± 10.7 | 2080 | 0.426 | 2518 | 0.428 |
| $CeO_2$-plain (NM-212) | - | 4.7 ± 1.4 | 1621 | 0.461 | 1331 | 0.480 |
| ZnO-plain (NM-110) | - | 48.8 - 87.7[d] | 2023 | 0.767 | 2171 | 0.845 |
| ZnO-TECS (NM-111) | TECS [a] | 34.8 ± 21.4 | 3810 | 0.959 | 3067 | 0.907 |
| Ag NM-300K | Tween 20, PEG trioleate | 21.1 ± 7.4 | 28.0 | 0.395 | 29.7 | 0.632 |

PS-$NH_2$: amine-modified polystyrene, PVP: poly(N-vinylpyrrolidone), F127: Block copolymer composed of ethylene and propylene oxide, Dispex AA4040: ammonium salt of an acrylic polymer, TECS: triethoxycaprylylsilane, Tween 20: polyoxyethylene (20) sorbitan mono-laurat, *: Dimethicone: Polydimethylsiloxane, [a] hydrophobic, [b] 100% rutile, [c] anatase, [d] shortest and longest length of particles; [1] Bangs laboratories, [2] Sigma. Note that in case of agglomerated MNMs, 100 % of the signal (peak area intensity) is attributed to agglomerates and no peaks at the size of primary particles could be detected.

**Table S2** The LOAELs, NOAELs, $EC_{10}$, and $EC_{50}$ values for interference with hatching and mortality of zebrafish embryos due to exposure to Ag NPs [µg/mL] at 120 hpf are shown.

| **Effects** | **LOAEL** µg/mL | **NOAEL** µg/mL | **$EC_{10}$** µg/mL | **$EC_{50}$** µg/mL |
|---|---|---|---|---|
| hatching | 3.9 | 2.0 | 3.0 | 7.8 |
| mortality | 7.8 | 3.9 | 4.2 | 23.5 |

**Table S3** The LOAELs, NOAELs, $EC_{10}$, and $EC_{50}$ values for interference with hatching of zebrafish embryos due to exposure to ZnO-plain, ZnO-TECS, and $ZnCl_2$ [µg/mL] determined at 120 hpf. Values are shown as the nominal or applied dose and in parentheses as the equivalent Zn concentration.

| **MNM** | **LOAEL** µg/mL | **NOAEL** µg/mL | **$EC_{10}$** µg/mL | **$EC_{50}$** µg/mL |
|---|---|---|---|---|
| ZnO-plain | 1.0 (0.8) | 0.5 (0.4) | 0.56 (0.44) | 0.95 (0.8) |
| ZnO-TECS | 1.0 (0.8) | 0.5 (0.4) | 0.58 (0.46) | 1.15 (0.95) |
| $ZnCl_2$ | 0.82 (0.4) | 0.41 (0.2) | 0.5 (0.22) | 0.95 (0.45) |

**Table S4** Amount of amino groups at the surface of plastic nanoparticles.

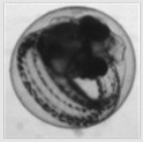
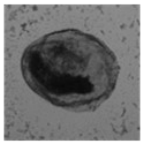
| **MNM** | **Primary amine concentration** [µmol/g] | **Numbers of amine groups/surface area** [# $NH_2/nm^2$] |
|---|---|---|
| PS-$NH_2$[1] | 1025 ± 176 | 5.0 ± 0.9 |
| PS-$NH_2$[2] | 3150 ± 353 | 11.7 ± 1.3 |
| PMMA-$NH_2$ | 3625 ± 530 | 16.5 ± 2.4 |

[1] Bangs laboratories, [2] Sigma

**A**

| Day 0 | Day 1 | Day 2 – 5 | Image analysis |
|---|---|---|---|
| Crossing setup | Spawning, embryo sorting, NP exposure at 6 hpf in 96-well plates, 12 embryos per condition | Automated image acquisition at 24, 48, 72, 96, 120 hpf<br>500 µm brightfield image stack (6 images), 100 µm distance | by the naked eye<br>> 4,000 images per MNM |

