Developmental effects of polystyrene nanoparticles in the chicken embryo

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Chapter 5. Summary, discussion and perspective

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Summary

Plastics are used as the main material for fabricating every-day items which have brought great convenience to our lives. On the downside, however, 60% of plastics end up dumped in landfills or as plastic waste in the environment (Geyer et al., 2017). This waste slowly releases fragments called small plastic particles which can be divided into microplastics and nanoplastics (MPs and NPs) according to their size. Small plastic particles have become ubiquitous in the environment where they pose potential dangers to wildlife and to humans (Allen et al., 2022; Zolotova et al., 2022).

MPs and NPs have been demonstrated to transfer from mother to offspring in zebrafish (Pitt et al., 2018). Furthermore, they are even found to be able to transfer through food webs (Chae et al., 2018; Kim et al., 2022). MPs and NPs are suspected to be a potential health risk to humans due to toxicity to cell cultures (Aguilar-Guzmán et al., 2022; Cortés et al., 2020; Gopinath et al., 2021; Ramsperger et al., 2020), and the fact that they have been detected in multiple human tissues body fluids (Jenner et al., 2022; Leslie et al., 2022; Ragusa et al., 2021; Zhao et al., 2023). Indirect evidence of potential toxicity to humans is the fact that small plastic particles are toxic in several whole animal models (Yin et al., 2021; Yong et al., 2020). For example, the toxicity of MPs and NPs has been widely studied in aquatic animals including *Daphnia magna* (Abdolahpur Monikh et al., 2020; Kelpsiene et al., 2020) the zebrafish (Lee et al., 2022; Torres-Ruiz et al., 2021) and the Pacific Oyster (Cole and Galloway, 2015). However, little is known about their potential risk to warm-blooded vertebrates.

In **Chapter 1**, we have summarized the currently findings about MPs and NPs, and their potential risk to humans. Additionally, we have reviewed the literature on using the chick embryo as a model for testing toxic agents for their specific toxicity towards embryonic development. Indeed chick embryos have already been used for several toxicity studies of nanoparticles, particularly metal and carbon particles.

In **Chapter 2**, I have shown that PS-NPs can cause neural tube defects in the head, trunk, tail, or a combination of these regions in the chick embryo. Neural tube defects represent a failure of neural tube closure (Greene and Copp, 2014), leading to increased morbidity and mortality (Copp and Greene, 2010; Madrid et al., 2023). We suggest that this failure of closure is due to toxic effects of PS-NPs on the neural crest — a population of neuroectodermal cells adjacent to the fusion zone of the closing neural tube (Creuzet, 2009; Green et al., 2015; Le Douarin et al., 2012; Le Douarin and Kalcheim, 1999; Martik and Bronner, 2021; Waldo et al., 1998). This model (which we summarize in Fig. 5-1) is consistent with a study showing that the neural crest is essential for neural tube closure, at least in the cranial region (Creuzet, 2009; Creuzet et al., 2006; Wang et al., 2023).

Next, in **Chapter 3**, we have provided the first evidence that exposure of chick embryos to PS-NPs can cause heart malformations. Specifically, we have detected cardiovascular malformations including ventricular septal defect, supernumerary arteries, persistent truncus arteriosus, abnormal blood vessels and excess cardiac jelly. In addition, we have shown that PS-NPs cause impaired cardiac function. These findings make sense in the light of previous reports that neural crest cells play an essential role of the cardiovascular development (Le Douarin and Kalcheim, 1999). For example, after neural crest cells were ablated surgically, the resulting embryo phenotype displayed cardiovascular abnormalities (Keyte and Hutson, 2012; Kirby and Waldo, 1995; Waldo et al., 1999).

In addition to the cardiovascular malformations described in **Chapter 3**, we have also noticed craniofacial defects in treated embryos. These defects include malformation (hypoplasia or aplasia) of the upper beak, and failure of Meckel's cartilage to fuse with its contralateral partner. These malformations, like others mentioned above may also be explained by the results of NPs affecting neural crest cells. This is because neural crest cells play an essential role in craniofacial development (Martik and Bronner, 2021).

