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**EVOLIUCINĖ MEDICINA:
ŽMOGAUS RAIDOS PERSPEKTYVOS IR SVEIKATA
BESIKEIČIANČIOJE APLINKOJE**

**EVOLUTIONARY MEDICINE:
PERSPECTIVES ON HUMAN DEVELOPMENT AND HEALTH
CONSEQUENCES IN A CHANGING ENVIRONMENT**

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Vilniaus universiteto Medicinos ir sveikatos mokslų
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THE IMPACT OF PHTHALATES ON EMBRYONIC MALFORMATIONS AND LONG BONE LENGTH ACROSS TWO RAT GENERATIONS

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Background and Aim: Phthalates are widely used plasticisers associated with embryonic malformations and disrupted prenatal skeletal development. This study aimed to evaluate and compare the effects of di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and their mixture on embryonic malformations and long bone length across two generations (F1 and F2).

Materials and Methods: Thirty-six female Wistar rats were randomly divided into six groups: control (K), DEHP200 mg/kg, DEHP1000 mg/kg, DBP100 mg/kg, DBP500 mg/kg, and a combination group (200 mg/kg DEHP + 100 mg/kg DBP). Animals were exposed to phthalates via diet. The same exposure protocol was applied to the F1 generation offspring. A subset of F1 females was bred to produce the F2 generation. Embryonic malformations (internal and external) and long bones were assessed by dissection and measured using a stereomicroscope. Groups were statistically compared. The study was approved by Lithuanian SFVS (No. G2-221).

Results: Embryonic malformation rates were highest in the combination group across both generations (F1: 22.73%, F2: 28.00%), indicating a potential mixture-related enhancement of developmental toxicity. Although overall malformation frequency decreased in F2, the observed defects were more severe, including anasarca and encephalocele. Long bone development showed generation-dependent differences. In F1 offspring, phthalate exposure was associated with reduced tibia and fibula lengths, particularly at lower doses. In contrast, F2 offspring showed significant alterations primarily in forelimb bones. Ulna and radius lengths were significantly affected by low-dose DEHP200 exposure, while humerus length differed between the DBP500 and DEHP + DBP groups.

Conclusions: Combined exposure to DEHP and DBP resulted in the highest teratogenic effects across both generations, with more severe malformations observed in F2 offspring. DEHP had a greater impact on long bone development than DBP, showing a non-monotonic dose-response relationship. Trans-generational exposure was associated with altered patterns of skeletal development, particularly in forelimb long bones.

Keywords: embryonic malformations, long bone length, phthalates, trans-generational effects