**B**

| Endpoint | Parameters | Example | Description |
|---|---|---|---|
| Hatching | The embryo/larva is located inside or outside of the chorion |  | Zebrafish embryo was exposed to 3.9 µg/ml ZnO-TECS at 6 hpf and monitored at 120 hpf. The larva/embryo is viable but not hatched |
| Mortality | • Coagulation of the embryo<br>• No heart beat<br>• No movement after tactile stimulation |  | Zebrafish embryo was exposed to 62.5 µg/mL PS-$NH_2$ and monitored at 120 hpf. Shown is a coagulated embryo. |
| Malformation | Abnormal morphology:<br>a = lordosis<br>b = pericardial edema<br>c = altered head morphology<br>d = loss of tissue integrity (lesions, hemorrage)<br>e = others |  | Zebrafish embryo was exposed to 7.8 µg/ml Ag NPs at 6 hpf and monitored at 120 hpf after dechorionation. |

**C**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | NP 1 conc. 1 | | | | | | | | | | | |
| B | NP 1 conc. 2 | | | | | | | | | | | |
| C | NP 2 conc. 1 | | | | | | | | | | | |
| D | NP 2 conc. 2 | | | | | | | | | | | |
| E | NP 3 conc. 1 | | | | | | | | | | | |
| F | NP 3 conc. 2 | | | | | | | | | | | |
| G | pos. ctrl | | | | | | | | | | | |
| H | neg. ctrl | | | | | | | | | | | |

**Fig. S1** (A) Setup of the zebrafish acute toxicity screening assay. (B) Acute toxicity endpoints, parameters and examples. Note that while hatching and mortality was quantified, malformations were only qualitatively assessed. (C) Plate layout for zebrafish acute toxicity pre-screening. MNMs were tested at two concentrations (1 and 125 µg/mL), positive controls (pos. ctrl) were exposed to PS-$NH_2$ at 62.5 µg/ml, negative controls (neg. ctrl) were exposed to Holtfreter´s medium alone.

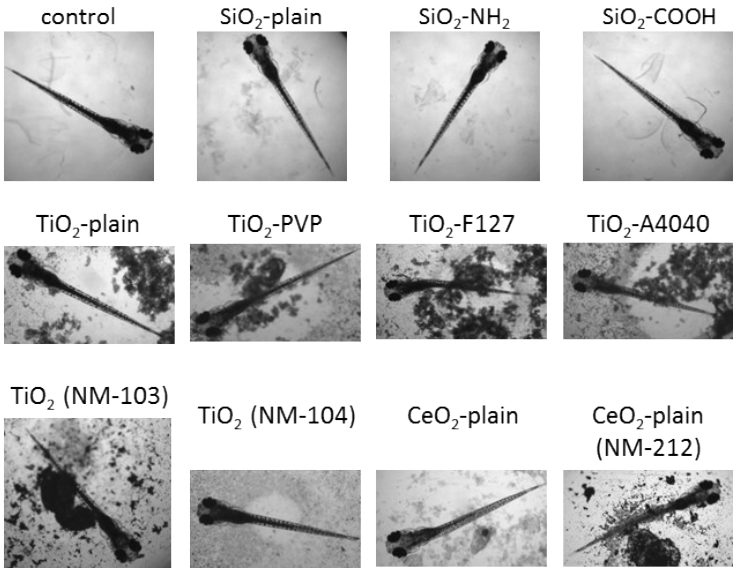



**Fig. S2** Representative images of zebrafish embryos exposed to non-toxic MNMs. Embryos were treated at 6 hpf with 125 µg/mL of MNMs or zebrafish water alone (control). Images of the embryos were acquired with an Olympus IX81 microscope at 120 hpf. Neither inhibition of hatching nor malformations or mortality are observed in exposed larvae. Note the presence of large particle agglomerates / aggregates in the case of titania and ceria NPs.

