

# Toxicity of Nanoplastics for zebrafish embryos, what we know and where to go next

Monica Torres-Ruiz<sup>1\*</sup>, Antonio De la Vieja<sup>2,3</sup>, Mercedes de Alba Gonzalez<sup>1</sup>, Marta Esteban Lopez<sup>1</sup>, Argelia Castaño Calvo<sup>1</sup>, Ana Isabel Cañas Portilla<sup>1</sup>

<sup>1</sup> Environmental Toxicology. Centro Nacional de Sanidad Ambiental (CNSA), Instituto de Salud Carlos III (ISCIII). Carretera Majadahonda-Pozuelo Km. 2,2., Majadahonda, Madrid 28220, Spain.

<sup>2</sup> Endocrine Tumors Unit (Unidad Funcional de Investigación en Enfermedades Endocrinas (UFIEC), Instituto de Salud Carlos III (ISCIII). Carretera Majadahonda-Pozuelo Km. 2,2., Majadahonda, Madrid 28220, Spain.

<sup>3</sup> Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto de Salud Carlos III (ISCIII), Madrid 28220, Spain.

\*Corresponding author: E-mail: [mtorres@isciii.com](mailto:mtorres@isciii.com)

## Supplementary material index:

Table A.1. Complementary information about reviewed articles.

Table A.2. Number of endpoints by study used to calculate % effects.

Table A.3. Results of endpoints per study.

Table A.4. Results of Locomotion and Heart Rate effects.

Table A.5. NP observation techniques in ZF embryos.

Table A.6. Full names and functions of validated gene transcripts used for analyses.

Table A.7. Full names and functions of validated enzymes/metabolites used for analyses.

Fig. A.1. Percent studies in which endpoints were observed (black) or not observed (grey).

Fig. A.2. Percent studies in which locomotion (a) and heart rate (b) were affected.

Fig. A.3. Percent studies in which NP accumulated in different ZF organs.

## Supplementary Information

### Toxicity of Nanoplastics for zebrafish embryos, what we know and where to go next.

**Table A.1.** Complementary information about reviewed articles

| Reference                    | NP Type and Solvent used   | Excipients of Stock Solution        | Mean manufacturer Size, Shape Route and mean z potential/charge | Measured Size range   | Aggregation/Dispersion | [NP]  | Exposure time  | Replicates for analyses   | Statistical Analysis Used   |
|------------------------------|--|-------------------------------------|---|---|------------------------|---|--|---|---|
| Brun et al. 2018<br>Medium   | PS Thermo Fisher Scientific Co.<br>Dyed beads (red)<br><br>Solvent: Embryo water   | Not stated                          | 25 nm<br><br>Route: Medium<br><br>- 25 mV                       | Average size in the medium of 19.3±0.6 nm after 0 h or 18.8±0.9 nm after 24 h | Low aggregation        | 10 µg/ml  | Mortality, development 0-120 hpf<br>Gene expression: 72-120 hpf<br><br>MR: every 24h | -Mortality, development: 5R, 10L<br>- Gene expression: 1R, 5 pooled (15L) samples<br>- Live Imaging: 1R, 10L  | - Homogeneity of Variance: Bartlett's<br>- One-way ANOVA, Bonferroni post hoc<br>- Significance p < 0.05  |
| Brun et al. 2019             | PS Thermo Fisher Scientific Co.<br>Dyed beads (Firefli™ green)<br><br>Solvent: Embryo water                              | Not stated                          | 25 nm<br>Round<br><br>Route: Medium<br><br>- 25 mV              | Average size in the medium of 19.3±0.6 nm after 0 h or 18.8±0.9 nm after 24 h | Low aggregation        | Assays: 0.2, 2, and 20 µg/ml<br><br>Mortality: 10 – 100 µg/ml | 72 - 120 hpf<br>Total: 48 h<br><br>MR: every 24h                                     | - Mortality: 4R, 10L<br>- Development: 3R, 10L<br>- Cortisol: 1R, 6 pooled (15L) samples<br>- Luciferase: 1R, 5 pooled (3L) samples<br>- Insulin: 1R, 7L<br>- Glucose: 1R, 5 pooled (10L) samples<br>- qPCR: 1R, 5 pooled (5L) samples<br>- Locomotion: 3R, 24L | - Normality check: Shapiro-Wilk<br>- Two-way ANOVA, Bonferroni post hoc<br>- One-way ANOVA<br>- t-test<br>- Significance p < 0.05   |
| Pitt et al. 2018a            | PS Bangs Laboratories Inc. (USA)<br>Dyed beads (Dragon Green)<br>Cat. # FSDG001<br><br>Solvent: ZF water (Instant Ocean) | 0.1 % SDS<br>0.09% NaN <sub>3</sub> | 40 nm<br>Round<br><br>Route: Medium<br><br>- 21 mV              | Range: 20 to 100 nm   | Low aggregation        | 0.1, 1, and 10 µg/ml  | 6 to 168 hpf<br>Total: 162 h<br><br>MR: No   | Mortality: 3R, 240L each<br>Hatching: 3R, 50L each<br>Heart rate: 3R, 5L each<br>Deformities: 1R, 5 L each<br>Fluorescence: 1R, 5 L each<br>Locomotion: 3R, 72L each  | - Fluorescence: One/two –way ANOVA with Fisher's post hoc. Fluorescence data log transformed<br>- Heart rate: Two way ANOVA (treatment and cohort as factors) and Fisher's post hoc<br>- Locomotion: Mixed-design repeated measures ANOVA (treatment and cohort factors) and Dunnett's post hoc<br>- p < 0.05<br>- GraphPadPrism 6.0 and StatView 5.0.1 |
| Hu et al. 2020               | PS Bangs Laboratories Inc. (USA)<br>Cat. # PS02002 (not dyed) and FSDG001 (green)<br><br>Solvent: Unknown                | Not stated                          | 40 nm<br>Round<br><br>Route: Medium<br><br>Negative charge      | Data not available  | Data not available     | 0.1, 1, and 10 µg/ml  | 5 – 120 hpf<br><br>Total: 115 h<br><br>MR: No  | -Mortality, development, hatching: 1R, 30L<br>-Heart rate: 1R, 10L<br>-Locomotion: 1R, 15L<br>-Accumulation: 1R, 3L<br>-Transcriptome: 1R, 3 pooled samples, number of larvae per sample unknown  | One-way ANOVA<br>Significance p < 0.05  |
| Zhang et al. 2020a<br>Medium | PS Bangs Laboratories Inc. (USA)<br>Dyed green<br>Cat. # FSDG001<br><br>Solvent: Embryo medium                           | 2mM NaN <sub>3</sub>                | 40 nm<br><br>Route: Medium<br><br>Negative charge               | 70±9.21   | Slight aggregation     | 0.5 and 5 µg/ml   | 1 – 96 hpf<br><br>Total: 95h<br><br>MR: every 24h                                    | -Mortality, development: 1R, 9L<br>- Locomotion: 3R, 15L<br>- Gene expression: 1R, 6 pooled (3L) samples<br>- Fluorescence: 2R, 5L<br>*In some instances, number of replicates  | -One-way ANOVA, Tukey's post hoc<br>- Significance p < 0.05   |

