Medizinische Fakultät Charité – Universitätsmedizin Berlin
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A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on reproductive development and function of female offspring rats

Inaugural-Dissertation
zur Erlangung des Grades
Doctor rerum medicarum
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Inhaltsverzeichnis

1 Abstract

Phthalates, a class of chemicals used as plasticizers, have attracted special attention because their high-volume production, ubiquitous environmental presence and possible association with adverse reproductive health outcomes. Di-(2-ethylhexyl) phthalate (DEHP) is the most common phthalate plasticizer used in the manufacture of a broad range of consumer goods. Although there is some evidence of female reproductive toxicity in adult animals, little is known about the effects of developmental exposure to these compounds on females. We performed an extensive dose response study following *in utero* and lactational exposure to DEHP and evaluated the reproductive effects on female offspring rats. Two wide ranges of doses which included dose levels relevant for human exposure, as well as high doses typically used in toxicological studies were tested. Female Wistar rats were treated daily with DEHP and peanut oil (vehicle control) by gavage from gestation day 6 to lactation day 21. The low-doses were: 0.015, 0.045, 0.135, 0.405 and 1.215 mg DEHP/kg bw/day and the high-doses were: 5, 15, 45, 135 and 405 mg DEHP/kg bw/day. At the dose levels tested, no signs of maternal toxicity were observed. A significant delay in the age at vaginal opening (approximately 2 days) was observed at 15 mg DEHP/kg bw/day and higher doses, as well as a trend for a delay in the age at first estrus at 135 and 405 mg DEHP/kg bw/day (approximately 2 days). Anogenital distance and nipple development were unaffected. Liver enlargement (characteristic of phthalate exposure in rats) was limited to the 135 and 405 mg DEHP/kg bw/day doses. In addition, an increase in liver and kidney weight was detected in dams (PND 22) at the highest dose (405 mg/kg/day). At adulthood, no effects on organ (liver, kidney, spleen, thymus, thyroid, ovary and uterus) or body weights were detected. Females presented a normal pattern of estrous cyclicity with no hormonal alterations (serum estradiol and progesterone). A statistically significant increase in tertiary atretic follicles was observed at the highest dose (405 mg/kg DEHP/day). Morphometric analysis of uterus and vagina luminal epithelial cell height were unaffected by treatment. Overall, these results indicate that developmental DEHP exposure can delay the onset of puberty in female offspring rats at doses that induce similar effects in males. However, the reproductive endpoints investigated during adulthood were largely unaffected in female offspring.

10 Annex

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. Lilienthal, H., Hack, A., Roth-Härer, A., Grande, S. W., Talsness, C. E. (2006). Effects of developmental exposure to 2,2',4,4',5-Pentabromodiphenyl Ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. Environmental Health Perspectives, 114, 194-201.

8. Talsness, C. E., Shakibaei M., Kuriyama, S. N**.,** Grande, S. W., Sterner-Kock, A., Schnitker, P., Souza, C., Grote, K., Chahoud, I. (2005). Ultrastructural changes observed in rat ovaries following in utero and lactational exposure to low doses of a polybrominated flame retardant. Toxicology Letters 157, 189-202.

9. Higuti, I. H., Grande, S. W., Sacco, R., Nascimento A. J. (2003) Isolation of Alkalophilic CGTase-Producing Bacteria and Characterization of Cyclodextrin-Glycosyltransferase. Brazilian Archives of Biology and Technology 46, 183-186.

10.3 Anteilserklärung

Ich, Simone Wichert Grande, erkläre, dass ich die Erstautorin der folgenden Publikationen war:

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.

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.

Die Publikationen fassen die Ergebnisse einer ausführlichen Dosis-Wirkungs-Studie an weiblichen Ratten, die gegenüber Di-(2-ethylhexyl) phthalat (DEHP) während der Trächtigkeit und Laktationsperiode exponiert waren, zusammen. Die Experimente des Anteils der Studie zur Wirkung auf die weiblichen Nachkommen und die Analyse der Ergebnisse habe ich als hauptsächlich beteiligte Forscherin durchgeführt.

Die Publikation mit dem Titel

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

habe ich als Co-Autorin mitverfasst. In diesem Teil der Studie war ich hauptverantwortlich für die Messung der Aromatase Aktivität bei den weiblichen exponierten Nachkommen.

Alle anderen Mitautoren der Publikationen waren an der Studie beteiligt.

Toxicological Sciences impact factor: 3.088
Rank 10 aus 75 Toxikologischen Zeitschriften (Quelle: ISI Web of Knowlegde)

Toxicology impact factor: 2.584 (Quelle: ISI Web of Knowlegde)
Rank 13 aus 75 Toxikologischen Zeitschriften (Quelle: ISI Web of Knowlegde)

Simone Wichert Grande — **Promovendin**

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

10.4 Eidesstattliche Erklärung

Ich, Simone Wichert Grande, 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 female 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.