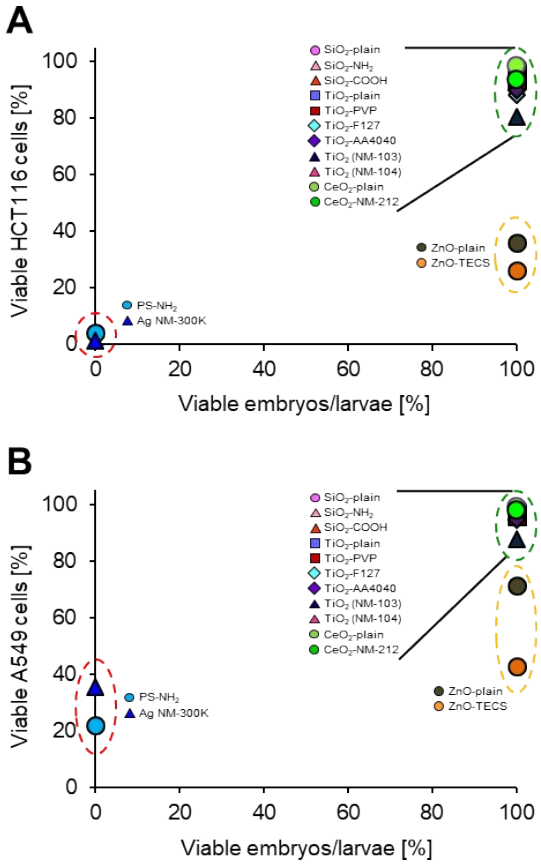



**Fig. S3** Comparison of *in vitro* results in human epithelial cells and *in vivo* results in zebrafish embryos/ larvae. Lethality of different MNMs (except for ZnO NPs) to zebrafish embryos/larvae correlates well with cytotoxicity in mammalian cells. The percentage of viable colon epithelial cells (A) and lung epithelial cells (B) after 24 h exposure to MNMs at 125 µg/mL (data from Hansjosten *et al.*, 2018) is compared to the percentage of viable zebrafish embryos/larva after 5 days incubation with MNMs at 125 µg/mL. Whereas non-toxic (dashed green circle) and toxic (dashed red circle) MNMs cluster together, only ZnO NPs (dashed orange circle) induce cytotoxicity in cell culture but do not induce mortality in zebrafish embryos.

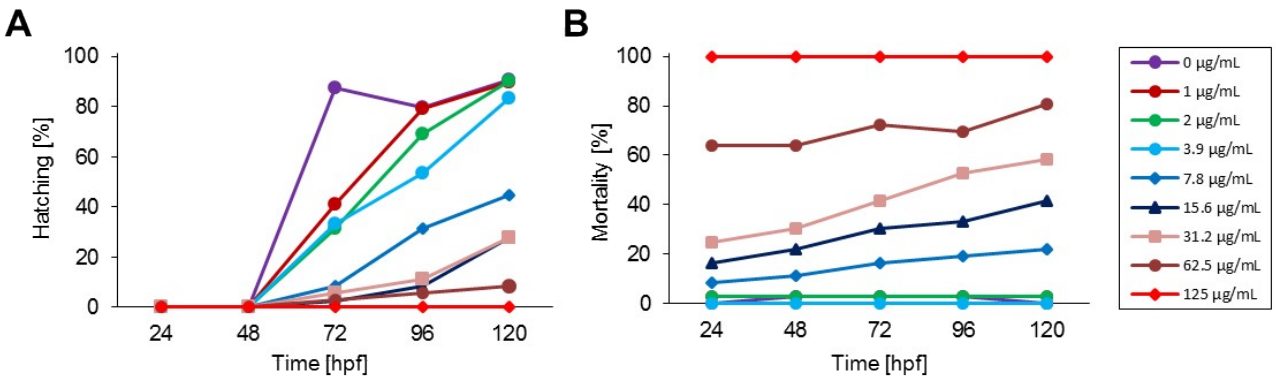



**Fig. S4** Time dependent effects of Ag NPs on hatching (A) and mortality (B) of zebrafish embryos. Zebrafish embryos at 6 hpf were treated with increasing Ag NP concentrations and analysed at the indicated time points. Controls were exposed to Holtfreter´s medium only (0 µg/mL). Hatching and mortality, normalised to the total number of analysed embryos, are shown, and are the means of 2-3 independent experiments. Note that for the 3.9 µg/mL concentration only one experiment was available.