|                      |   |                                     |   |                                   |                    |  |  |   |  |
|----------------------|---|-------------------------------------|---|-----------------------------------|--------------------|--|--|---|--|
|                      |   |                                     |   |                                   |                    |  |  | explained in figures does not coincide with methods section   |  |
| Trevisan et al. 2019 | PS Bangs Laboratories Inc. (USA)<br><br>Solvent: Danieau médium (30%)   | 0.1 % SDS<br>0.05% NaN <sub>3</sub> | 44 nm Round<br><br>Route: Medium<br><br>- 38 mV   | Average size in medium: 44.73 nm  | Low aggregation    | 10 µg/ml   | 6 to 96 hpf<br><br>Total: 90 h<br><br>MR: No   | -Mortality and malformations: 3R, 20L each<br>- Heart rate: 2R, 10L each<br>- EROD: 3R, 10L each<br>- Blood vessel formation: 3R, 4L each<br>- OCR 24h: 4R, 5-6L each<br>- NADH production: 3R, 10L each<br>- Mitochondrial content: 3R, 10L each<br><br>*In some instances, number of replicates explained in figures does not coincide with methods section | - Normality check with Shapiro-Wilk and Kolmogorov-Smirnov<br>- ANOVA with Tukey post hoc for parametric variables<br>- Kruskal-Wallis with Dunn's post hoc for non-parametric variables<br>- Significance p < 0.05                            |
| Trevisan et al. 2020 | PS Bangs Laboratories Inc. (USA)<br><br>Solvent: Danieau médium (30%)   | 0.1 % SDS<br>0.05% NaN <sub>3</sub> | 44 nm Round<br><br>Route: Medium<br><br>- 38 mV   | Average size in medium: 44.73 nm  | Low aggregation    | 1 µg/ml  | 6 to 96 hpf<br><br>Total: 90 h<br><br>MR: No   | -Mortality and malformations: 3R, 30L each<br>- Heart rate: 3R, 12L each<br>- OCR and CO <sub>2</sub> : 3R, 7-8L each<br>- NADH production: 3R, 10L each<br>*In some instances, number of replicates explained in figures does not coincide with methods section  | - Normality check: Shapiro-Wilk<br>-Homogeneity of variance check: Bartlett's<br>- One-way ANOVA with Tukey post hoc for parametric variables<br>- Kruskal-Wallis with Dunn's post hoc for non-parametric variables<br>- Significance p < 0.05 |
| Chen et al. 2017     | PS Polysciences Co. (PA, USA)<br><br>Solvent: DMSO 0.05% in artificial freshwater                                 | Not stated                          | 50 nm Round<br><br>Route: Medium<br><br>- 30 mV   | Average size in medium: 47±0.2 nm | No aggregation     | 1 µg/ml  | 3 to 120 hpf<br><br>Total: 117 h<br><br>MR: every 24h  | -Swimming: 6R, 10L each<br>-Length: 3R, 20L each<br>- RNA: 3R, 30L each<br>- Enzymes: 3R, 30L each  | - Normality check with Shapiro-Wilk<br>- One-way ANOVA with Tukey's post-hoc (SPSS)<br>- PCA to estimate contribution to locomotor activity<br>- Significance p < 0.05   |
| Lee et al. 2019      | PS Polyscience Inc. (USA)<br>Undyed (toxicity) and Dyed (accumulation) beads (green)<br><br>Solvent: Embryo water | Not stated                          | 50 (toxicity and accumulation), 200 and 500 nm (only accumulation)<br>Round<br><br>Route: Medium<br><br>- 40 mV | Data not available                | No aggregation     | 100 µg/ml  | 24 to 48 hpf<br><br>Total: 24 h  | -NP Accumulation: Not clear<br>- Mortality, hatching, malformations: 3R, 10L each<br>- Cell death (AO): Not clear<br>- RNA: Not clear<br>- ROS: Not clear<br>- Electron microscopy: Not clear   | - No Normality check<br>- ANOVA<br>- Significance p < 0.05   |
| Zhao et al. 2020     | Baseline Chromtech Research Center (China)<br><br>Solvent: Embryo water E3  | Not stated                          | 100nm and 65 nm Round, dyed (accumulation) and undyed (toxicology)<br><br>Route: Medium<br><br>Negative charge  | Data not available                | Data not available | Range of 0.02 – 200 µg/ml for heart rate, hatching and body length (100nm)<br><br>All other assays (65 nm): 20 µg/ml | 4-168 hpf<br><br>Total: 72, 164 depending on assay<br>Up to 12dpf for vertebrae assay<br>MR: every 24h | -Preliminary analysis with 100 nm NP: 1R, 20 L<br>Mortality, development, hatching, heart rate: 1R, 60L<br>-Accumulation: 1R, 15L<br>-Thyroid hormones: 1R, 3 pooled (20L)<br>-Metabolome: 1R, 5 pooled (200L)<br>- Vertebrae calcification: 1R, 10L  | - ANOVA with Tukey post hoc<br>- Significance p < 0.05<br>- PLS-DA for metabolomics analysis   |

|                       |   |   |  |  |  |  |   |  |   |
|-----------------------|---|---|--|--|--|--|---|--|---|
| Duan et al. 2020      | PS Bangs Laboratories Inc. (USA)<br><br>Solvent: Embryo water   | 0.1 % SDS<br>0.05 – 0.09 % NaN <sub>3</sub>                     | 100 nm<br>Round, dyed red and undyed<br><br>Route: Medium<br><br>Negative charge | Average size in medium: 100 nm   | Data not available   | 200 µg/ml  | 0-120 hpf<br><br>Total: 24, 48, 60, 72 h depending on assay<br><br>MR: No                   | - Mortality, hatching, heart rate, blood flow: 3R, 20L<br>-Metabolome:1R, 3 pooled (50L) samples   | t-test<br>PCA<br>Significance p < 0.05  |
| Liu et al. 2021       | Tiajin Baseline ChemTech Research Center (China)<br><br>Solvent: MilliQwater, 0.1% DMSO   | Not stated  | 100 nm<br>Round<br>Route: Medium<br><br>Negative charge                          | Data not available   | Data not available   | Survival, heart rate and locomotion: 0.01 µg/ml<br>Genetic analysis: 0.001, 0.01, 0.1 µg/ml                        | Exposed: 2- 12hpf<br>Total: 10 h<br>Genetic analysis at 12 hpf<br>Others: 120 hpf<br>MR: No | - Mortality, heart rate, locomotion: 3R, 24L<br>- qPCRA and transcriptome:3R, 12 pooled (40L) samples  | - ANOVA with Tukey post hoc<br>- Significance p < 0.05<br>- Transcriptome: PCA; UMAP, Go functional enrichment, KEGG pathway                                      |
| Parenti et al 2019    | PS Bangs Laboratories Inc. (USA)<br>Dyed beads (red)<br>Cat. # FSFR003<br><br>Solvent: ZF water (Instant Ocean, 0.1% methylene blue)  | 0.1 % Tween 20 and 0.09 % NaN <sub>3</sub>                      | 500 nm<br>Round<br>Route: Medium<br><br>0.13 mV                                  | Average size in medium<br>533.17±17.8 nm                                       | Data not available   | 1 µg/ml  | 72 to 120 hpf<br><br>Total: 48 h<br><br>MR: every 24h                                       | Locomotion: 3R, 12L each<br>P-gp: 1R, 30L pooled and 3 aliquots measured<br>COX: 1R, 70L pooled and 3 aliquots measured<br>GST, CAT, GPx, SOD: 1R, 60L pooled and 3 aliquots measured<br>PCC: 1R, 80L pooled and 3 aliquots measured<br>ROS: 1R, 12L | Normality check with Shapiro-Wilk<br>- One-way ANOVA with Duncan post-hoc (STATISTICA 7.0)<br>- Locomotion: t-test<br>- Significance p < 0.05                     |
| Zhang and Goss 2020   | PS Thermo Fisher Scientific Co.<br>Dyed beads (green)<br>Cat. # F8813 and F88787<br><br>Solvent: Embryo water   | 2mM NaN <sub>3</sub><br><br>Dialysis was performed to remove it | 20 and 500 nm<br>Round<br>Route: Medium<br><br>- 13 mV                           | Average sizes in medium<br>35.5±2 nm and 522.6±17 nm                           | No aggregation   | Assays: 10 µg/ml<br>Cl <sub>50</sub> : 3.125 – 50 µg/ml  | 24 – 120 hpf<br>Total: 96 h<br><br>MR: every 48h  | -CL <sub>50</sub> : 1R, 4wells of a 6 well plate (not known how many embryos in each)<br>- Malformations and EROD: 1R, 10L   | Normality check: Shapiro-Wilk<br>-Homogeneity of variance check: Breusch-Pagan<br>- Two-way ANOVA with Tukey post hoc<br>- Significance p < 0.05                  |
| VanPommen et al. 2017 | 25 and 50nm PS Thermo Fisher Scientific Co.<br>Dyed beads (red)<br>Cat. # R25 and R50<br>250 and 700nm<br>Corpuscular Inc. (USA)<br>Dyed beads (red)<br>Cat. # 103127-05 and 103129-05<br>Solvent: Embryo water (instant ocean) | Not stated  | 25, 50, 250 and 700 nm<br>Round<br>Route: Medium<br><br>- 5 mV to -30 mV         | Average sizes in medium:<br>38.1±7.1<br>70.6±18.1<br>298.4±24.7<br>943.6±108.2 | 25 nm NP tended to aggregate after 24h<br>All others showed little aggregation | 25 and 50 nm NP → 0-48 hpf: 25 µg/ml; 24-72 hpf and 72-120 hpf: 50 µg/ml<br>250 and 700 nm NP → 5 µg/ml all stages | 0-120 hpf<br>Total: 48 h per exposure<br><br>MR: every 24h                                  | -Mortality and malformations: 1R, 10L<br>- Microscopy: 1R, 10L? not clear<br>- Eye size: 1R, 4L  | - One-way ANOVA<br>- Significance p < 0.05  |
| Liu et al. 2019       | PS Aladdin Industrial (China)<br><br>Solvent:E3 medium  | Not stated  | 100 nm<br>Round<br>Route: Medium<br><br>- 20 mV                                  | Average sizes in medium:<br>100 nm   | Low aggregation  | 0.001 and 1 µg/ml  | 0 -96 hpf<br>Total 96 h<br><br>MR: No   | -Mortality, development: 2R, 20-24L<br>-ROS and SOD: 3R, 20-24L  | - One-way ANOVA<br>- Significance p < 0.05  |
| Pedersen et al. 2020  | PS Bangs Laboratories Inc. (USA)<br>Dyed beads (Dragon Green)<br><br>Solvent: ZF water (Instant Ocean)  | 0.1 % Tween 20 and 2mM NaN <sub>3</sub>                         | 50 and 200 nm<br>Round<br>Route: Medium<br><br>Negative charge                   | Average sizes in medium 42 and 194 nm  | Data not available   | 0.01, 0.1, 1 and 10 µg/ml  | 6 to 120 hpf<br><br>Total: 114 h<br><br>MR: every 24h                                       | Locomotion: 3R, 20L each<br>Fluorescence: 1R, 20L<br>Transcriptomic: 3R, 3 pooled (5L) samples each but only 5 analyzed  | - Normality check with Shapiro-Wilk<br>- One-way ANOVA with Tuckey post-hoc (R)<br>- Significance p < 0.05<br>- Abnormalities, mortality and hatching: Chi-Square |

