Medizinische Fakultät Charité – Universitätsmedizin Berlin
Campus Benjamin Franklin
aus dem Institut für Klinische Pharmakologie und Toxikologie
Direktor (kommissarisch): Prof. Dr. Ralf Stahlmann

A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on reproductive development and function of male offspring rats

Inaugural-Dissertation
zur Erlangung des Grades
Doctor rerum medicarum
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Campus Benjamin Franklin

vorgelegt von Anderson Joel Martino Andrade
aus Curitiba (Brasilien)

Gutachter

1.: Prof. Dr. I. Chahoud

2.: Prof. Dr. H. Hörtnagl

3.: Prof. Dr. rer. nat. habil. A. Hartwig

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Inhaltsverzeichnis

1 Abstract

In the present study, the reproductive effects of *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP) were investigated in male offspring rats. A large number of low and high doses were used to adequately characterize the dose-response relationship for different endpoints. Female Wistar rats were treated daily with DEHP and peanut oil (control) by gavage from gestation day 6 to lactation day 21 at doses of 0.015, 0.045, 0.135, 0.405 and 1.215 mg DEHP/kg body weight (bw)/day (low doses) and at 5, 15, 45, 135 and 405 mg DEHP/kg bw/day (high doses). A significant delay in the age at puberty onset (preputial separation) was observed in male offspring exposed to 15, 45, 135 and 405 mg/kg/day. Nipple retention and reduced anogenital distance, both sensitive markers of anti-androgenic effects during development, were only observed at the highest DEHP dose. In addition, a transient increase in testis weight (an effect that qualitatively differs from high dose exposures) was detected in weaning rats (postnatal day 22) exposed to 5, 15, 45, and 135 mg/kg/day. The activity of brain aromatase, the enzyme that catalyzes the conversion of testosterone to estradiol and plays a critical role in the sexual differentiation of the central nervous system, was significantly changed in male and female rats. In newborn males (postnatal day 1), aromatase activity was inhibited at low doses and increased at high doses resulting in a nonmonotonic dose response profile which resembled a J-shaped curve. In contrast to the results on PND 1, males were largely unaffected at weaning (PND 22), indicating that the observed effects on aromatase activity were transient. At adulthood, a reduction in daily sperm production of 19-25% relative to control was observed in male offspring exposed to 15 mg/kg/day and higher doses. In addition, a low incidence of cryptorchidism was observed in DEHP exposed groups with a lowest observed adverse effect level of 5 mg/kg/day. No adverse effects were observed in male sexual behaviour. Overall, the present results indicate that *in utero* and lactational DEHP exposure can induce reproductive tract abnormalities and impair the sexual development and testicular function of male offspring rats. The fact that some endpoints (testis weight at weaning and brain aromatase activity in newborn males) showed biphasic responses indicates that a full picture of DEHP effects can only be observed when a wide range of low and high doses are used.

10 Anexx

10.1 Curriculum vitae

Mein Lebenslauf wird aus Datenschutzgründen in der elektronischen Version meiner Arbeit nicht mit veröffentlicht.

10.2 List of publications

1. Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2007). A dose response study following *in utero* and lactational exposure to Di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult female offspring rats. Toxicology 229, 114-122.

2. Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following *in utero* and lactational exposure to Di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228, 85-97.

3. Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose response study following *in utero* and lactational exposure to Di-(2-ethylhexyl) phthalate (DEHP): Nonmonotonic dose response and low dose effects on rat brain aromatase activity. Toxicology 227, 185-192.

4. Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225, 64-74.

5. Grande, S. W., Andrade, A. J., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose-response study following *in utero* and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. Toxicol. Sci. 91, 247-254.

6. Grote, K., Andrade, A. J., Grande, S. W., Kuriyama, S. N., Talsness, C. E., Appel, K. E., Chahoud, I. (2006). Effects of peripubertal exposure to triphenyltin on female sexual development of the rat. Toxicology 222, 17-24.

7. Dalsenter, P. R., Cavalcanti, A. M., Andrade, A. J., Araújo, S. L., Marques M. C. (2004). Reproductive evaluation of aqueous crude extract of Achillea millefolium L. (Asteraceae) in Wistar rats. Reprod. Toxicol. 18, 819-23.

8. Ohi, M., Dalsenter, P. R., Andrade A. J., Nascimento, A. J. (2004). Reproductive adverse effects of fipronil in Wistar rats. Toxicol. Lett. 146, 121-7.

9. Dalsenter, P. R., Araújo, S. L., de Assis, H. C., Andrade, A. J., Dallegrave, E. (2003). Pre and postnatal exposure to endosulfan in Wistar rats. Hum. Exp. Toxicol. 22, 171-5.

10. Andrade, A. J., Araújo, S. L., Santana, G. M., Ohi, M., Dalsenter, P R. (2002). Reproductive effects of deltamethrin on male offspring of rats exposed during pregnancy and lactation. Regul. Toxicol. Pharmacol. 36, 310-7.

11. Andrade, A. J., Araújo, S. L., Santana, G. M., Ohi, M., Dalsenter, P R. (2002). Screening for in vivo (anti)estrogenic and (anti)androgenic activities of technical and formulated deltamethrin. Regul. Toxicol. Pharmacol. 35, 379-82.

10.3 Anteilserklärung

Ich, Anderson Joel Martino Andrade, erkläre, dass ich der Hauptautor der vorliegenden Publikationen war. Die Publikationen fassen die Ergebnisse einer ausführlichen Dosis-Wirkungs-Studie an männlichen Ratten, die gegenüber Di-(2-ethylhexyl) phthalat (DEHP) während der Trächtigkeit und Laktationsperiode exponiert waren, zusammen. Als Hauptforscher des Anteils der Studie zur Wirkung auf die männlichen Nachkommen habe ich die Experimente durchgeführt, die Ergebnisse analysiert und die Publikationen mit folgenden Titeln als Erstautor selbst verfasst:

Andrade, A. J., Grande, S. W., Talsness, C. E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose response study following *in utero* and lactational exposure to Di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. Toxicology 228, 85-97.

Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Chahoud, I. (2006). A dose response study following *in utero* and lactational exposure to Di-(2-ethylhexyl) phthalate (DEHP): Nonmonotonic dose response and low dose effects on rat brain aromatase activity. Toxicology 227, 185-192.

Andrade, A. J., Grande, S. W., Talsness, C. E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I. (2006). A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225, 64-74.

Alle anderen Mitautoren der Publikationen waren an der Studie beteiligt.
Toxicology impact factor: 2.584 (Quelle: ISI Web of Knowlegde)
Rank 13 aus 75 Toxikologischen Zeitschriften (Quelle: ISI Web of Knowlegde)

Anderson Joel Martino Andrade — **Promovend**

Prof. Dr. Ibrahim Chahoud — **Betreuer**

10.4 Eidesstattliche Erklärung

Ich, Anderson Joel Martino Andrade, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: „A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on reproductive development and function of male offspring rats“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“