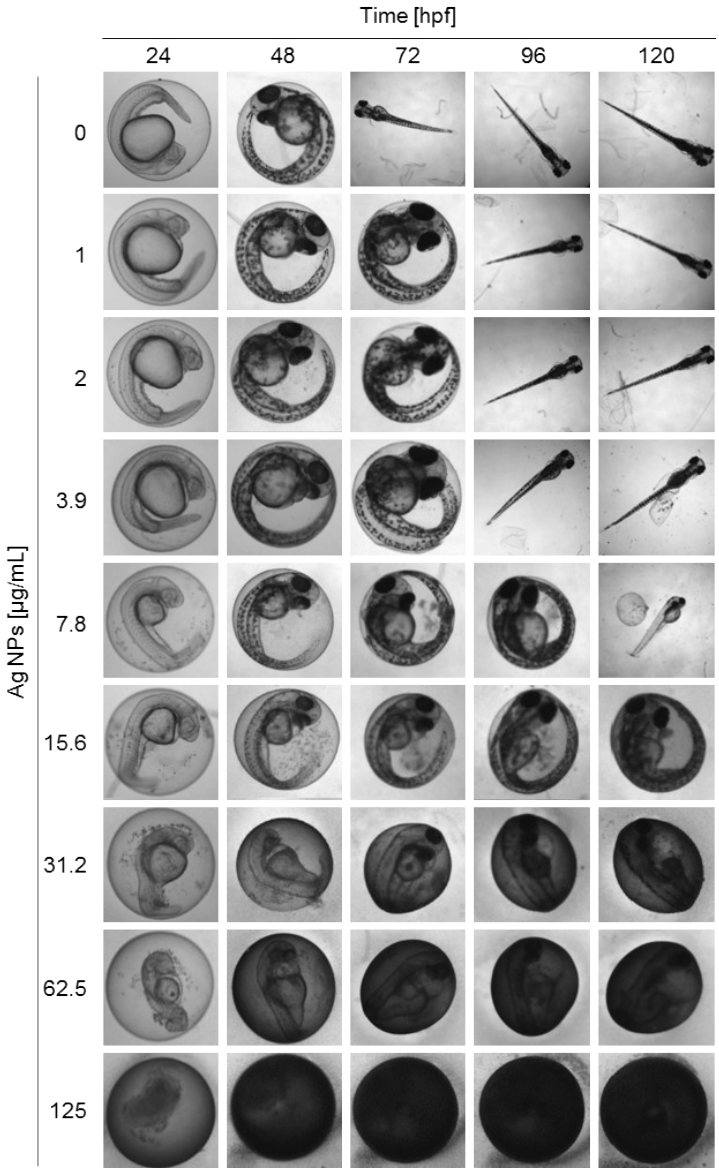



**Fig. S5** Images of zebrafish embryos and larvae treated with Ag NPs at increasing concentrations and different time points show delay of hatching, malformations and mortality. Note that at high concentrations, silver NPs coat the chorion and due to their light reflecting properties block the view and prevent proper imaging of the embryos.

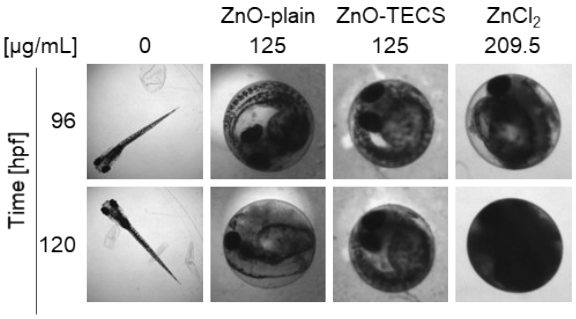



**Fig. S6** Images of zebrafish embryos and larvae treated with high concentrations of ZnO NPs and $ZnCl_2$ at different time points.