|                                 |  |  |  |   |                    |  |  |  |   |
|---------------------------------|--|--|--|---|--------------------|--|--|--|---|
|                                 |  |  |  |   |                    |  |  |  | - Differential gene expression: DESeq <sup>2</sup>  |
| Sökmen et al. 2020              | PS Thermo Fisher Scientific Co.<br>Dyed beads (red)<br>Cat. # R25<br><br>Stock solution injected   | SDS and NaN <sub>3</sub><br><br>Controlled for                                     | 25 nm Round<br><br>Route: Injection<br><br>Negative charge             | Average size in medium 20±3.2 nm  | Data not available | 0.81 ng injected                               | 4 to 120 hpf<br>Total: 116 h   | - Mortality and hatching: 3R, 2000L each<br>- Apoptosis, ROS: 1R, 50L<br>- NP detection: 1R, 150L<br>- Immunofluorescence: 1R, 20L   | -ANOVA with Tukey's post hoc<br>- Significance p < 0.05   |
| Brun et al. 2018<br>Injection   | PS Thermo Fisher Scientific Co.<br>Dyed beads (red)<br><br>Solvent: MilliQ water   | Not stated   | 25 nm Round<br><br>Route: Injection<br><br>Negative charge             | Average size in the medium of 19.3±0.6 nm after 0 h or 18.8±0.9 nm after 24 h                             | Low aggregation    | 0.01 ng injected per embryo                    | Injected at 30 hpf<br><br>Total: 24 h                                  | - Body imaging: 1R, 10L<br>- Gene expression: 1R, 5 pooled (15L) samples   | - Homogeneity of Variance: Bartlett's<br>- One-way ANOVA, Bonferroni post hoc<br>- Significance p < 0.05  |
| Zhang et al. 2020a<br>Injection | PS Bangs Laboratories Inc. (USA)<br>Solvent: RO water<br>Dyed beads (green)  | 2mM NaN <sub>3</sub>   | 40 nm Round<br><br>Route: Injection<br><br>Negative charge             | 70±9.21   | Slight aggregation | 0.52 ng, 1.56 ng or 2.6 ng injected per embryo | Injected at 0 hpf 1 – 96 hpf<br><br>Total: 95h<br><br>MR: every 24h    | -Mortality, development: 1R, 9L<br>- Locomotion: 3R, 15L<br>- Gene expression: 1R, 6 pooled (3L) samples<br>- Fluorescence: 2R, 5L<br>*In some instances, number of replicates explained in figures does not coincide with methods section | -One-way ANOVA, Tukey's post hoc<br>- Significance p < 0.05   |
| Veneman et al. 2017             | PS Corpuscular Inc. (USA)<br>Dyed beads (red)<br>Cat. # 103125-05<br><br>Solvent: Embryo water (instant ocean)                                   | Not stated   | 700 nm Round<br><br>Route: Injection<br><br>Negative charge            | Data not available  | Data not available | 5 ng injected per embryo                       | 1-120 hpf<br>Injected at 1 hpf and 48 hpf<br>Sampled at 72 and 120 hpf | -Mortality and malformations: 1R,150L<br>-RNAseq: 1R, 10L pooled and 3 aliquots measured   | - Differential gene expression: DESeq<br>- Significance p < 0.05  |
| Pitt et al. 2018b               | PS Bangs Laboratories Inc. (USA)<br>Dyed beads (green) and non-dyed beads<br>Cat. # FSDG001 and PS02002<br><br>Solvent: ZF water (Instant Ocean) | 0.1 % SDS and 0.05 - 0.09 % NaN <sub>3</sub><br><br>Ultrafiltration to remove them | 40 nm Round<br><br>Route: Maternal Paternal Co-parental<br><br>- 25 mV | Non-dyed beads average size in medium: 30.67±8.97 nm<br>Dyed beads average size in medium: 34.5 ± 10.8 nm | Low aggregation    | Unknown  | 6 to 144 hpf<br><br>Total: 138   | Mortality and development: 2R, 30L each<br>Antioxidant enzymes: 3R, 6L each<br>Heart rate: 2R, 10L each<br>Fluorescence: 2R, 3L each<br>Locomotion: 2R, 12-24L each  | - Normality check with Shapiro-Wilk<br>- ANOVA with Tukey post hoc or t-test for parametric variables<br>- Kruskal-Wallis with Dunn's post hoc or Mann-Whitney U for non-parametric variables<br>- Locomotion: Two-way ANOVA (treatment and cohort factors)<br>- p < 0.05<br>- GraphPadPrism6.0 |

