

1 **Supplemental Information**

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3 **Metal accumulation and distribution in the zebrafish (*Danio rerio*) embryo:**
4 **differences between nanoparticles and metal ions**

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30 A comparison between nominal and real exposure concentrations at the start of the experiment
 31 measured by neb-ICP-MS is shown in **Tab. S1**. Loss of substance from the exposure solutions may
 32 result from adsorption, agglomeration and degradation of particles taking place during handling and
 33 storage of suspensions.

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35 **Tab. S1:** Comparison between nominal and measured nanoparticle exposure concentrations.

Nominal NP exposure concentrations	Real NP exposure concentrations	Recovery
60 µg Ag/L	16.2 µg Ag/L	27 %
60 µg Au/L	34.2 µg Au/L	57 %
60 µg Cu/L	52 µg Cu/L	87 %
60 µg Zn/L	55.5 µg Zn/L	93 %

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37 Quantification of total element concentrations

38 Visualization by Laser Ablation ICP-MS

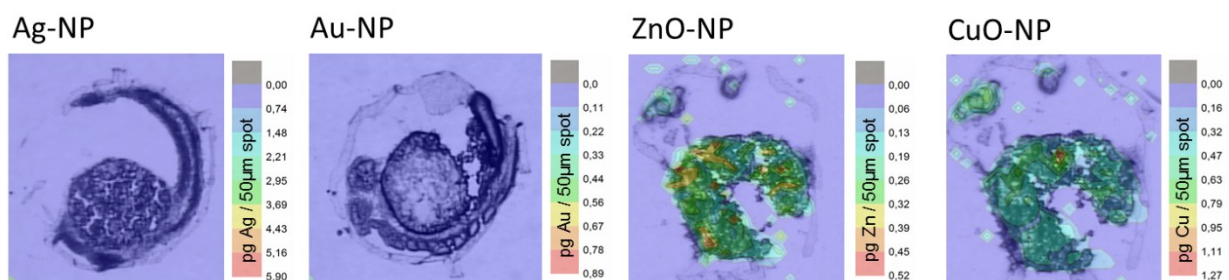
39 Prior to the testing of the NPs, untreated organisms were investigated to obtain information on the
 40 natural background of the respective elements after digestion (**SI, Tab. S2**) and for LA-ICP-MS (**SI,**
 41 **Fig. S1**). The background values were subtracted from all subsequent measurements.

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43 **Tab. S2:** Natural background of respective elements obtained by neb-ICP-MS measurement of untreated
 44 individual organisms (control) after digestion.

Nanomaterial	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)
Ag-NP	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Au-NP	0.20 ± 0.01	0.18 ± 0.02	0.11 ± 0.01
CuO-NP	1.81 ± 0.23	1.06 ± 0.17	0.54 ± 0.18
ZnO-NP	2.73 ± 0.84	2.88 ± 0.94	2.65 ± 0.31

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46 **Fig. S1:** Natural background of respective elements obtained by LA-ICP-MS measurement of untreated
 47 organisms (control).

48 **Results**

49 Characterization of nanoparticle powders, suspensions, and exposure solutions

50 The hydrodynamic particle sizes measured for the exposure solutions in ISO-WATER differ in the
51 following range: 18 ± 2 nm (Au-NP) < 80 ± 1 nm (ZnO-NP) < 117 ± 24 nm (Ag-NP) < 132 ± 2 nm
52 (CuO-NP) at the start of the experiments. After 24 h exposure, sizes of 204 nm (Ag-NP), 420 nm
53 (CuO-NP), and > 5000 nm for Au and ZnO-NPs were measured in the exposure solutions, indicating a
54 high agglomeration of particles within the media. The dissolved fraction of the respective particle
55 solutions was determined to be 48.3 ± 7.2 % Ag (Ag-NP), 9.8 ± 5.5 % Cu (CuO-NP), and 58.1 ± 2.6 %
56 Zn (ZnO-NP) (SI, Tab. S3).

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58 Impact of the chemical identity of NPs with respect to ionic fractions

59 In addition to the exposure with nanomaterials, the respective ionic substances (AgNO_3 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$,
60 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) were tested to allow comparing to Ag-NP, CuO-NP, and ZnO-NP results. The obtained
61 concentrations after an exposure to 60 μg element/l for 24 h are 6.4 ± 1.8 ng/organism, $2.1 \pm$
62 0.4 ng/organism, and 0.4 ± 0.1 ng/organism for AgNO_3 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, respectively
63 (SI, Fig. S2).