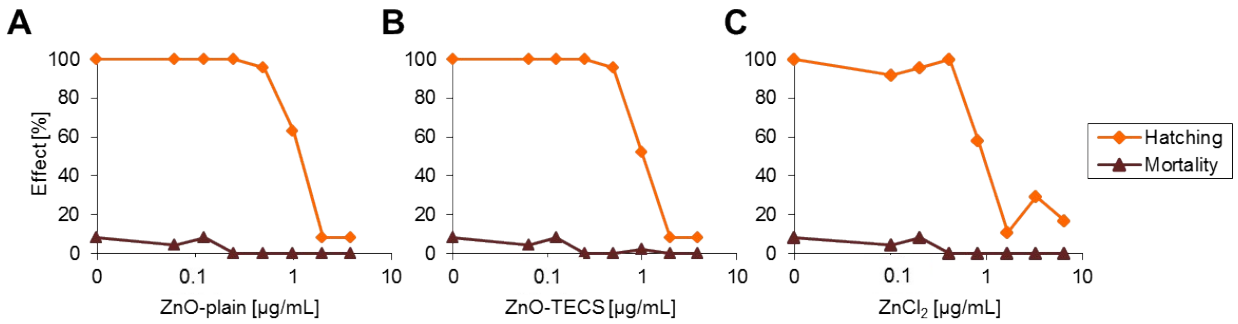



**Fig. S7** ZnO NPs inhibit hatching of zebrafish embryos dependent on concentration but do no induce mortality. Zebrafish embryos were treated with increasing concentrations of ZnO-plain NPs (A), ZnO-TECS NPs (B) and $ZnCl_2$ (C) at 6 hpf and were analyzed at 120 hpf. The controls were exposed to Holtfreter´s medium only (0 µg/mL). Hatching and mortality were normalized to the total number of analyzed embryos. Data shown are mean values from two independent experiments.

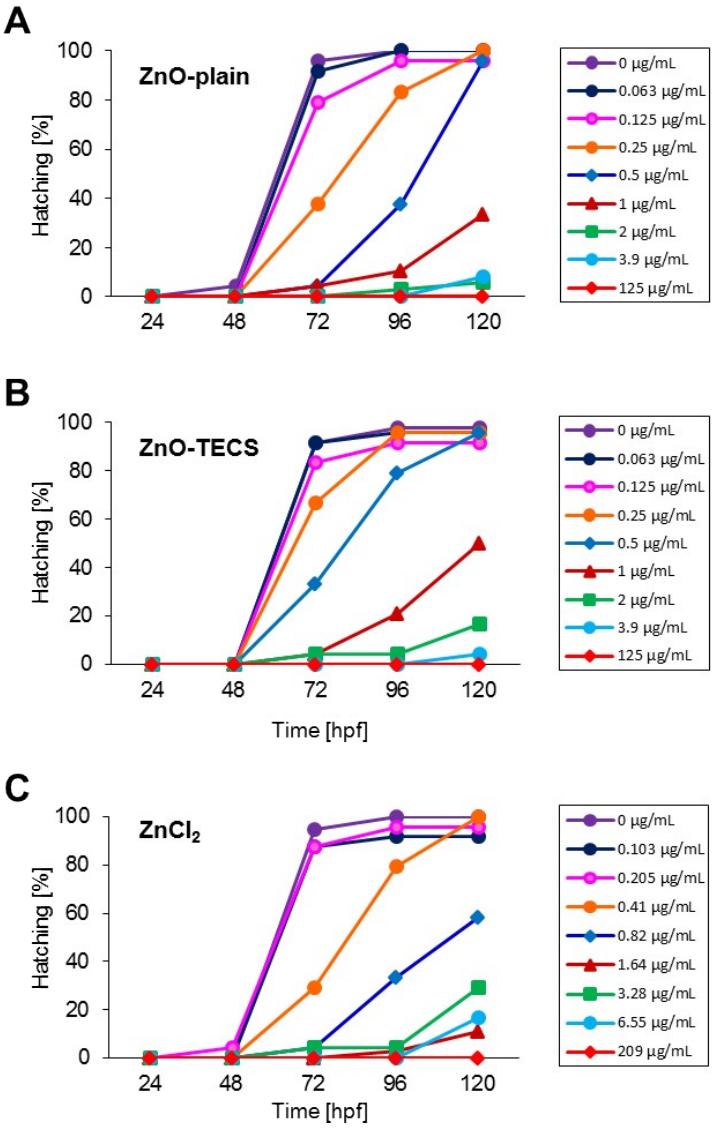



**Fig. S8** Time-dependent effect of ZnO-plain NPs (A), ZnO-TECS NPs (B) and $ZnCl_2$ (C) on hatching of zebrafish embryos. Zebrafish embryos at 6 hpf were treated with increasing concentrations of NPs and $ZnCl_2$ and were analysed at the indicated time points. The control was exposed to Holtfreter´s medium only (0 µg/mL). The number of hatched embryos has been normalised to the total number of analysed embryos. Data are shown as mean values from 2-4 independent experiments.