**Table A.2.** Number of endpoints by study used to calculate % effects. Total: total number of endpoints studied. Significant: Number of endpoints that were significantly affected by NP. Non-significant: Number of endpoints that were not significantly affected by NP. I: injection exposure.

|                      | Concentration (µg/ml) | Size (nm) | Total | Significant | Non-significant | % Significant Effects |
|----------------------|-----------------------|-----------|-------|-------------|-----------------|-----------------------|
| Brun et al. 2018     | 10                    | 25        | 18    | 9           | 9               | 50.00                 |
| Brun et al. 2019     | 20                    | 25        | 13    | 10          | 3               | 76.92                 |
| Pitt et al. 2018a    | 0.1                   | 40        | 8     | 3           | 5               | 37.50                 |
|                      | 1                     | 40        | 8     | 3           | 5               | 37.50                 |
|                      | 10                    | 40        | 8     | 3           | 5               | 37.50                 |
| Hu et al. 2020       | 0.1                   | 40        | 14    | 5           | 9               | 35.71                 |
|                      | 1                     | 40        | 14    | 7           | 7               | 50.00                 |
|                      | 10                    | 40        | 15    | 12          | 3               | 80.00                 |
| Zhang et al. 2020a   | 0.5                   | 40        | 13    | 6           | 7               | 46.15                 |
|                      | 5                     | 40        | 11    | 6           | 5               | 54.55                 |
| Trevisan et al. 2019 | 10                    | 44        | 16    | 5           | 11              | 31.25                 |
| Trevisan et al. 2020 | 1                     | 44        | 15    | 2           | 13              | 13.33                 |
| Chen et al. 2017     | 1                     | 50        | 11    | 7           | 5               | 63.64                 |
| Lee et al. 2019      | 100                   | 50        | 10    | 4           | 6               | 40.00                 |
|                      | 100                   | 200       | 2     | 1           | 1               | 50.00                 |
|                      | 100                   | 500       | 2     | 1           | 1               | 50.00                 |
| Zhao et al. 2020     | 0.02                  | 100       | 3     | 0           | 3               | 0.00                  |
|                      | 0.2                   | 100       | 3     | 0           | 3               | 0.00                  |
|                      | 2                     | 100       | 3     | 0           | 3               | 0.00                  |
|                      | 20                    | 100       | 3     | 0           | 3               | 0.00                  |
|                      | 200                   | 100       | 3     | 2           | 1               | 66.67                 |
|                      | 20                    | 65        | 10    | 2           | 8               | 20.00                 |
| Duan et al. 2020     | 200                   | 100       | 7     | 5           | 2               | 71.43                 |
| Liu et al. 2021      | 0.001                 | 100       | 10    | 5           | 5               | 50.00                 |
|                      | 0.01                  | 100       | 17    | 10          | 7               | 58.82                 |
|                      | 0.1                   | 100       | 10    | 5           | 5               | 50.00                 |
| Paranti et al 2019   | 1                     | 500       | 8     | 3           | 5               | 37.50                 |
| Zhang and Goss 2020  | 10                    | 20        | 5     | 1           | 4               | 20.00                 |

|                         |          |     |    |   |   |        |
|-------------------------|----------|-----|----|---|---|--------|
|                         | 10       | 500 | 5  | 1 | 4 | 20.00  |
| Van Pomeran et al. 2017 | 50       | 25  | 3  | 1 | 2 | 33.33  |
|                         | 50       | 50  | 3  | 1 | 2 | 33.33  |
|                         | 5        | 250 | 3  | 1 | 2 | 33.33  |
|                         | 5        | 700 | 3  | 1 | 2 | 33.33  |
| Liu et al. 2019         | 0.001    | 100 | 5  | 1 | 4 | 20.00  |
|                         | 1        | 100 | 5  | 1 | 4 | 20.00  |
| Pedersen et al. 2020    | 0.01     | 50  | 5  | 0 | 5 | 0.00   |
|                         | 0.1      | 50  | 5  | 1 | 4 | 20.00  |
|                         | 1        | 50  | 6  | 1 | 5 | 16.67  |
|                         | 10       | 50  | 6  | 2 | 4 | 33.33  |
|                         | 0.01     | 200 | 5  | 0 | 5 | 0.00   |
|                         | 0.1      | 200 | 5  | 1 | 4 | 20.00  |
|                         | 1        | 200 | 6  | 3 | 3 | 50.00  |
|                         | 10       | 200 | 6  | 3 | 3 | 50.00  |
| Sökmen et al. 2020      | 0.81     | 25  | 7  | 7 | 0 | 100.00 |
| Brun et al. 2018 I      | 0.01     | 25  | 11 | 4 | 7 | 36.36  |
| Zhang et al. 2020a I    | 0.52     | 40  | 13 | 4 | 9 | 30.77  |
|                         | 1.56     | 40  | 13 | 4 | 9 | 30.77  |
|                         | 2.6      | 40  | 13 | 6 | 7 | 46.15  |
| Veneman et al. 2017     | 5        | 700 | 4  | 2 | 2 | 50.00  |
| Pitt et al. 2018b       | Maternal | 40  | 10 | 5 | 5 | 50.00  |

**Table A.3.** Results of endpoints per study. Yes: This endpoint was studied and effect was found. No: This endpoint was studied and effect was not found. NA: This endpoint was not studied. I: injection exposure.

|                      | Concentration (µg/ml) | Size (nm) | Mortality | Hatching | Development | Heart rate | Locomotion | Accumulation | Genetic | Metabolic |
|----------------------|-----------------------|-----------|-----------|----------|-------------|------------|------------|--------------|---------|-----------|
| Brun et al. 2018     | 10                    | 25        | No        | No       | No          | NA         | NA         | Yes          | Yes     | NA        |
| Brun et al. 2019     | 20                    | 25        | No        | NA       | Yes         | NA         | Yes        | Yes          | Yes     | Yes       |
| Pitt et al. 2018a    | 0.1                   | 40        | No        | No       | No          | Yes        | Yes        | Yes          | NA      | No        |
|                      | 1                     | 40        | No        | No       | No          | Yes        | Yes        | Yes          | NA      | No        |
|                      | 10                    | 40        | No        | No       | No          | Yes        | Yes        | Yes          | NA      | No        |
| Hu et al. 2020       | 0.1                   | 40        | No        | No       | No          | Yes        | Yes        | Yes          | Yes     | NA        |
|                      | 1                     | 40        | No        | No       | No          | Yes        | Yes        | Yes          | Yes     | NA        |
|                      | 10                    | 40        | No        | No       | No          | Yes        | Yes        | Yes          | Yes     | NA        |
| Zhang et al. 2020a   | 0.5                   | 40        | No        | No       | Yes         | NA         | No         | Yes          | Yes     | NA        |
|                      | 5                     | 40        | No        | No       | NA          | NA         | Yes        | Yes          | Yes     | NA        |
| Trevisan et al. 2019 | 10                    | 44        | No        | NA       | No          | No         | NA         | NA           | NA      | Yes       |
| Trevisan et al. 2020 | 1                     | 44        | No        | No       | No          | No         | NA         | NA           | NA      | Yes       |
| Chen et al. 2017     | 1                     | 50        | No        | NA       | Yes         | NA         | Yes        | NA           | Yes     | Yes       |
| Lee et al. 2019      | 100                   | 50        | No        | No       | No          | NA         | NA         | Yes          | No      | Yes       |
|                      | 100                   | 200       | No        | NA       | NA          | NA         | NA         | Yes          | NA      | NA        |
|                      | 100                   | 500       | No        | NA       | NA          | NA         | NA         | Yes          | NA      | NA        |
| Zhao et al. 2020     | 0.02                  | 100       | NA        | No       | No          | No         | NA         | NA           | NA      | NA        |
|                      | 0.2                   | 100       | NA        | No       | No          | No         | NA         | NA           | NA      | NA        |
|                      | 2                     | 100       | NA        | No       | No          | No         | NA         | NA           | NA      | NA        |
|                      | 20                    | 100       | NA        | No       | No          | No         | NA         | NA           | NA      | NA        |
|                      | 200                   | 100       | NA        | Yes      | Yes         | No         | NA         | NA           | NA      | NA        |
|                      | 20                    | 65        | No        | No       | No          | No         | NA         | Yes          | NA      | Yes       |
| Duan et al. 2020     | 200                   | 100       | No        | Yes      | NA          | Yes        | NA         | Yes          | NA      | Yes       |
| Liu et al. 2021      | 0.001                 | 100       | NA        | NA       | NA          | NA         | NA         | NA           | Yes     | NA        |
|                      | 0.01                  | 100       | No        | NA       | NA          | Yes        | Yes        | NA           | Yes     | NA        |
|                      | 0.1                   | 100       | NA        | NA       | NA          | NA         | NA         | NA           | Yes     | NA        |
| Paranti et al 2019   | 1                     | 500       | NA        | NA       | NA          | NA         | NA         | Yes          | NA      | Yes       |
| Zhang and Goss 2020  | 10                    | 20        | No        | No       | No          | NA         | NA         | Yes          | NA      | No        |
|                      | 10                    | 500       | No        | No       | No          | NA         | NA         | Yes          | NA      | No        |