Our finding of both cardiovascular and craniofacial defects is consistent with the concept of a 'cardiocraniofacial module' (Gans and Northcutt, 1983; Keyte and Hutson, 2012; Martik and Bronner, 2021) which is dependent on neural crest cells for its normal development. Interestingly, it was previously shown that the exposure of chicken embryos to metal nanoparticles (zinc oxide) causes craniofacial abnormalities, which was suggested to result from disruption of neural crest development (Yan et al., 2020). We should be cautious in interpreting those findings because many types of metal nanoparticles shed ions, and so the toxicity of zinc oxide nanoparticles observed in that study is not necessarily mediated by the same mechanisms nanoplastic toxicity. It should also be noted that craniofacial defects that we observed here are not necessarily a reflection of abnormal neural crest development.

In a few NP treated embryos, the tailbud was hypoplastic or aplastic; one embryo showed agenesis of the tail and phocomelia of the hindlimbs (**Chapters 2** and **3**). We suggest that these effects reflect an effect of PS-NPs on surface-exposed mesenchymal populations in the primitive streak of the tailbud. During gastrulation, and the process of ingression, the dorsal surface epithelium undergoes an epithelial-mesenchymal transition with local loss of the basal lamina (Bellairs, 1986; Shook and Keller, 2003). This process is still active at the stage when we introduced the PS-NPs to the egg (Knezevic et al., 1998).

Another potential source of mesenchymal cells in the caudal region, that might be affected by PS-NP exposure, is the junction between regions of primary and secondary neurulation (Dady et al., 2014). This process starts around stage 8 (Dady et al., 2014). Binding of the PS-NPs to these junctional mesenchymal cells could explain the gross dysplasia of the neural tube in the caudal region of some embryos (**Chapters 2** and **3**). We exposed embryos to PS-NPs at stage 8. Both gastrulation and neurulation are still active at this stage (Keibel and Abraham, 1900).

In **Chapter 4**, our experiments, with fluorescent PS-NPs added to live embryos, show strong labelling in the neural crest, but little or none in any other tissues (**Chapter 4**, Fig. 4-1). Furthermore, we find that that fluorescence is seen in the cytoplasm within 2 h and is still there at 6 h. However, these experiments should be interpreted with caution because: single 25 nm particles are too small to be directly visualized with optical microscopes; and it is possible that the fluorescence we saw was partly due to leakage of the fluorochrome from the particles. In any case, it is evident that strong fluorescence is specifically found in neural crest cells.

Our TUNEL-labelling experiments showed enhanced cell death in both the dorsal midline of the neural tube (**Chapter 4**, Fig. 4-7), and in ectopic clumps of cells in the neural tube lumen that we identified as neural crest cells with neural crest markers (**Chapter4**, Fig. 4-2 to 4). The same markers also identify a population of neural crest cells in the dorsal midline of PS-NP treated embryos that have failed to migrate. We also see evidence of reduced neural crest cell migration into the pharyngeal arches when using TFAP2A expression as a marker of migrating cardiac crest cells (**Chapter 3**, Fig.3-6).

Together, these data suggest that neural crest cells are damaged or undergo cell death after binding PS-NPs and fail to migrate (Fig. 5-1; (Wang et al., 2023). Candidate mechanisms of PS-NPs cell-damage include the denaturation of proteins (Gopinath et al., 2019; Hollóczki and Gehrke, 2019), and the accumulation of PS-NPs in the cytoplasm, after being taken up by endocytosis, and then resisting degradation by lysosomal enzymes (Nie et al., 2021). Our hypothesis, that PS-NPs inhibit neural crest migration, is consistent with experiments on other migratory cell types. For example, it has been shown that PS-NPs supress the migration and dispersion of aggregated CT26 murine carcinoma cells *in vitro* (Beaune et al., 2019).

Our study shows that PS-NPs have harmful effects on early chicken embryos, producing a range of malformations. We find that the toxicity of PS-NPs is due to their ‘targeting’ a specific subpopulation of embryonic cells. The targeting is passive,

in the sense that PS-NPs appear to strongly bind to neural crest cells and not to other cells. The fact that NPs cause multi-system malformations is of great concern; given the extensive environmental exposure of humans to small plastic particles, the reported presence of small plastic particles in human tissues, and the current development of a new generations of nanomedicines intended for human therapeutic use.