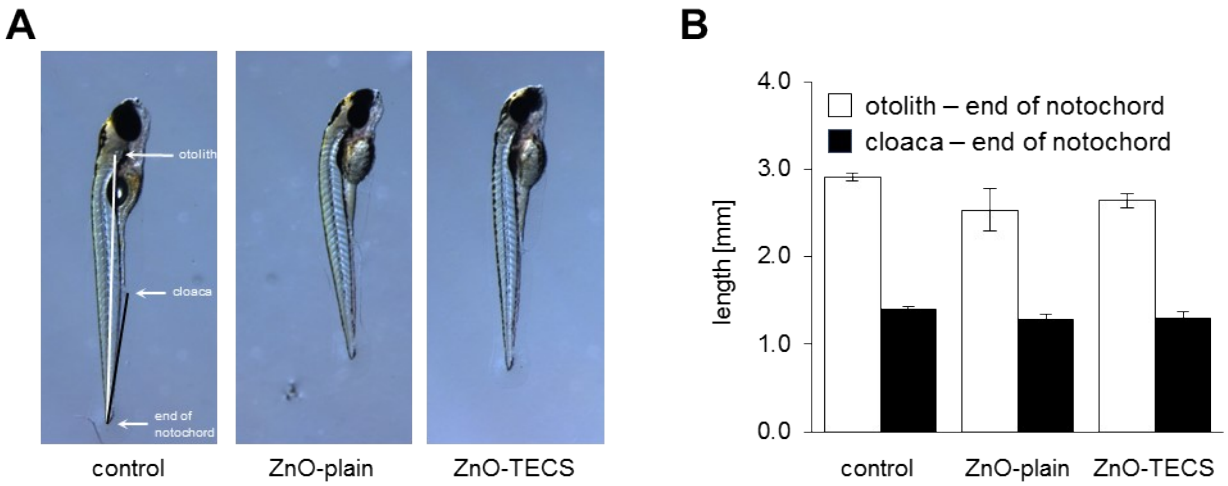



**Fig. S9** Exposure to ZnO NPs (1 µg/mL) reduces the body length of unhatched larvae. (A) Representative images of untreated and treated larvae at 5 dpf. Morphological hallmarks are indicated. The white line illustrates the distance between the posterior end of the notochord and the otolith whereas the black line indicates the distance between the cloaca and the posterior end of the notochord. Also note the incomplete consumption of the yolk sac as a further sign of delayed development upon NP exposure. (B) Quantification of length was performed with ImageJ (version 1.52a, National Institutes of Health, USA, https://imagej.nih.gov/ij/) analyzing 3-4 embryos per condition.