|   |          |     |             |             |             |             |             |             |             |             |
|---|----------|-----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Van Pomeran et al. 2017                   | 50       | 25  | No          | NA          | No          | NA          | NA          | Yes         | NA          | NA          |
|   | 50       | 50  | No          | NA          | No          | NA          | NA          | Yes         | NA          | NA          |
|   | 5        | 250 | No          | NA          | No          | NA          | NA          | Yes         | NA          | NA          |
|   | 5        | 700 | No          | NA          | No          | NA          | NA          | Yes         | NA          | NA          |
| Liu et al. 2019                           | 0.001    | 100 | No          | NA          | No          | NA          | NA          | NA          | NA          | Yes         |
|   | 1        | 100 | No          | NA          | No          | NA          | NA          | NA          | NA          | Yes         |
| Pedersen et al. 2020                      | 0.01     | 50  | No          | No          | No          | NA          | No          | No          | NA          | NA          |
|   | 0.1      | 50  | No          | No          | No          | NA          | No          | Yes         | NA          | NA          |
|   | 1        | 50  | No          | No          | No          | NA          | No          | Yes         | No          | NA          |
|   | 10       | 50  | No          | No          | No          | NA          | No          | Yes         | Yes         | NA          |
|   | 0.01     | 200 | No          | No          | No          | NA          | No          | No          | NA          | NA          |
|   | 0.1      | 200 | No          | No          | No          | NA          | No          | Yes         | NA          | NA          |
|   | 1        | 200 | No          | No          | No          | NA          | Yes         | Yes         | Yes         | NA          |
| Sökmen et al. 2020                        | 10       | 200 | No          | No          | No          | NA          | Yes         | Yes         | Yes         | NA          |
|   | 0.81     | 25  | Yes         | Yes         | Yes         | NA          | NA          | Yes         | NA          | Yes         |
| Brun et al. 2018 Inj                      | 0.01     | 25  | NA          | NA          | NA          | NA          | NA          | NA          | Yes         | NA          |
| Zhang et al. 2020a Inj                    | 0.52     | 40  | No          | No          | NA          | NA          | No          | Yes         | Yes         | NA          |
|   | 1.56     | 40  | No          | No          | NA          | NA          | No          | Yes         | Yes         | NA          |
|   | 2.6      | 40  | No          | No          | No          | NA          | No          | Yes         | Yes         | NA          |
| Veneman et al. 2017                       | 5        | 700 | No          | NA          | No          | NA          | NA          | Yes         | Yes         | NA          |
| Pitt et al. 2018b                         | Maternal | 40  | No          | NA          | Yes         | Yes         | No          | Yes         | NA          | Yes         |
| <b>No</b>                                 |          |     | <b>40</b>   | <b>29</b>   | <b>33</b>   | <b>8</b>    | <b>11</b>   | <b>2</b>    | <b>2</b>    | <b>5</b>    |
| <b>Yes</b>                                |          |     | <b>1</b>    | <b>3</b>    | <b>6</b>    | <b>9</b>    | <b>12</b>   | <b>34</b>   | <b>19</b>   | <b>12</b>   |
| <b>Percent No</b>                         |          |     | <b>97.6</b> | <b>90.6</b> | <b>84.6</b> | <b>47.1</b> | <b>47.8</b> | <b>5.6</b>  | <b>9.5</b>  | <b>29.4</b> |
| <b>Percent Yes</b>                        |          |     | <b>2.4</b>  | <b>9.4</b>  | <b>15.4</b> | <b>52.9</b> | <b>52.2</b> | <b>94.4</b> | <b>90.5</b> | <b>70.6</b> |
| <b>Total number that studied endpoint</b> |          |     | <b>41</b>   | <b>32</b>   | <b>39</b>   | <b>17</b>   | <b>23</b>   | <b>36</b>   | <b>21</b>   | <b>17</b>   |
| <b>% studies that tested endpoint</b>     |          |     | <b>82</b>   | <b>64</b>   | <b>78</b>   | <b>34</b>   | <b>46</b>   | <b>72</b>   | <b>42</b>   | <b>34</b>   |

**Table A.4.** Results of Locomotion and Heart Rate effects. Yes: This effect was found. No: This effect was not found. NA: This endpoint was not studied. M: medium exposure. I: injection exposure.

|                      |                       |           | Locomotion    |              | Heart Rate  |              |            |            |
|----------------------|-----------------------|-----------|---------------|--------------|-------------|--------------|------------|------------|
|                      | Concentration (µg/ml) | Size (nm) | Hyperactivity | Hypoactivity | Increase HR | Decreased HR | Both       | No effect  |
| Brun et al. 2018     | 10                    | 25        | NA            | NA           | NA          | NA           | NA         | NA         |
| Brun et al. 2019     | 20                    | 25        | <b>Yes</b>    | <b>No</b>    | NA          | NA           | NA         | NA         |
| Pitt et al. 2018a    | 0.1                   | 40        | <b>No</b>     | <b>Yes</b>   | <b>No</b>   | <b>Yes</b>   | NA         | NA         |
|                      | 1                     | 40        | <b>No</b>     | <b>Yes</b>   | <b>No</b>   | <b>Yes</b>   | NA         | NA         |
|                      | 10                    | 40        | <b>No</b>     | <b>Yes</b>   | <b>No</b>   | <b>Yes</b>   | NA         | NA         |
| Hu et al. 2020       | 0.1                   | 40        | <b>No</b>     | <b>Yes</b>   | <b>No</b>   | <b>Yes</b>   | NA         | NA         |
|                      | 1                     | 40        | <b>No</b>     | <b>Yes</b>   | <b>No</b>   | <b>Yes</b>   | NA         | NA         |
|                      | 10                    | 40        | <b>No</b>     | <b>Yes</b>   | <b>No</b>   | <b>Yes</b>   | NA         | NA         |
| Zhang et al. 2020a   | 0.5                   | 40        | <b>No</b>     | <b>No</b>    | NA          | NA           | NA         | NA         |
|                      | 5                     | 40        | <b>No</b>     | <b>Yes</b>   | NA          | NA           | NA         | NA         |
| Trevisan et al. 2019 | 10                    | 44        | NA            | NA           | NA          | NA           | NA         | <b>Yes</b> |
| Trevisan et al. 2020 | 1                     | 44        | NA            | NA           | NA          | NA           | NA         | <b>Yes</b> |
| Chen et al. 2017     | 1                     | 50        | <b>No</b>     | <b>Yes</b>   | NA          | NA           | NA         | NA         |
| Lee et al. 2019      | 100                   | 50        | NA            | NA           | NA          | NA           | NA         | NA         |
|                      | 100                   | 200       | NA            | NA           | NA          | NA           | NA         | NA         |
|                      | 100                   | 500       | NA            | NA           | NA          | NA           | NA         | NA         |
| Zhao et al. 2020     | 0.02                  | 100       | NA            | NA           | <b>No</b>   | <b>No</b>    | <b>No</b>  | <b>Yes</b> |
|                      | 0.2                   | 100       | NA            | NA           | <b>No</b>   | <b>No</b>    | <b>No</b>  | <b>Yes</b> |
|                      | 2                     | 100       | NA            | NA           | <b>No</b>   | <b>No</b>    | <b>No</b>  | <b>Yes</b> |
|                      | 20                    | 100       | NA            | NA           | <b>No</b>   | <b>No</b>    | <b>No</b>  | <b>Yes</b> |
|                      | 200                   | 100       | NA            | NA           | <b>No</b>   | <b>No</b>    | <b>No</b>  | <b>Yes</b> |
|                      | 20                    | 65        | NA            | NA           | NA          | NA           | NA         | <b>Yes</b> |
| Duan et al. 2020     | 200                   | 100       | NA            | NA           | NA          | NA           | <b>Yes</b> | NA         |
| Liu et al. 2021      | 0.001                 | 100       | NA            | NA           | NA          | NA           | NA         | NA         |
|                      | 0.01                  | 100       | <b>No</b>     | <b>Yes</b>   | <b>Yes</b>  | <b>No</b>    | <b>No</b>  | NA         |
|                      | 0.1                   | 100       | NA            | NA           | NA          | NA           | NA         | NA         |
| Paranti et al 2019   | 1                     | 500       | NA            | NA           | NA          | NA           | NA         | NA         |
| Zhang and Goss 2020  | 10                    | 20        | NA            | NA           | NA          | NA           | NA         | NA         |