In the future, it will be important to explore the underlying mechanism of the selective binding of nanoplastics to neural crest cells. One hypothesis is that nanoplastics bind to specific cell adhesion molecules, known to be expressed by neural crest cells, such as cadherin 6B. Cadherin 6B is associated with epithelial mesenchymal transformation in both embryonic cells and some cancer cells. If this hypothesis is confirmed, the finding would contribute to both our understanding of the embryonic toxicity of nanoplastics and possibly also to cancer research.

Discussion and perspective

Our data show that 25 nm PS-NPs selectively attach to neural crest cells, and possibly other cell populations undergoing epithelial-mesenchymal transformation on the dorsal surface of the embryo. By contrast, PS-NPs show little or no attachment to intact embryonic epithelia. It is possible that this reflects the differential expression of adhesion molecules on different cell populations on the surface of the embryo. For example, sugar residues in the cell coat of neural crest cells in mouse and rat embryos differ considerably from neurectoderm and epithelial ectoderm (Smits-van Prooije et al., 1986). Furthermore the cell adhesion molecule cadherin 6B is differentially expressed on pre-migratory crest cells and cadherin 7 is expressed on migratory crest cells (Taneyhill and Schiffmacher, 2017). Note that the switch between the expression of different cadherin molecules (the ‘cadherin switch’) accompanies the segregation of the neural crest lineage from the neurectoderm (Dady et al., 2012). Our hypothesis is that PS-NPs may interact with the cadherins

during neurulation, possibly changing the protein structure and functions (Hollóczki and Gehrke, 2019; Kihara et al., 2021). The interaction of proteins with nanoplastics is the basis of the 'corona' which is sometimes observed on nanoparticles exposed to proteins .

The concentration of nanoparticles used in this study (5 mg/mL) is higher than that reported, for example, in human blood. (Leslie et al., 2022). However, it should be remembered that PS-NPs and other nanomedicines are likely to be used in high concentrations. Furthermore, it has been shown that nanoplastics can be transmitted to offspring in several animal models (Pitt et al., 2018; Zhao et al., 2017); if this applies to humans, then we might expect a cumulative increase in particles in human tissues over the generations.

The highly selective effect of PS-NPs on embryonic neural crest cells may mean that they are capable of disrupting development even in low levels exposures. Furthermore, detecting nanoplastics in the environment and in human tissues, is extremely difficult, and the associated analytics are in their infancy. As a result, it is likely that human exposure to PS-NPs has been substantially underestimated. Finally, it is worth remembering that even if society stops now with all plastic pollution, the weathered nanoplastic debris levels from existing plastics in the environment will still increase. In the future, we will need more information about the teratogenicity of nanoplastics — and possibly of nanomaterials in general.

Our study shows that PS-NPs cause effects on development that closely resemble those produced by a surgical ablation of the neural crest (Bockman et al., 1987; Hutson and Kirby, 2007; Kirby, 1999; Kirby et al., 1989; Kirby et al., 1985). This means that nanoplastics can have catastrophic effects on development by 'targeting' a specific subpopulation of embryonic cells. The targeting is passive, in the sense that PS-NPs may simply show strong binding to certain cell-surface molecules on neural crest cells. In any case, the highly selective effect of PS-NPs on certain exposed embryonic cell populations may mean that they are capable of disrupting

development even in low-level exposures. The production of malformations by NPs are of concern, given the extensive environmental exposure of humans to small plastic particles (Anagnosti et al., 2021; Cox et al., 2019; Koelmans et al., 2019; Vighi et al., 2021; Zhang et al., 2020), the reported presence of small plastic particles in human tissues (Jenner et al., 2022; Leslie et al., 2022; Ragusa et al., 2021), and the current development of a new generations of nanomedicines intended for human therapeutic use (Boehnke et al., 2022). In summary, we believe that PS-NPs of primary or secondary origin pose a potential danger to living embryos of humans and other vertebrates. Because of this, the increasing burden of environmental nanoplastics is a matter for concern.

