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**UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
FACULDADE DE MEDICINA**

Leandro Alves dos Santos

**Exposição materna a mistura de ftalatos: efeitos
sobre o desenvolvimento e função da tireoide na
prole de machos e fêmeas Sprague-Dawley**

Dissertação apresentada à
Faculdade de Medicina,
Universidade Estadual Paulista “Júlio
de Mesquita Filho”, Câmpus de
Botucatu, para obtenção do título de
Mestre em Patologia.

Orientador: Prof. Dr. Luís Fernando Barbisan

**Botucatu
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Palavras-chave: Descendência; Exposição materna; Ftalatos; Poluentes orgânicos persistentes; Tireoide.

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DE LEANDRO ALVES DOS SANTOS, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA, DA FACULDADE DE MEDICINA.

Aos 01 dias do mês de fevereiro do ano de 2022, às 14:00 horas, por meio de Videoconferência, realizou-se a defesa de DISSERTAÇÃO DE MESTRADO de LEANDRO ALVES DOS SANTOS, intitulada **Exposição materna a mistura de ftalatos: efeitos sobre o desenvolvimento e função da tiroide na prole de machos e fêmeas Sprague-Dawley**. A Comissão Examinadora foi constituída pelos seguintes membros: Prof. Dr. LUIS FERNANDO BARBISAN (Orientador(a) - Participação Virtual) do(a) Depto. de Biologia Estrutural e Funcional / UNESP - Câmpus de Botucatu - IB/Unesp, Prof. Dr. CLEVERTON ROBERTO DE ANDRADE (Participação Virtual) do(a) Depto. de Fisiologia e Patologia / FO/Araraquara - Unesp, Prof. Dr. LUCAS TADEU BIDINOTTO (Participação Virtual) do(a) Centro de Pesquisa em Oncologia Molecular (CPOM) / Hospital de Câncer de Barretos. Após a exposição pelo mestrando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final: Aprovado. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.


Prof. Dr. LUIS FERNANDO BARBISAN

Dedicatória

Dedico este trabalho ao meu grande amigo, conselheiro e “pai profissional” José Lucas de Carvalho, (*in memoriam*)
Que um dia possamos nos reencontrar, jogar conversa fora e claro, comer uma paçoca.

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Resumo

Os Desreguladores Endócrinos (DEs) são substâncias químicas naturais ou produzidas pela ação humana que possuem o potencial de interferir no sistema endócrino, afetando o crescimento/desenvolvimento e a reprodução dos organismos vivos, incluindo os seres humanos. Eles afetam principalmente a função de hormônios esteroides, podendo ter atividade anti-estrogênica/estrogênica ou anti-androgênica/androgênica, ou outros órgãos como a tireoide, alterando seu desenvolvimento e função. Os ftalatos são uma classe de substâncias utilizadas, dentre outros fins, como amaciantes plásticos, e que são agrupados em baixo e de alto peso molecular. Assim, os ftalatos e seus metabólitos são detectados na população de recém-nascidos, de jovens e adultos em diversas matrizes biológicas. Na literatura, a exposição aos ftalatos foi associada à disgenesia testicular, malformação genital, diminuição de produção de hormônios sexuais, alterações ovarianas, dentre outras. O efeito transgeracional da exposição aos ftalatos ainda não é bem estabelecido, porém estudos pré-clínicos tem indicado que ftalatos podem levar a distúrbios reprodutivos e endócrinos na descendência, mas poucos estudos testam animais expostos a misturas complexas de DEs. Assim, o objetivo deste estudo foi o de avaliar se a exposição materna a uma mistura de seis diferentes ftalatos [19% DEHP (Bis(2-etilexil) ftalato), 36% DEP (Dietilftalato), 15% DBP (Di-n-butilftalato), 10% DiBP (Diisobutilftalato), 8% BBzP (Butilbenzilftalato), e 10% DiNP (Diisonilftalato)] é capaz de alterar o desenvolvimento e função da glândula tireoide na prole de machos e fêmeas Sprague-Dawley (SD) pré-púberes e adultas (F1). Ratas SD prenhes receberam o tratamento com a mistura de ftalatos do dia gestacional 12 (DG12) ao dia pós-natal 21 (DPN 21), por gavagem diária, nas doses de 20µg, 200µg e 200mg/kg. Nos dias pós-natal (DPN) 21 e 120, as tireoides da prole (8 animais por ninhada, macho ou fêmea) foram removidas, processadas histologicamente para análises morfológicas e imunoistoquímicas. Foram observadas poucas alterações morfológicas na tireoide nos dois períodos analisados (DPN 21 e 120), tais como, alterações no volume relativo de compartimento estromal e de altura de epitélio tireoidiano, em especial nas fêmeas no DPN 21 e alterações hormonais em machos no DPN 21. Os níveis de proliferação celular pelo antígeno nuclear de proliferação celular (PCNA) foram semelhantes entre os grupos nos dois períodos analisados. Os resultados indicam que a exposição materna a uma mistura complexa de ftalatos e em doses relevantes não causou alterações importantes da morfologia da tireoide dos descendentes.