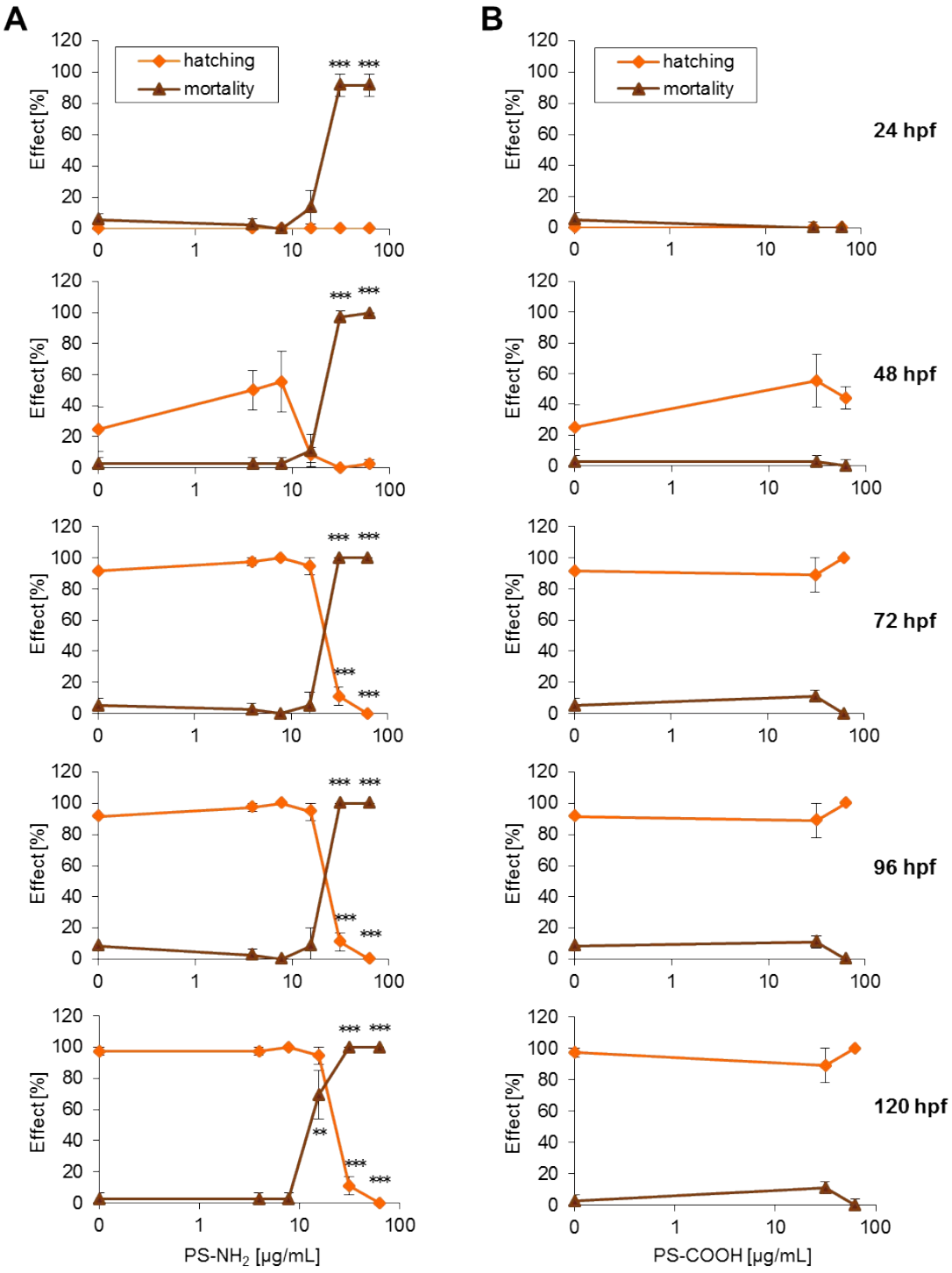



**Fig. S10** PS-$NH_2$ NPs but not PS-COOH NPs dose-dependently inhibit hatching and induce mortality in zebrafish embryos. Zebrafish embryos were treated at 6 hpf with increasing concentrations of PS-$NH_2$ NPs or PS-COOH NPs at the indicated time points. The control was exposed to Holtfreter´s medium only (0 µg/mL). The number of hatched or dead embryos/larvae has been normalised to the total number of analysed embryos. Results of three independent experiments were averaged and error bars show s.e.m. (** $p < 0.01$, *** $p < 0.001$).