|                         |          |     |     |    |    |     |    |    |    |
|-------------------------|----------|-----|-----|----|----|-----|----|----|----|
|                         | 10       | 500 | NA  | NA | NA | NA  | NA | NA | NA |
| Van Pomeran et al. 2017 | 50       | 25  | NA  | NA | NA | NA  | NA | NA | NA |
|                         | 50       | 50  | NA  | NA | NA | NA  | NA | NA | NA |
|                         | 5        | 250 | NA  | NA | NA | NA  | NA | NA | NA |
|                         | 5        | 700 | NA  | NA | NA | NA  | NA | NA | NA |
| Liu et al. 2019         | 0.001    | 100 | NA  | NA | NA | NA  | NA | NA | NA |
|                         | 1        | 100 | NA  | NA | NA | NA  | NA | NA | NA |
| Pedersen et al. 2020    | 0.01     | 50  | No  | No | NA | NA  | NA | NA | NA |
|                         | 0.1      | 50  | No  | No | NA | NA  | NA | NA | NA |
|                         | 1        | 50  | No  | No | NA | NA  | NA | NA | NA |
|                         | 10       | 50  | No  | No | NA | NA  | NA | NA | NA |
|                         | 0.01     | 200 | No  | No | NA | NA  | NA | NA | NA |
|                         | 0.1      | 200 | No  | No | NA | NA  | NA | NA | NA |
|                         | 1        | 200 | Yes | No | NA | NA  | NA | NA | NA |
|                         | 10       | 200 | Yes | No | NA | NA  | NA | NA | NA |
| Sökmen et al. 2020      | 0.81     | 25  | NA  | NA | NA | NA  | NA | NA | NA |
| Brun et al. 2018 I      | 0.01     | 25  | NA  | NA | NA | NA  | NA | NA | NA |
| Zhang et al. 2020a I    | 0.52     | 40  | No  | No | NA | NA  | NA | NA | NA |
|                         | 1.56     | 40  | No  | No | NA | NA  | NA | NA | NA |
|                         | 2.6      | 40  | No  | No | NA | NA  | NA | NA | NA |
| Veneman et al. 2017     | 5        | 700 | NA  | NA | NA | NA  | NA | NA | NA |
| Pitt et al. 2018b       | Maternal | 40  | No  | No | No | Yes | NA | NA | NA |

|  |                            |             |             |             |             |             |             |
|--|----------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
|  | <b>No</b>                  | <b>20</b>   | <b>14</b>   | <b>12</b>   | <b>6</b>    | <b>6</b>    | <b>0</b>    |
|  | <b>Yes</b>                 | <b>3</b>    | <b>9</b>    | <b>1</b>    | <b>7</b>    | <b>1</b>    | <b>8</b>    |
|  | <b>Total studied</b>       | <b>23</b>   | <b>23</b>   | <b>17</b>   | <b>17</b>   | <b>17</b>   | <b>17</b>   |
|  | <b>Total did not study</b> | <b>27</b>   | <b>27</b>   | <b>33</b>   | <b>33</b>   | <b>33</b>   | <b>33</b>   |
|  | <b>Percent No</b>          | <b>87.0</b> | <b>60.9</b> | <b>70.6</b> | <b>35.3</b> | <b>35.3</b> | <b>0.0</b>  |
|  | <b>Percent Yes</b>         | <b>13.0</b> | <b>39.1</b> | <b>5.9</b>  | <b>41.2</b> | <b>5.9</b>  | <b>47.1</b> |

**Table A.5.** NP observation techniques in ZF embryos. Only articles that studied PSNP accumulation were used for percent calculations. FI: fluorescence microscopy, E.M.: electron microscopy, C.M.: confocal microscopy. Two articles that used both medium and injection exposures were counted only once for this analysis. Yes: This technique was used. No: This technique was not used. M: medium exposure. I: injection exposure.

|                         | <b>FI</b>          | <b>E. M.</b> | <b>C. M.</b> |
|-------------------------|--------------------|--------------|--------------|
| Brun et al. 2018 M      | <b>Yes</b>         | No           | No           |
| Brun et al. 2019        | <b>Yes</b>         | No           | No           |
| Pitt et al. 2018a       | <b>Yes</b>         | No           | No           |
| Hu et al. 2020          | <b>Yes</b>         | No           | No           |
| Zhang et al. 2020a M    | <b>Yes</b>         | No           | No           |
| Trevisan et al. 2019    | NA                 | NA           | NA           |
| Trevisan et al. 2020    | NA                 | NA           | NA           |
| Chen et al. 2017        | <b>Yes</b>         | No           | No           |
| Lee et al. 2019         | <b>Yes</b>         | <b>Yes</b>   | No           |
| Zhao et al. 2020        | <b>Yes</b>         | No           | No           |
| Duan et al. 2020        | <b>Yes</b>         | <b>Yes</b>   | No           |
| Liu et al. 2021         | NA                 | NA           | NA           |
| Paranti et al 2019      | No                 | No           | <b>Yes</b>   |
| Zhang et al. 2020b      | <b>Yes</b>         | No           | No           |
| Van Pomeran et al. 2017 | <b>Yes</b>         | No           | No           |
| Liu et al. 2019         | NA                 | NA           | NA           |
| Pedersen et al. 2020    | <b>Yes</b>         | No           | No           |
| Sökmen et al. 2020      | <b>Yes</b>         | <b>Yes</b>   | No           |
| Brun et al. 2018 I      | <b>Yes</b>         | No           | No           |
| Zhang et al. 2020aI     | <b>Yes</b>         | No           | No           |
| Veneman et al. 2017     | <b>Yes</b>         | No           | No           |
| Pitt et al. 2018b       | <b>Yes</b>         | No           | No           |
|                         | <b>No</b>          | <b>5</b>     | <b>17</b>    |
|                         | <b>Yes</b>         | <b>15</b>    | <b>3</b>     |
|                         | <b>Total</b>       | <b>20</b>    | <b>20</b>    |
|                         | <b>Percent No</b>  | <b>25.0</b>  | <b>85.0</b>  |
|                         | <b>Percent Yes</b> | <b>75.0</b>  | <b>15.0</b>  |