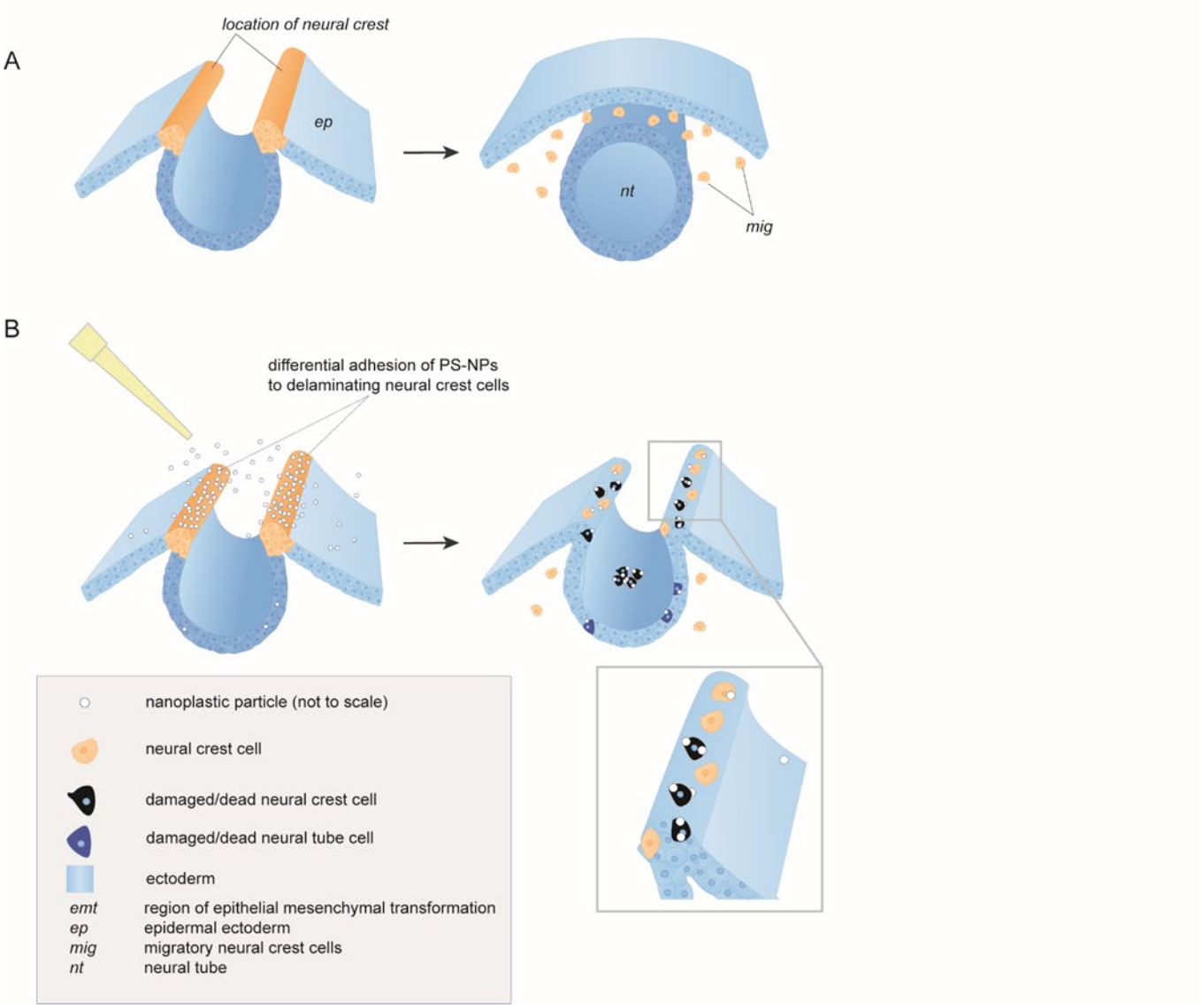

Fig. 5-1. **Our hypothesis of PS-NP-induced developmental toxicity, based on disruption of neural crest development. A**, normal development of the neural tube and neural crest. **B**, development of the neural tube and crest after exposure of the embryo to 25 nm PS-NPs. We hypothesize that the nanoparticles adhere selectively to the neural crest cells undergoing epithelial-mesenchymal transition, and induce cell death in at least a subpopulation of those neural crest cells. This reduces the number of normal neural crest cells, disrupts their migration and interferes with the normal closure of the neural tube. It also often leaves a clump of putative neural crest cells in the lumen of the neural tube (or the neural groove, in the case of a neural tube defect). We suggest that these events lead to neural tube defects, microphthalmia, craniofacial and cardiac malformations. Note that we saw some evidence of very limited cell death in the neural tube induced by PS-NPs (as shown in this figure).