64 **Tab. S3:** Chemical and physical properties of investigated nanomaterials (powder, suspension, exposure solution).

Nanomaterial	Characterization of powders		Characterization of suspensions					Characterization of exposure solutions (ISO water)	
	X _{TEM} (nm)	BET (m ² /g)	Concentration (g/l)	Coating (c) / stabilizer (s)	Smoluchovski Zeta potential (mV)	X _{DLS} (nm)	Dissolved fraction (%)	X _{DLS} (nm) t ₀	X _{DLS} (nm) t ₂₄
AgNP	21 ± 8	-	4.0	PVP (c)	-19	117 ± 24	48.3 ± 7.2	135	204
AuNP	13 ± 1	-	0.06	Sodium citrate (s)	-38	18 ± 2	-	22	> 5000
CuO-NP	22-25	28	0.09	Tetrasodium pyrophosphate (s)	-64	132 ± 2	9.8 ± 5.5	131	420
ZnO-NP	10-15	60	5.6	-	+34	80 ± 1	58.1 ± 2.6	81	> 5000

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67 **Tab. S4:** Percentage distribution and concentrations measured for the zebrafish embryo and chorion structures for a 60 µg element/l exposure.

68 **RESULTS OF EXPOSURE WITH NANOPARTICLES**

Ag-NP							
Exposure period (h)	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)	Calculated conc. PVS (ng)	Time-average 2-24 h (ng/organism)	Time-average 2-24 h (ng/chorion)	Time-average 2-24 h (ng/embryo)
0.5	3.07 ± 0.34	1.36 ± 0.73	0.08 ± 0.07	1.63 ± 0.81	8.46 ± 1.78	5.63 ± 1.38	0.43 ± 0.14
1	2.37 ± 0.64	1.91 ± 0.49	0.20 ± 0.07	0.73 ± 0.56			
2	5.64 ± 0.58	3.59 ± 0.81	0.33 ± 0.22	1.73 ± 0.76			
4	8.22 ± 1.33	5.82 ± 1.15	0.65 ± 0.19	1.75 ± 0.19			
8	10.01 ± 1.27	7.49 ± 2.43	0.44 ± 0.21	2.08 ± 1.53			
24	9.95 ± 0.80	5.64 ± 2.83	0.31 ± 0.05	4.00 ± 2.56			

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Au-NP							
Exposure period (h)	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)	Calculated conc. PVS (ng)	Time-average 2-24 h (ng/organism)	Time-average 2-24 h (ng/chorion)	Time-average 2-24 h (ng/embryo)
0.5	5.46 ± 4.63	1.87 ± 2.12	1.05 ± 0.89	3.74 ± 2.25	12.04 ± 7.82	7.54 ± 6.69	0.51 ± 0.28
1	4.66 ± 1.91	1.28 ± 0.73	0.98 ± 0.98	2.50 ± 0.20			
2	5.43 ± 1.35	2.44 ± 1.13	0.98 ± 0.67	2.60 ± 1.67			
4	5.84 ± 2.01	3.00 ± 2.09	0.38 ± 0.28	4.58 ± 1.93			
8	12.10 ± 1.84	5.80 ± 2.95	0.30 ± 0.12	5.99 ± 3.37			
24	24.79 ± 0.68	18.91 ± 5.11	0.38 ± 0.24	5.50 ± 3.92			

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CuO-NP							
Exposure period (h)	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)	Calculated conc. PVS (ng)	Time-average 2-24 h (ng/organism)	Time-average 2-24 h (ng/chorion)	Time-average 2-24 h (ng/embryo)
0.5	0.27 ± 0.02	0.35 ± 0.11	0.36 ± 0.08	-0.53 ± 0.04	0.92 ± 0.12	0.58 ± 0.11	0.42 ± 0.10
1	0.42 ± 0.04	0.35 ± 0.10	0.43 ± 0.13	-0.35 ± 0.06			
2	0.45 ± 0.03	0.64 ± 0.25	0.38 ± 0.08	-0.56 ± 0.33			
4	0.99 ± 0.28	0.47 ± 0.08	0.45 ± 0.17	0.62 ± 0.00			
8	0.78 ± 0.07	0.57 ± 0.07	0.34 ± 0.00	0.18 ± 0.00			
24	1.47 ± 0.09	1.10 ± 0.05	0.54 ± 0.14	0.77 ± 0.00			