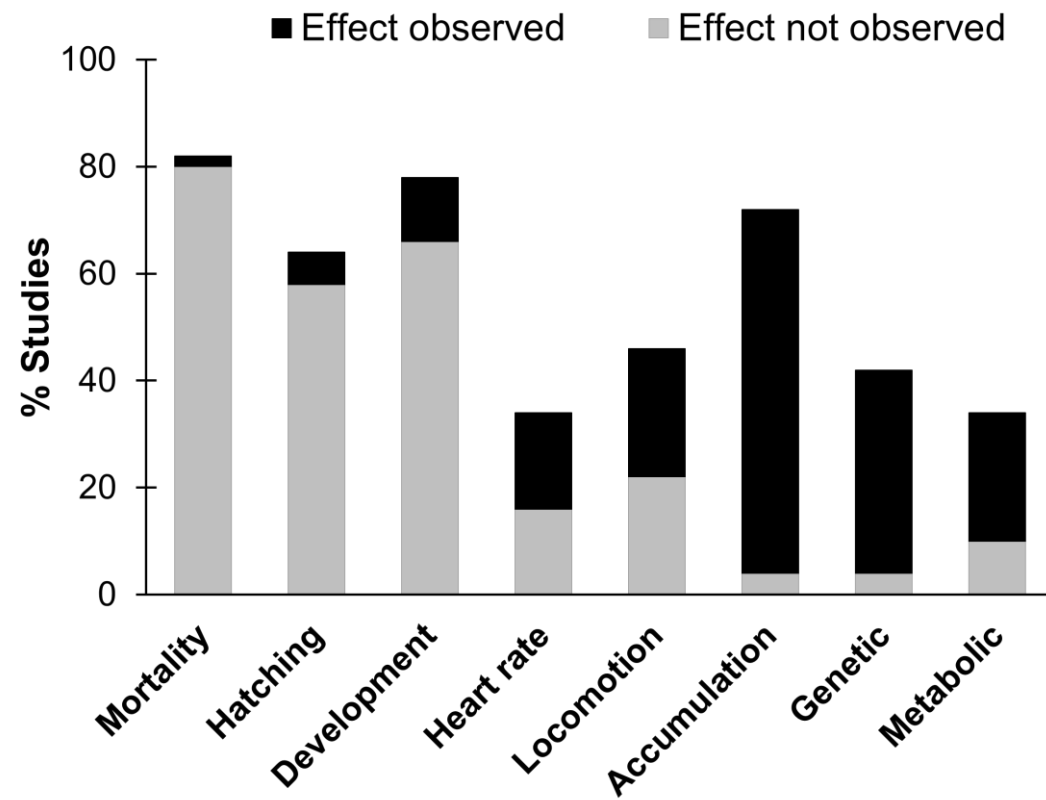
**Table A.6.** Full names and functions of validated gene transcripts used for analyses. Genes are in alphabetical order.

| Gene          | Name for analysis | Full Name                                | Function  |
|---------------|-------------------|--|---|
| aox5          | aox1              | Aldehyde oxidase 5                       | Produces hydrogen peroxide and, under certain conditions, can catalyze the formation of superoxide                              |
| apoa2         | apoa2             | Apolipoprotein A-II                      | High density lipoprotein, hypercholesterolemia  |
| arrdc3a       | arrdc3            | Arrestin Domain Containing 3             | Regulates G protein-mediated signaling, involved in cancer  |
| cat           | cat               | Catalase                                 | Protects cell from oxidative damage by reactive oxygen species (ROS)  |
| ccl20a        | ccl20             | Chemokine (C-C motif) ligand 20          | Chemokine (immunity)  |
| cyp19a1b      | cyp19a1           | Cytochrome P450 aromatase                | Converts androgens into estrogens, pivotal for reproduction and sex determination. Role in neuroprotection                      |
| dnmt1         | dnmt1             | DNA (cytosine-5)-methyltransferase 1     | Maintenance of DNA methylation, development   |
| dnmt3aa       | dnmt3a            | DNA (cytosine-5)-methyltransferase 3A    | De novo DNA methylation   |
| dnmt3bb1      | dnmt3b            | DNA methyltransferase beta, fish paralog | De novo DNA methylation, development  |
| fgf21         | fgf21             | Fibroblast growth factor 21              | Stimulates glucose uptake in adipocytes but not in other cell   |
| g6pca         | g6pc              | Glucose-6-phosphatase                    | Pathway gluconeogenesis, accumulation of glycogen and fat in the liver, glycogen storage disease                                |
| gfap          | gfap              | Glial fibrillary acidic protein          | Involved in many important CNS processes, upregulation causes neurodegenerative disease   |
| gpx           | gpx1              | Glutathione peroxidase                   | Detoxification of hydrogen peroxide, and is one of the most important antioxidant enzymes                                       |
| gr            | gsr               | Glutathione reductase                    | Critical enzyme in resisting oxidative stress and maintaining the reducing environment of the cell                              |
| gstp          | gstp1             | Glutathione S-transferase P              | Role in detoxification, thought to function in xenobiotic metabolism  |
| ifn- $\gamma$ | ifng              | Interferon gamma                         | Cellular response to viral and microbial infections   |
| il1- $\beta$  | il1b              | Interleukin 1 beta                       | Cytokine (immunity)   |
| il6           | il6               | Interleukin                              | Pro-inflammatory cytokine and an anti-inflammatory myokine, role in chronic inflammation, autoimmune disease, cancer            |
| irg1l         | acod1             | Immune-Responsive Gene 1                 | Regulates inflammation through its inhibitory effects on cytokine and reactive oxygen species production                        |
| ldha          | ldha              | Lactate dehydrogenase A                  | Catalyzes the inter-conversion of pyruvate and L-lactate. many human cancers have higher LDHA levels compared to normal tissues |
| mbp           | mbp               | Myelin basic protein                     | Myelination nervous system, role in demyelinating diseases such as multiple sclerosis   |
| mucms1        | muc1              | Mucin                                    | Mucus, respiratory system, altered in cancer  |
| opn1lw2       | opn1lw            | Red-sensitive opsin-2, <i>D. rerio</i>   | Visual pigments are the light-absorbing molecules that mediate vision   |
| opn1mw1       | opn1mw            | Green-sensitive opsin-1                  | Visual pigment  |