References

Abdolahpur Monikh, F., Vijver, M. G., Guo, Z., Zhang, P., Darbha, G. K. and Peijnenburg, W. J. G. M. (2020). Metal Sorption onto Nanoscale Plastic Debris and Trojan Horse Effects in Daphnia Magna: Role of Dissolved Organic Matter. *Water Research* **186**, 116410.

Aguilar-Guzmán, J. C., Bejtka, K., Fontana, M., Valsami-Jones, E., Villezcas, A. M., Vazquez-Duhalt, R. and Rodríguez-Hernández, A. G. (2022). Polyethylene Terephthalate Nanoparticles Effect on Raw 264.7 Macrophage Cells. *Microplastics and Nanoplastics* **2**, 9.

Allen, D., Allen, S., Abbasi, S., Baker, A., Bergmann, M., Brahney, J., Butler, T., Duce, R. A., Eckhardt, S., Evangeliou, N., et al. (2022). Microplastics and Nanoplastics in the Marine-Atmosphere Environment. *Nature Reviews Earth & Environment* **3**, 393-405.

Anagnosti, L., Varvaresou, A., Pavlou, P., Protopapa, E. and Carayanni, V. (2021). Worldwide Actions against Plastic Pollution from Microbeads and Microplastics in Cosmetics Focusing on European Policies. Has the Issue Been Handled Effectively? *Marine Pollution Bulletin* **162**, 111883.

Beaune, G., Nagarajan, U., Brochard-Wyart, F. and Winnik, F. M. (2019). Polymeric Nanoparticles Limit the Collective Migration of Cellular Aggregates. *Langmuir* **35**, 7396-7404.

Bellairs, R. (1986). The Primitive Streak. *Anat Embryol (Berl)* **174**, 1-14.

Bockman, D. E., Redmond, M. E., Waldo, K., Davis, H. and Kirby, M. L. (1987). Effect of Neural Crest Ablation on Development of the Heart and Arch Arteries in the Chick. *Am.J.Anat.* **180**, 332-341.

Boehnke, N., Straehla, J. P., Safford, H. C., Kocak, M., Rees, M. G., Ronan, M., Rosenberg, D., Adelmann, C. H., Chivukula, R. R., Nabar, N., et al. (2022). Massively Parallel Pooled Screening Reveals Genomic Determinants of Nanoparticle Delivery. *Science* **377**, eabm5551.

Chae, Y., Kim, D., Kim, S. W. and An, Y.-J. (2018). Trophic Transfer and Individual Impact of Nano-Sized Polystyrene in a Four-Species Freshwater Food Chain. *Scientific reports* **8**, 284.

Cole, M. and Galloway, T. S. (2015). Ingestion of Nanoplastics and Microplastics by Pacific Oyster Larvae. *Environmental Science & Technology* **49**, 14625-14632.

Copp, A. J. and Greene, N. D. (2010). Genetics and Development of Neural Tube Defects. *J Pathol* **220**, 217-230.

Cortés, C., Domenech, J., Salazar, M., Pastor, S., Marcos, R. and Hernández, A. (2020). Nanoplastics as a Potential Environmental Health Factor: Effects of Polystyrene Nanoparticles on Human Intestinal Epithelial Caco-2 Cells. *Environmental Science: Nano* **7**, 272-285.

Cox, K. D., Covernton, G. A., Davies, H. L., Dower, J. F., Juanes, F. and Dudas, S. E. (2019). Human Consumption of Microplastics. *Environmental Science & Technology* **53**, 7068-7074.

Creuzet, S. E. (2009). Regulation of Pre-Otic Brain Development by the Cephalic Neural Crest. *Proceedings of the National Academy of Sciences* **106**, 15774-15779.