ZnO-NP							
Exposure period (h)	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)	Calculated conc. PVS (ng)	Time-average 2-24 h (ng/organism)	Time-average 2-24 h (ng/chorion)	Time-average 2-24 h (ng/embryo)
0.5	3.19 ± 0.15	0.28 ± 0.03	2.71 ± 0.17	0.40 ± 0.15	3.75 ± 0.13	0.51 ± 0.14	2.78 ± 0.36
1	3.34 ± 0.09	1.06 ± 0.23	2.90 ± 0.18	-0.61 ± 0.47			
2	3.76 ± 0.30	0.63 ± 0.09	2.79 ± 0.15	0.67 ± 0.16			
4	3.57 ± 0.13	0.37 ± 0.08	3.36 ± 0.08	0.00 ± 0.00			
8	3.75 ± 0.82	0.69 ± 0.13	2.55 ± 0.91	1.01 ± 0.31			
24	3.94 ± 1.22	0.38 ± 0.07	2.42 ± 0.71	1.15 ± 0.69			

72 RESULTS OF EXPOSURE WITH IONIC SUBSTANCES

AgNO ₃							
Exposure period (h)	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)	Calculated conc. PVS (ng)	Time-average 2-24 h (ng/organism)	Time-average 2-24 h (ng/chorion)	Time-average 2-24 h (ng/embryo)
0.5	1.13 ± 0.28	0.82 ± 0.24	0.12 ± 0.05	0.65 ± 0.00	4.57 ± 1.17	1.80 ± 0.36	0.22 ± 0.09
1	1.54 ± 0.13	1.46 ± 0.41	0.31 ± 0.30	0.38 ± 0.00			
2	3.55 ± 0.83	1.58 ± 0.93	0.23 ± 0.07	1.75 ± 0.33			
4	3.81 ± 0.51	1.33 ± 0.54	0.12 ± 0.05	2.36 ± 0.77			
8	4.41 ± 1.37	2.08 ± 1.91	0.18 ± 0.19	3.97 ± 0.12			
24	6.52 ± 1.76	2.22 ± 1.42	0.36 ± 0.29	3.94 ± 1.35			

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CuSO ₄ *5H ₂ O							
Exposure period (h)	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)	Calculated conc. PVS (ng)	Time-average 2-24 h (ng/organism)	Time-average 2-24 h (ng/chorion)	Time-average 2-24 h (ng/embryo)
0.5	1.77 ± 0.11	1.42 ± 0.20	0.50 ± 0.04	0.34 ± 0.00	2.74 ± 0.50	2.20 ± 0.50	0.25 ± 0.09
1	1.67 ± 0.16	1.32 ± 0.22	0.44 ± 0.66	0.50 ± 0.07			
2	1.93 ± 0.04	1.39 ± 0.19	0.16 ± 0.09	0.90 ± 0.63			
4	2.81 ± 0.43	2.20 ± 0.13	0.40 ± 0.34	1.07 ± 1.11			
8	2.89 ± 0.21	2.51 ± 0.46	0.24 ± 0.09	0.49 ± 0.14			
24	3.32 ± 0.42	2.69 ± 0.67	0.21 ± 0.09	0.87 ± 0.71			

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ZnSO₄*7H₂O

Exposure period (h)	Concentration egg (ng/organism)	Concentration chorion (ng/chorion)	Concentration embryo (ng/embryo)	Calculated conc. PVS (ng)	Time-average 2-24 h (ng/organism)	Time-average 2-24 h (ng/chorion)	Time-average 2-24 h (ng/embryo)
0.5	3.20 ± 0.29	0.87 ± 0.10	2.47 ± 0.23	0.47 ± 0.08	2.35 ± 0.28	0.35 ± 0.16	2.95 ± 0.28
1	3.20 ± 0.54	0.78 ± 0.26	2.37 ± 0.50	0.36 ± 0.10			
2	2.27 ± 0.16	0.61 ± 0.41	1.75 ± 0.26	0.37 ± 0.00			
4	2.53 ± 0.34	0.26 ± 0.01	2.06 ± 0.26	1.30 ± 0.00			
8	2.67 ± 0.32	0.45 ± 0.18	1.64 ± 0.10	0.57 ± 0.42			
24	1.92 ± 0.10	0.07 ± 0.03	2.35 ± 0.78	0.01 ± 0.00			

