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Di-n-Butyl phthalate or DBP, a Common Ingredient in Plastics, Induces Craniofacial Defects During Embryonic Development

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**Abstract**

Phthalates comprise a large chemical family utilized in numerous household products including synthetic leather, vinyl flooring, blood transfusion bags, adhesives, soaps, shampoos, cosmetics, nail polishes, pharmaceuticals and food packaging (Schechter, 2006). Specifically, phthalates are utilized to provide plasticity to rigid materials allowing for more pliability and act as lubricants as well as solvents. Globally, more than three million metric tons of phthalates are produced. Although phthalates demonstrate various biotic and abiotic degradation pathways, concerning concentrations are routinely found in aquatic ecosystems, soil and groundwater as phthalates readily leach from products. Several studies have detected DBP levels in aquatic environments at mg/L and in soil and sediment at mg/kg levels (Xu et al., 2015). DBP’s ubiquitous presence in the environment has led the European Commission to label DBP as a priority substance and the US EPA as a priority environmental pollutant.

Common routes of exposure to phthalates include ingestion, inhalation and absorption through the skin and phthalates have been detected in human breast milk and blood plasma. The endocrine disrupting effects of DBP have been noted. Differentiation of male gonads was adversely effected by DBP in Rana nigromaculata tadpoles at 0.1µM (Oehlmann et al., 2009). Enhanced estrogenic activity in zebrafish has also been reported (Chen et al., 2014). Recently, the immunotoxicity of DBP on zebrafish was assessed and found to inhibit neutrophils and macrophage formation (Xu et al., 2015).

Currently, there is limited data regarding exposure to DBP during the window of embryonic development. Here we seek to investigate the effects of DBP during early development and hypothesize that DBP treatments will negatively affect the developing body.

**Introduction and Hypothesis**

**Di-n-Butyl phthalate (DBP)** is a high production volume plasticizer added to increase the flexibility of synthetic polymers. DBP is found in a variety of everyday items like food packaging, cosmetics, cleaning materials, lubricants, waves and mastectomies. DBP readily leaches from products into soil and groundwater and its ubiquitous presence in the environment has led the European Commission to label DBP as a priority substance. Sediment and water analysis has noted high levels of DBP and the endocrine disrupting effects of DBP are well noted. Given the widespread uses and exposure to DBP, studies on developmental toxicity are needed. To that end, we sought to investigate the developmental effects of DBP exposure during early development utilizing the zebrafish vertebrate model system. We treated gastrula staged embryos with increasing concentrations of DBP and noted concentration dependent defects in craniofacial development, but the effect was specific with no other developmental defects noted. Overall cranial size in DBP treated embryos, as measured vertically from cranial vault tip to development, but the effect was specific with no other developmental defects noted. Overall cranial size in DBP treated embryos, as measured vertically from cranial vault tip to jaw and horizontally from nose to pectoral fin, was significantly less than controls, but the intraocular distance was increased. Subsequent analysis of jaw bone development demonstrated loss of and/or disorganization of cartilage development with concomitant defects in vascular innervation and neuronal patterning. Furthermore, vasculization of the cranial cavity also became disorganized or completely lost. We conclude that DBP at environmentally relevant doses, is toxic to craniofacial development in zebrafish.

**Results**

**Figure 1: DBP induces neural and craniofacial defects.** Control embryos in lateral (A) or dorsal (B) views at 96hrs. DBP treated embryos (C-F) demonstrate decreased cranial development and jaw defects (arrows) with a range of phenotypes from moderate (D,E) to severe (F). Eyes appear smaller and intraocular distance altered.

**Figure 2: DBP induces loss of and/or disorganization of jaw cartilage development.** Alcian blue staining at 96hrs showing cartilage structure in control embryos in ventral (A,C) and lateral views (B,D). Anatomy as shown: N: nasal, OA: opercular arch, PO: preorbital ossicle, RA: rhamphotheca, TC: trigeminal, TM: transverse maxillary, VM: vertical mandible, UM: upper mandible, VM: vertical mandible, VP: ventral pharynx, Z: zygomatic arch. DBP treatments show disorganization and/or loss of cartilage (D-F).

**Figure 3: DBP induces loss of and/or disorganization of neural innervation of the jaw.** RM044 anti-acetylated tubulin immunohistochemistry showing neurite loss and neurite retraction in control embryos in ventral (A,C) and lateral views (B,D). Anatomy as shown: PV: trigeminal nerve innervating intramuscular tissues, V: trigeminal nerve innervating the 1st branchial arch (Mandibular arch), VII: facial nerve innervating 2nd branchial arch (Pharyngeal arch) x: pH/po outer lateral line nerve. SCMN: spinal cord motor neurons. DBP treatments show disorganization and/or loss of neural patterning (E-H).

**Conclusions and Future Directions**

CONCLUSIONS: Here we utilize a commonly used ingredient found in plastic at sublethal concentrations. Embryos treated with 2.5µM of Di-n-butyl phthalate before the onset of gastrulation and treated at 96hpf demonstrate:

- A decrease in the size of the cranial vault as measured from the tip of the cranial cavity to the base of the jaw, a decrease in the length of the cranium from the tip of the nose to the posterior fin, a decrease in the distance from lens to lens, but an increase in the intraocular distance
- A loss of and/or disorganization of the jaw cartilage as determined by Alcian Blue staining
- A loss of and/or disorganization of neuronal innervation of the jaw as determined by RM044 anti-acetylated tubulin immunohistochemistry
- A loss of and/or disorganization of vasculization of the jaw and the brain as determined by live fli-1 mCherry transgenes

Thus, we conclude at environmentally present concentrations that are non-lethal to zebrafish, DBP is teratogenic to craniofacial development.

FUTURE DIRECTIONS: We are quite interested in the eye phenotypes noted during our treatments and our future studies will focus on DBP effects on eye development from 24-96hpf.

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**References**