Creuzet, S. E., Martinez, S. and Le Douarin, N. M. (2006). The Cephalic Neural Crest Exerts a Critical Effect on Forebrain and Midbrain Development. *Proc Natl Acad Sci USA* **103**, 14033-14038.

Dady, A., Blavet, C. and Duband, J. L. (2012). Timing and Kinetics of E- to N-Cadherin Switch During Neurulation in the Avian Embryo. *Dev Dyn* **241**, 1333-1349.

Dady, A., Havis, E., Escriou, V., Catala, M. and Duband, J. L. (2014). Junctional Neurulation: A Unique Developmental Program Shaping a Discrete Region of the Spinal Cord Highly Susceptible to Neural Tube Defects. *J Neurosci* **34**, 13208-13221.

Gans, C. and Northcutt, R. G. (1983). Neural Crest and the Origin of Vertebrates: A New Head. *Science* **220**, 268-273.

Geyer, R., Jambeck, J. R. and Law, K. L. (2017). Production, Use, and Fate of All Plastics Ever Made. *Sci Adv* **3**, e1700782.

Gopinath, P. M., Saranya, V., Vijayakumar, S., Mythili Meera, M., Ruprekha, S., Kunal, R., Pranay, A., Thomas, J., Mukherjee, A. and Chandrasekaran, N. (2019). Assessment on Interactive Prospectives of Nanoplastics with Plasma Proteins and the Toxicological Impacts of Virgin, Coronated and Environmentally Released-Nanoplastics. *Scientific Reports* **9**, 8860.

Gopinath, P. M., Twayana, K. S., Ravanan, P., John, T., Mukherjee, A., Jenkins, D. F. and Chandrasekaran, N. (2021). Prospects on the Nano-Plastic Particles Internalization and Induction of Cellular Response in Human Keratinocytes. *Particle and Fibre Toxicology* **18**, 35.

Green, S. A., Simoes-Costa, M. and Bronner, M. E. (2015). Evolution of Vertebrates as Viewed from the Crest. *Nature* **520**, 474-482.

Greene, N. D. and Copp, A. J. (2014). Neural Tube Defects. *Annu Rev Neurosci* **37**, 221-242.

Hollóczki, O. and Gehrke, S. (2019). Nanoplastics Can Change the Secondary Structure of Proteins. *Scientific Reports* **9**, 16013.

Hutson, M. R. and Kirby, M. L. (2007). Model Systems for the Study of Heart Development and Disease. Cardiac Neural Crest and Conotruncal Malformations. *Semin Cell Dev Biol* **18**, 101-110.

Jenner, L. C., Rotchell, J. M., Bennett, R. T., Cowen, M., Tentzeris, V. and Sadofsky, L. R. (2022). Detection of Microplastics in Human Lung Tissue Using Muftir Spectroscopy. *Sci Total Environ* **831**, 154907.

Keibel, F. and Abraham, K. (1900). Normentafeln Zur Entwicklungsgeschichte Des Huhnes (Gallus Domesticus). In *Normentafeln zur Entwicklungsgeschichte der Wirbelthiere.* (ed F. Keibel), pp. 132p. Jena: Fischer.

Kelpsiene, E., Torstensson, O., Ekvall, M. T., Hansson, L.-A. and Cedervall, T. (2020). Long-Term Exposure to Nanoplastics Reduces Life-Time in Daphnia Magna. *Scientific Reports* **10**, 5979.

Keyte, A. and Hutson, M. R. (2012). The Neural Crest in Cardiac Congenital Anomalies. *Differentiation* **84**, 25-40.

Kihara, S., Ashenden, A., Kaur, M., Glasson, J., Ghosh, S., van der Heijden, N., Brooks, A. E., Mata, J. P., Holt, S. and Domigan, L. J. (2021). Cellular Interactions with Polystyrene Nanoplastics—the Role of Particle Size and Protein Corona. *Biointerphases* **16**, 041001.

Kim, L., Cui, R., Il Kwak, J. and An, Y.-J. (2022). Trophic Transfer of Nanoplastics through a Microalgae–Crustacean–Small Yellow Croaker Food Chain: Inhibition of Digestive Enzyme Activity in Fish. *Journal of Hazardous Materials* **440**, 129715.