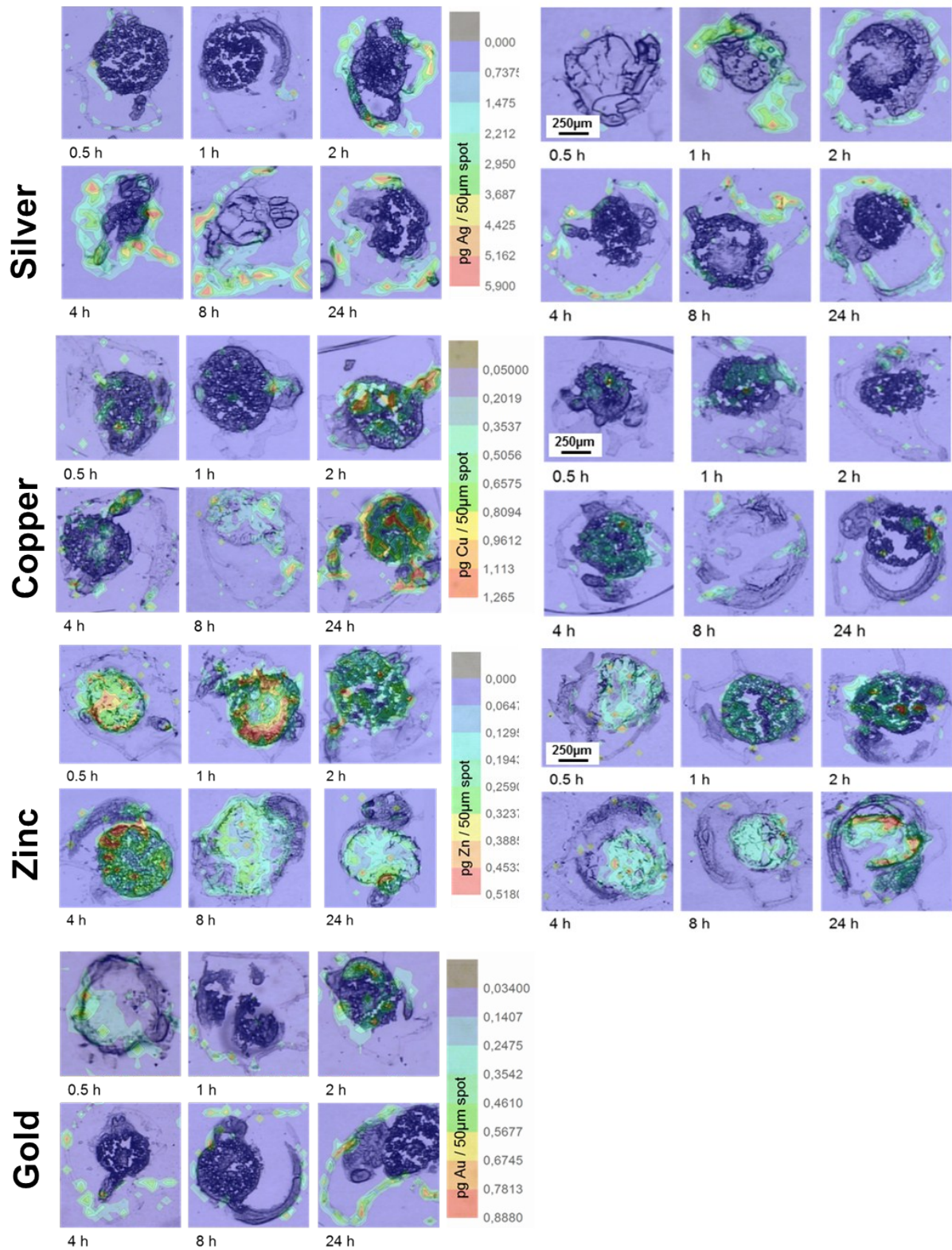
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78 **Tab. S5:** Internal concentrations of the respective metals in the different zebrafish embryo compartments.

Metal	Egg (ng/μl)	Chorion (ng/μl)	Embryo (ng/μl)	Calculated conc. PVS (ng/μl)
nAg	820.57	6.44	13.82	14.26
Ag ⁺	268.65	6.46	1.22	7.81
nAu	1544.79	12.17	1.54	26.35
nCuO	94.69	0.19	2.29	1.60
Cu ²⁺	324.79	0.75	1.01	4.43
nZnO	66.43	1.76	11.27	5.49
Zn ²⁺	32.79	0.07	9.08	3.28

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82 **Fig. S2:** Visualization of the time course of metal accumulation for nanoparticles and the respective ions after
 83 determination of the elemental distribution by LA-ICP-MS (right column: nanoparticles, left column: metal ions)

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85 **References**

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87 NanoValid, EU-project (2015). Dispersion of NNV-011 (CuO) nanoparticle suspensions for
88 toxicological testing.

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