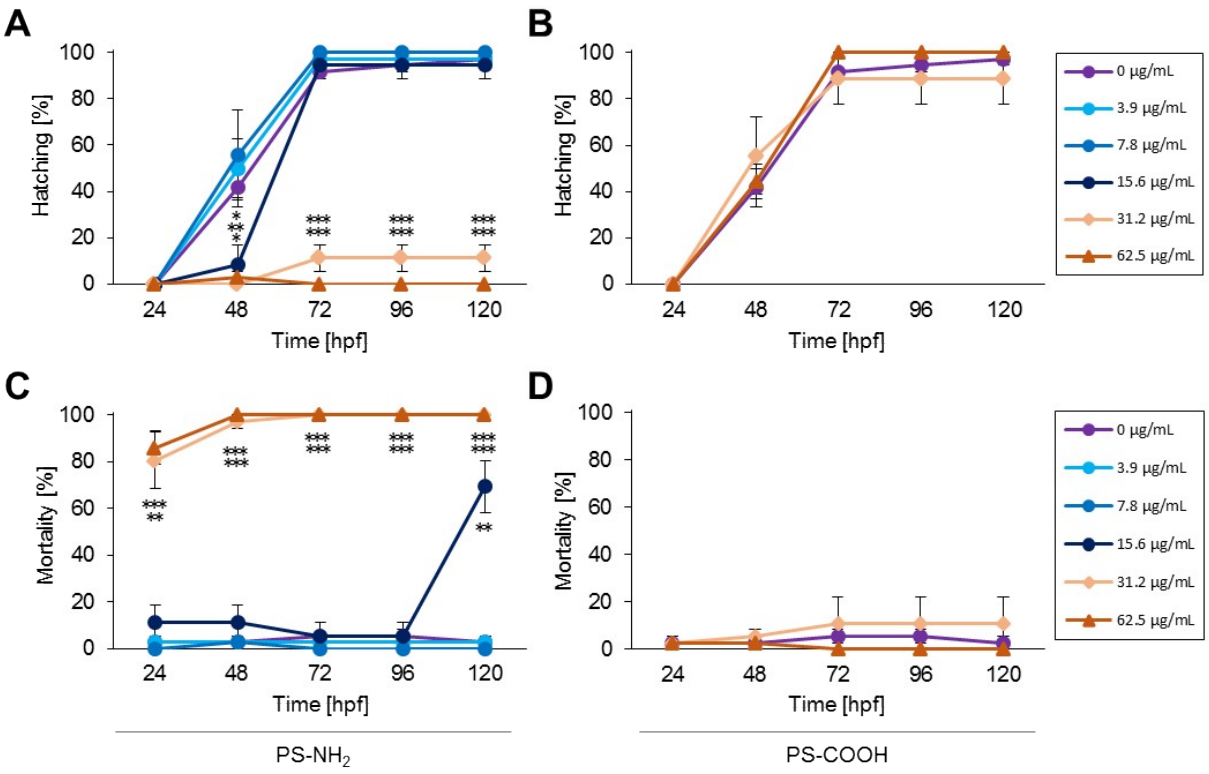



**Fig. S11** PS-$NH_2$ NPs inhibit hatching (A) and induce mortality (C) in a time-dependent manner while PS-COOH NPs (B, D) have no effect. Zebrafish embryos were treated and analysed as described in Fig. S8. Results of three independent experiments were averaged and error bars show s.e.m. (** $p < 0.01$, *** $p < 0.001$). Note that in (A) asterisks from top to bottom refer to 15.6, 31.2 and 62.5 µg/mL at 48 hpf and to 31.2 and 62.5 µg/mL at 72, 96 and 120 hpf. In (C) asterisks from top to bottom refer to 62.5 and 31.2 µg/mL, respectively, at all time points. At 120 hpf also in the case of 15.6 µg/mL asterisks indicate significance.

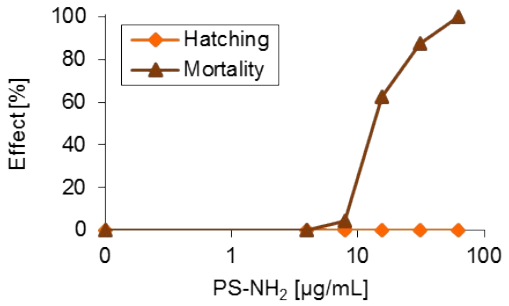



**Fig. S12** PS-$NH_2$ NPs from a different source also inhibit hatching and trigger mortality. For verification of adverse effects of PS-$NH_2$ NPs (Bangs Laboratories) shown in Figs. 1 and 5 and Figs. S10 and S11, PS-$NH_2$ NPs from another manufacturer (Sigma-Aldrich) were investigated. Zebrafish embryos were treated with increasing concentrations of Sigma PS-$NH_2$ NPs as described in Fig. S8 and analysed at 24 hpf. Data are the means of two independent experiments.

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