Kirby, M. L. (1999). 11 - Contribution of Neural Crest to Heart and Vessel Morphology. In *Heart Development* (ed. R. P. Harvey & N. Rosenthal), pp. 179-193. San Diego: Academic Press.

Kirby, M. L., Creazzo, T. L. and Christiansen, J. L. (1989). Chronotropic Responses of Chick Atria to Field Stimulation after Various Neural Crest Ablations. *Circ.Res.* **65**, 1547-1554.

Kirby, M. L., Tumage, K. L. and Hays, B. M. (1985). Characterization of Conotruncal Malformations Following Ablation of Cardiac Neural Crest. *Anatomical Records* **213**, 87-87.

Kirby, M. L. and Waldo, K. L. (1995). Neural Crest and Cardiovascular Patterning. *Circ.Res.* **77**, 211-215.

Knezevic, V., De Santo, R. and Mackem, S. (1998). Continuing Organizer Function During Chick Tail Development. *Development* **125**, 1791-1801.

Koelmans, A. A., Nor, N. H. M., Hermsen, E., Kooi, M., Mintenig, S. M. and De France, J. (2019). Microplastics in Freshwaters and Drinking Water: Critical Review and Assessment of Data Quality. *Water research*.

Le Douarin, N. M., Couly, G. and Creuzet, S. E. (2012). The Neural Crest Is a Powerful Regulator of Pre-Otic Brain Development. *Dev Biol* **366**, 74-82.

Le Douarin, N. M. and Kalcheim, C. (1999). *The Neural Crest*. Cambridge: Cambridge University Press.

Lee, W. S., Kim, H., Sim, Y., Kang, T. and Jeong, J. (2022). Fluorescent Polypropylene Nanoplastics for Studying Uptake, Biodistribution, and Excretion in Zebrafish Embryos. *ACS Omega* **7**, 2467-2473.

Leslie, H. A., van Velzen, M. J. M., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J. and Lamoree, M. H. (2022). Discovery and Quantification of Plastic Particle Pollution in Human Blood. *Environ Int* **163**, 107199.

Madrid, L., Vyas, K. J., Kancherla, V., Leulseged, H., Suchdev, P. S., Bassat, Q., Sow, S. O., El Arifeen, S., Madhi, S. A., Onyango, D., et al. (2023). Neural Tube Defects as a Cause of Death among Stillbirths, Infants, and Children Younger Than 5 Years in Sub-Saharan Africa and Southeast Asia: An Analysis of the Champs Network. *The Lancet Global Health*.

Martik, M. L. and Bronner, M. E. (2021). Riding the Crest to Get a Head: Neural Crest Evolution in Vertebrates. *Nature Reviews Neuroscience* **22**, 616-626.

Nie, J.-h., Shen, Y., Roshdy, M., Cheng, X., Wang, G. and Yang, X. (2021). Polystyrene Nanoplastics Exposure Caused Defective Neural Tube Morphogenesis through Caveolae-Mediated Endocytosis and Faulty Apoptosis. *Nanotoxicology*, 1-20.

Pitt, J. A., Trevisan, R., Massarsky, A., Kozal, J. S., Levin, E. D. and Di Giulio, R. T. (2018). Maternal Transfer of Nanoplastics to Offspring in Zebrafish (Danio Rerio): A Case Study with Nanopolystyrene. *Sci Total Environ* **643**, 324-334.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M. C. A., Baiocco, F., Draghi, S., et al. (2021). Plasticenta: First Evidence of Microplastics in Human Placenta. *Environ Int* **146**, 106274.

Ramsperger, A., Narayana, V. K. B., Gross, W., Mohanraj, J., Thelakkat, M., Greiner, A., Schmalz, H., Kress, H. and Laforsch, C. (2020). Environmental Exposure Enhances the Internalization of Microplastic Particles into Cells. *Science Advances* **6**, eabd1211.

Shook, D. and Keller, R. (2003). Mechanisms, Mechanics and Function of Epithelial-Mesenchymal Transitions in Early Development. *Mech Dev* **120**, 1351-1383.