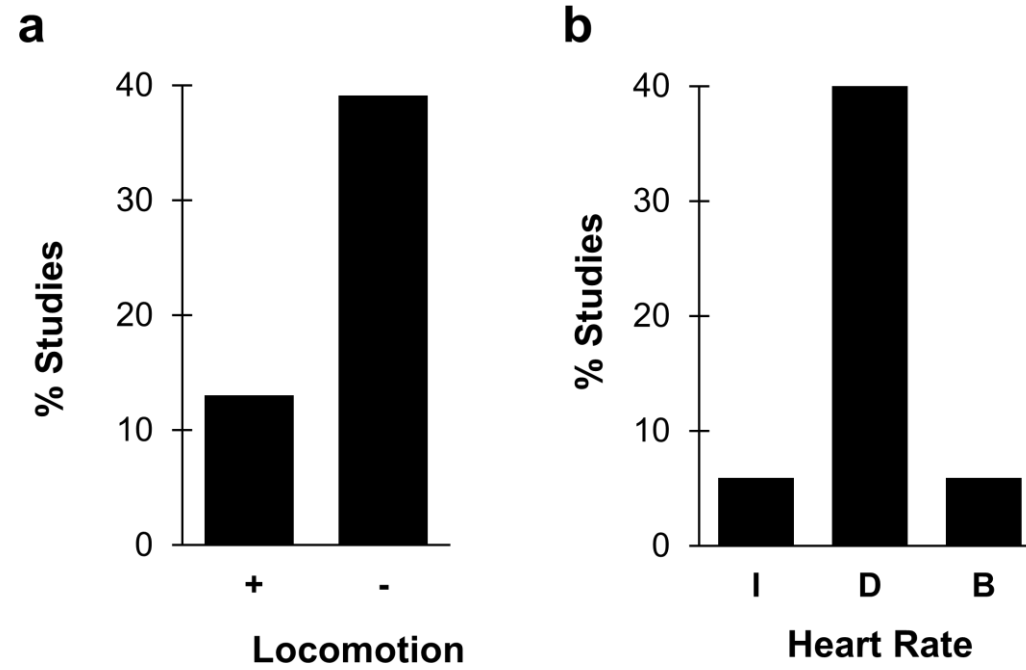
|          |        |   |   |
|----------|--------|---|---|
| opn1sw2  | opn1sw | Blue light receptor                                       | Visual pigment  |
| pck1     | pck1   | Piruvate carboxykinase                                    | Regulation of gluconeogenesis   |
| slc2a2   | slc2a2 | Glucose transporter 2                                     | Glucose transport. Defect glycogen disease, energy metabolism   |
| slc6a4   | slc6a4 | The serotonin transporter                                 | Neuron communication. changes in expression involved in mental disease  |
| socs3a   | socs3  | Suppressor of cytokine signaling 3                        | The expression of SOCS3 gene is induced by various cytokines, involved in insulin resistance, energy metabolism                           |
| sod1     | sod1   | Superoxide dismutase 1                                    | Responsible for destroying free superoxide radicals in the body, SOD1 is known to have a capacity to limit the detrimental effects of ROS |
| sod2     | sod2   | Superoxide dismutase 2                                    | Clears mitochondrial ROS and confer protection against cell death   |
| syn2a    | syn2   | Synapsin IIa  | Predicted to be involved in neurotransmitter secretion, synapsis. Knockouts learning disabilities   |
| tnfa     | tnf    | Tumor necrosis factor                                     | Secreted by macrophages, produce inflammation, involved in autoimmunity and cancer  |
| trpv6    | trpv6  | Transient Receptor Potential Vanilloid subfamily member 6 | Involved in the first step in Ca <sup>2+</sup> absorption in the intestine, many processes and diseases                                   |
| try      | prss1  | Trypsin   | Protease, digests food in small intestine   |
| zfrho    | rho    | Rhodopsin   | Photoreceptor activity and retinal binding activity. Involved in absorption of visible light  |
| atubulin | tuba1a | Tubulin   | Function in many processes, including structural support, intracellular transport, and DNA segregation.                                   |

**Table A.7.** Full names and functions of validated enzymes/metabolites used for analyses.

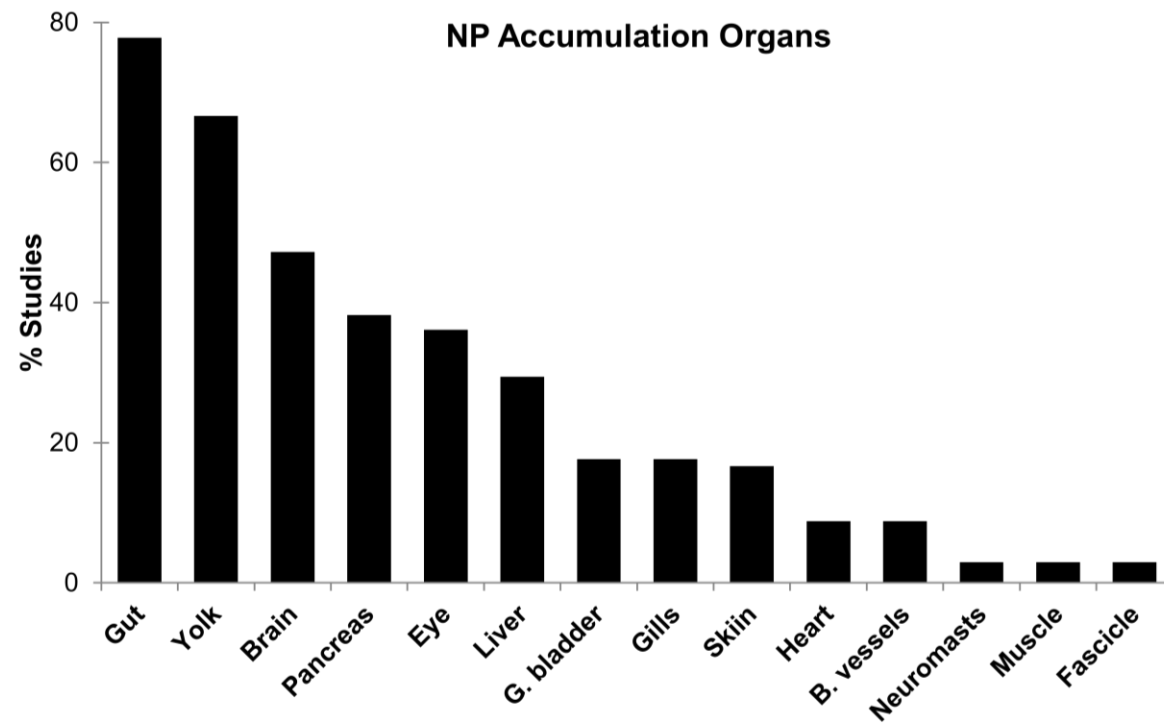
| Metabolite | Full Name   | Function   |
|------------|---|--|
| ROS        | Reactive oxygen species                           | Cellular damage  |
| SOD        | Superoxide dismutase                              | Responsible for destroying free superoxide radicals, SOD1 can limit the detrimental effects of ROS |
| P-gp       | p-glycoprotein                                    | Xenobiotic elimination   |
| CAT        | Catalase  | Protecting the cell from oxidative damage by reactive oxygen species (ROS)                         |
| GPx        | Glutathione peroxidase                            | Detoxification of hydrogen peroxide, and is one of the most important antioxidant enzymes          |
| GST        | Glutathione-S-transferase                         | Role in detoxification, thought to function in xenobiotic metabolism                               |
| EROD       | Ethoxyresorufin-O-deethylase                      | Measures activity of cytochrome P450 (involved in oxidation/reduction processes))                  |
| GR         | Glutathione reductase                             | Critical enzyme in resisting oxidative stress and maintaining the reducing environment of the cell |
| COX        | Cyclooxygenase                                    | Cellular stress response   |
| GSH        | Reduced form of glutathione                       | Low molecular weight antioxidant, substrate to GPx   |
| Thiol      | Organic compound containing the sulphhydryl group | Critical role in preventing the formation of any oxidative stress condition in the cells           |
| AchE       | Acetylcholinesterase                              | Hydrolyses Ach in synaptic cleft   |
| TSH        | Thyroid stimulating hormone                       | Stimulates thyroid hormone biosynthesis  |
| NADH       | Reduced nicotinamide adenine dinucleotide         | Fuels the electron transport chain. Important for catabolism but also anabolism                    |
| T3         | Triiodothyronine                                  | Growth and development, other processes  |
| T4         | Thyroxine   | Prohormone and reservoir of T3   |



**Fig. A.1.** Percent studies in which endpoints were observed (black) or not observed (grey).



**Fig. A.2.** Percent studies in which locomotion (a) and heart rate (b) were affected. Plus sign: hyperactivity; minus sign: hypoactivity; I: increased heart rate; D: decreased heart rate; B: both increase and decreased heart rate observed.



**Fig. A.3.** Percent studies in which NP accumulated in different ZF organs.