

Charité | Campus Benjamin Franklin | Garystrasse 5, 14195 Berlin

Dr. Michael D. Shelby
CERHR Director
National Institute of Environmental Health
Sciences
PO Box 12233 - MD EC-32
79 T.W. Alexander Dr. - Bldg. 4401
Research Triangle Park, NC 27709

Institut für Klinische Pharmakologie und Toxikologie
Abteilung Toxikologie

Prof. Dr. med. Gilbert Schönfelder

Unser Zeichen:
Tel. +49 30 8445 1704
Fax +49 30 8445 1761
Gilbert.schoenfelder@charite.de
www.charite.de/iklphatox



Dear Dr. Shelby,

gladly I shall respond to the comments made on Bisphenol A by the committee of the National Toxicology Program in their "interim draft" of April 2007. I hope that my comments to the statements help to clarify the points in question.

1. **Statement:** "Schönfelder *et al.* {Schönfelder, 2002 #535}, supported by the German Federal Ministry for Education and Research, examined the effects of prenatal bisphenol A exposure on the rat vagina. Sprague Dawley rats were gavaged on GD 6–21 with bisphenol A at 0 (2% corn starch vehicle), 0.1, or 50 mg/kg bw/day. A positive control group was treated with 0.2 mg/kg bw/day 17-estradiol in a peanut oil vehicle. **[No information was provided on the number of dams treated, the day of vaginal plug, purity of bisphenol A, or the type of chow, bedding, and caging materials used.]**"

Comment: This publication is a part of a study which was conducted in our institute (Talsness C, Fialkowski O, Gericke C, Merker H.J, Chahoud I (2000) The effects of low and high doses of Bisphenol A on the reproductive system of female and male rat offspring. Congenital Anomalies 40: 94-107). Here ist the requested information

- *No information was provided on the number of dams treated:* The litter size was: Mondamin =20, 0.1 mg BPA= 20, 50 mg BPA= 18 and 0.2 mg 17 α EE= 11.
- *The day of vaginal plug:* As a vaginal plug is not reliable in the rat, the day of a sperm positive smear was considered to be = gd 0.
- *Purity of bisphenol A:* The purity was \geq 98%.
- *The type of chow:* Rodent chow (altromin 1324) obtained from Altromin GmbH (Lage, FRG).
- *Bedding:* Wood shavings obtained from Altromin GmbH.
- *Caging materials used:* Type III macrolon cages.

2. **Statement:** “At 3 months of age, estrous cyclicity was evaluated for 3 weeks in 42 female offspring of the control group, 21 offspring of the 0.1 mg/kg bw/day group, 18 offspring of the 50 mg/kg bw/day group, and 24 offspring of the 17 α –estradiol group. **[The number of litters represented was not stated.]**”

Comment: This publication is a part of a study which was conducted in our institute (Talsness C, Fialkowski O, Gericke C, Merker H.J, Chahoud I (2000) The effects of low and high doses of Bisphenol A on the reproductive system of female and male rat offspring. *Congenital Anomalies* 40: 94-107). Here ist the requested information:

- *The number of litters represented was not stated:* The litter size was: Mondamin =20, 0.1 mg BPA= 20, 50 mg BPA= 18 and 0.2 mg 17 α EE= 11.

3. **Statement:** “At 4 months of age, female offspring were killed in either estrus or diestrus. Vaginas were fixed in Bouin solution and a histopathological evaluation was conducted. Western blot analyses were conducted to measure expression of ER and ER . **[No information was provided on the number of animals examined, and it does not appear that statistical evaluations were conducted.]**”

Comment: Here ist the requested information:

- *No information was provided on the number of animals examined:* This is not true. In the „materials and methods“ section the exact numbers of animal were provided by the authors.
- *It does not appear that statistical evaluations were conducted:* Indeed, there was no statistical analysis performed, because we did not observe significant deviation within each group.

4. **Statement:** “Strengths/Weaknesses: Vaginal histopathology of female offspring is of interest but the quality of the study cannot be judged due to unclear methodology. Uncertainty about the numbers of animals (7 or 8 dams may have been used in each group, but group size is uncertain) and the number of offspring examined render this study of marginal value.

Utility (Adequacy) of the CERHR Evaluation Process: This study is inadequate for the evaluation process.”

Comment: Given the exact number of offspring, which were analyzed should not render this study of marginal value, because we always used offspring from different mothers for our analysis. Each estrus group contained 22 offspring from 20 dams in the cornstarch group, 13 offspring from 13 dams in the 0.1 mg /kg / d and 12 offspring from 12 dams in the 50 mg / kg / d BPA group, as well as 19 offspring from 11 dams in the 0.2 mg / kg / d E2 group.

4. **Statement:** “Schönfelder et al. {Schönfelder, 2004 #687}, supported by the German Federal Ministry for Environmental Protection and Radiation Security, examined the effects of prenatal bisphenol A exposure on the rat uterus. **[No information was provided about composition of feed, caging, or bedding.]** Sprague Dawley rats [number treated not specified] were gavaged with bisphenol A **[purity not reported]** at 0 (2% corn starch vehicle), 0.1, or 50 mg/kg bw/day on GD 6–21. The high bisphenol A dose was selected because it was reported to be the no observed effect level (NOEL) recommended by the Society of the Plastics Industry.”

Comment: Here ist the requested information:

- *No information was provided about composition of feed, caging, or bedding:*
 - The type of chow: Rodent chow (altromin 1324) obtained from Altromin GmbH (Lage, FRG).
 - Bedding: Wood shavings obtained from Altromin GmbH.
 - Caging materials used: Type III macrolon cages.
- *Purity not reported:* The purity of bisphenol A was \geq 98%.

5. **Statement:** The high bisphenol A dose was selected because it was reported to be the no observed effect level (NOEL) recommended by the Society of the Plastics Industry. A positive control group was gavaged with 0.2 mg/kg bw/day ethinylestradiol on GD 6–21. Estrous cyclicity was examined for 3 weeks in 6 female offspring/group beginning at 3 months of age. Six female offspring/group were killed at 4 months of age on the day of estrus. Body and reproductive organ weights were measured. Uteri were fixed in methacarn solution and sectioned. Examinations of uterine morphology were conducted. Immunohistochemistry techniques were used to detect ER and ER in the uterus, and results were verified by Western blot. Data were analyzed by Mann-Whitney test. **[It was not clear if data were analyzed on a per litter or per offspring basis.]**

Comment: Each female came from a different litter so the data were analyzed on a per litter basis.

6. **Statement:** *“Table 71. Uterine Effects in Rats Exposed to Bisphenol A During Prenatal Development. Values were estimated by CERHR from a graph.”*

Comment: Why would CERHR want to estimate the values from a graph, when exact data were provided by the figure legends

7. **Statement:** *“Strengths/Weaknesses: A strength is the examination of effects on uterine indices in female offspring, however, this strength is overwhelmed by the weakness inherent in the data being based on 6 females/group, which is too few animals to reach a conclusion with certainty. There are also uncertainties about the number of litters examined.”*

Comment: The differences between the control and treated groups were statistically significant. Obviously, the magnitude of the effect was strong enough to be detected with a group size of six. It is noteworthy that another publication # 343 in the original CERHR analysis was deemed "very useful in the evaluation" that had n=5, n=6, n=7, n=11 litters for male organ weights. It is unclear why this "line of thinking" has been applied inconsistently. Even though the positive control in this 343 study showed no effect, here the number of statistical units was deemed sufficient.

Sincerely,



Gilbert Schönfelder