Smits-van Prooije, A. E., Poelmann, R. E., Gesink, A. F., van Groeningen, M. J. and Vermeij-Keers, C. (1986). The Cell Surface Coat in Neurulating Mouse and Rat Embryos, Studied with Lectins. *Anat Embryol (Berl)* **175**, 111-117.

Taneyhill, L. A. and Schiffmacher, A. T. (2017). Should I Stay or Should I Go? Cadherin Function and Regulation in the Neural Crest. *Genesis* **55**.

Torres-Ruiz, M., De la Vieja, A., de Alba Gonzalez, M., Esteban Lopez, M., Castaño Calvo, A. and Cañas Portilla, A. I. (2021). Toxicity of Nanoplastics for Zebrafish Embryos, What We Know and Where to Go Next. *Science of The Total Environment* **797**, 149125.

Vighi, M., Bayo, J., Fernández-Piñas, F., Gago, J., Gómez, M., Hernández-Borges, J., Herrera, A., Landaburu, J., Muniategui-Lorenzo, S. and Muñoz, A.-R. (2021). Micro and Nano-Plastics in the Environment: Research Priorities for the near Future. *Reviews of Environmental Contamination and Toxicology Volume 257*, 163-218.

Waldo, K., Miyagawa-Tomita, S., Kumiski, D. and Kirby, M. L. (1998). Cardiac Neural Crest Cells Provide New Insight into Septation of the Cardiac Outflow Tract: Aortic Sac to Ventricular Septal Closure. *Dev.Biol.* **196**, 129-144.

Waldo, K., Zdanowicz, M., Burch, J., Kumiski, D. H., Stadt, H. A., Godt, R. E., Creazzo, T. L. and Kirby, M. L. (1999). A Novel Role for Cardiac Neural Crest in Heart Development. *J Clin Invest* **103**, 1499-1507.

Wang, M., Rücklin, M., Poelmann, R. E., de Mooij, C. L., Fokkema, M., Lamers, G. E. M., de Bakker, M. A. G., Chin, E., Bakos, L. J., Marone, F., et al. (2023). Nanoplastics Causes Extensive Congenital Malformations During Embryonic Development by Passively Targeting Neural Crest Cells. *Environment International* **173**, 107865.

Yan, Y., Wang, G., Huang, J., Zhang, Y., Cheng, X., Chuai, M., Brand-Saberi, B., Chen, G., Jiang, X. and Yang, X. (2020). Zinc Oxide Nanoparticles Exposure-Induced Oxidative Stress Restricts Cranial Neural Crest Development During Chicken Embryogenesis. *Ecotoxicol Environ Saf* **194**, 110415.

Yin, K., Wang, Y., Zhao, H., Wang, D., Guo, M., Mu, M., Liu, Y., Nie, X., Li, B., Li, J., et al. (2021). A Comparative Review of Microplastics and Nanoplastics: Toxicity Hazards on Digestive, Reproductive and Nervous System. *Science of The Total Environment* **774**, 145758.

Yong, C. Q. Y., Valiyaveettil, S. and Tang, B. L. (2020). Toxicity of Microplastics and Nanoplastics in Mammalian Systems. *International Journal of Environmental Research and Public Health* **17**, 1509.

Zhang, J., Wang, L. and Kannan, K. (2020). Microplastics in House Dust from 12 Countries and Associated Human Exposure. *Environment International* **134**, 105314.

Zhao, L., Qu, M., Wong, G. and Wang, D. (2017). Transgenerational Toxicity of Nanopolystyrene Particles in the Range of Mg L− 1 in the Nematode Caenorhabditis Elegans. *Environmental Science: Nano* **4**, 2356-2366.

Zhao, Q., Zhu, L., Weng, J., Jin, Z., Cao, Y., Jiang, H. and Zhang, Z. (2023). Detection and Characterization of Microplastics in the Human Testis and Semen. *Science of The Total Environment* **877**, 162713.

Zolotova, N., Kosyreva, A., Dzhalilova, D., Fokichev, N. and Makarova, O. (2022). Harmful Effects of the Microplastic Pollution on Animal Health: A Literature Review. *PeerJ* **10**, e13503.