Palavras chaves: Misturas de Ftalatos; Exposição materna; Descendência (F1); Tireoide; Ratos Sprague-Dawley;

Abstract

Endocrine Disruptors (ED) are natural and synthetic chemicals produced by human action that have the potential to interfere with the endocrine system, affecting the growth and reproduction of living organisms, including the human. ED mainly affect the function of steroid hormones, and may have anti-estrogenic/estrogenic or anti-androgenic/androgenic activity or other organs since as thyroid development and function. Despite scarce epidemiological evidence, there are reports that ED, such as bisphenol A (BPA) and phthalates, can alter the levels of thyroid hormones in pregnant women, leading to hormonal imbalance in the embryo and fetus. Phthalates are a class of substances used, among other purposes, as plastic softeners, which are classified into low and high molecular weights. Thus, phthalates and their metabolites are detected in the young/adult population in several biological matrices. In the literature, maternal exposure to phthalates has been associated with testicular dysgenesis, genital malformation, decreased production of sex hormones, ovarian alterations, among other changes in descendent. The transgenerational effect of maternal exposure to phthalates is not yet well established, but preclinical studies have indicated that phthalates can lead to reproductive and endocrine disorders in the offspring, but few animal studies evaluate adverse effects of ED complex mixtures of. Thus, the aim of this study was to assess whether maternal exposure to a mixture of six different phthalates [19% DEHP (Bis (2-ethylexyl) phthalate), 36% DEP (Diethylphthalate), 15% DBP (Di-n- butyl phthalate), 10% DiBP (Diisobutyl phthalate), 8% BBzP (Butylbenzyl phthalate), and 10% DiNP (Diisononyl phthalate)] is able to alter the development and function of the thyroid gland in the offspring of pre-pubertal and adult males and females Sprague-Dawley rats.(F1). The pregnant rats received the phthalate mixture from gestational day 12 (DG12) to postnatal day 21 (PND 21), by daily gavage, in the doses of 20 μ g, 200 μ g and 200mg/kg. On PND 21 and 120, the thyroid (8 animals per litter, male or female) were removed and processed histologically for morphological and immunohistochemical analyzes. Few morphological changes were observed in the thyroid in both periods analyzed, such as changes in the relative volume of the stromal compartment and height of the thyroid epithelium, especially in females and TSH hormone changes in males.

Key words: Phthalate mixture; Maternal exposure; Offspring (F1); Thyroid; Sprague-Dawley rats;

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Abreviações:

BPA, Bisfenol A;

Des.....Desreguladores endócrinos

POPs.....Poluentes orgânicos persistentes

DEHP.....Bis(2-etilhexil) ftalato

DEP..... Dietilftalato

DBP.....Di-n-butilftalato

DiBP..... Diisobutilftalato

BBzP..... Butilbenzilftalato

DiNPDiisonilftalato

DPN..... Dias pós-natal

Pht Phthalates

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Conclusão

Os resultados obtidos permitem predizer que as doses de exposição materna utilizadas neste estudo, bem mais próximas das encontradas na exposição humana, também são capazes de causar algumas alterações na glândula tireoidiana, porém em menor intensidade que as doses mais altas encontradas na literatura.

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