Section 508–conformant HTML versions of these articles are available at http://dx.doi.org/10.1289/ehp.1511031 and http://dx.doi.org/10.1289/ehp.1511181.

The Correspondence section is not peer-reviewed. Personal opinions expressed herein are the sole responsibility of the authors. EHP *neither endorses nor disputes the content of the letters it publishes.*

Comment on "Effects of *in Utero* Exposure to Arsenic during the Second Half of Gestation on Reproductive End Points and Metabolic Parameters in Female CD-1 Mice"

http://dx.doi.org/10.1289/ehp.1511031
Refers to http://dx.doi.org/10.1289/ehp.1509703

Rodriguez et al. recently reported that the adult female offspring of pregnant CD-1 mice exposed to 10 ppb or 42.5 ppm arsenic (As) exhibited reproductive and metabolic effects. These findings are not consistent with those of others working in this field and did not show a dose response. As such, we urge caution against drawing conclusions based on this single study.

Rodriguez et al. reported that on postnatal day 21, female offspring in the 10-ppb and 42.5-ppm dose groups were approximately 11% and 7% heavier, respectively, than controls. At 6 months of age, the treated female offspring were ≥ 22% (the exact percentages are unclear from the study report) heavier than controls. The study authors mention data from other investigators using C57BL6/J mice showing no effects of gestational As exposure on offspring body weight (Ramsey et al. 2013; Kozul-Horvath et al. 2012). Other studies using a similar exposure paradigm also reported no effects on body weight of the female offspring of C3H mice (Waalkes et al. 2003, 2004), C57BL6/J mice (Markowski et al. 2011, 2012), or Tg.AC mice (Tokar et al. 2010).

The authors suggest that discrepancies between their results and those of others may be due to differences in the genetic background of the mice in the various studies. However, Waalkes et al. (2006) conducted a study in pregnant CD-1 mice exposed to 0 or 85 ppm sodium arsenite from gestational day (GD) 8 to GD18 with no effects of treatment on female offspring body weight. In another study (Tokar et al. 2011) involving whole-life exposure (from preconception into adulthood) of CD-1 mice to 6, 12, or 24 ppm sodium arsenite, body weights of treated mice were similar to those of controls at all time points assessed. It is also interesting to note that controls in the study by Tokar et al. (2011) weighed considerably more (42.4 g at 25 weeks of age) than controls in the Rodriguez et al. study (approximately 34.4 g at 26 weeks of age).

The reason for this discrepancy between the findings of Rodriguez et al. and those of other investigators is not known but may relate to differences in diet or husbandry. Alternatively, it is possible that the controls in the study by Rodriguez et al. are unusually small for their age, such that the observed effect of treatment may be a statistical anomaly; this could explain why a dose-related difference was not observed.

Rodriguez et al. also reported that both doses of As were associated with early vaginal opening; again, no dose response was evident. The authors mention the results of two other studies in their discussion—both of which showed delays in puberty rather than early onset (Reilly et al. 2014; Davila-Esqueda et al. 2012). We identified two other studies that examined vaginal opening with gestational-only As exposure. Markowski et al. (2012) exposed pregnant C57BL6/J mice to 0, 8, 25, or 80 ppm sodium arsenite from GD4 until birth with no effects on the onset of puberty. Gandhi et al. (2012) exposed pregnant albino rats to 0, 1.5, 3, or 4.5 mg As/kg/day from GD8 until birth; again, no effects on vaginal opening were observed.

The onset of puberty is positively correlated with body weight (Carney et al. 2004). Unfortunately, Rodriguez et al. did not report the mean weights at vaginal opening; therefore, we do not know if the early vaginal opening in the As-treated groups may have been a function of the increased body weights. However, animals in the 10-ppb and 42.5-ppm groups weighed more than controls and thus were likely to reach puberty earlier than controls.

In closing, the findings of Rodriguez et al. with regard to body weight and pubertal effects conflict with those of other investigators. Until other investigators can replicate the results reported by Rodriguez et al., we believe these findings should be viewed with extreme caution.

Financial support for this analysis was provided in part by the Arsenic Science Task Force. The analysis and conclusions presented in this letter are those of the authors.

Amy L. Williams and John M. DeSesso
Exponent, Inc., Alexandria, Virginia, USA

Address correspondence to A.L. Williams, Exponent, Inc., 1800 Diagonal Rd., Ste. 500, Alexandria, VA 22314 USA. E-mail: awilliams@exponent.com

References

Carney EW, Zablotny CL, Marty MS, Crissman JW, Anderson P, Woolhiser M, et al. 2004. The effects of feed restriction during *in utero* and postnatal development in rats. Toxicol Sci 82(1):237–249, PMID:15310860.

Davila-Esqueda ME, Jiménez-Capdeville ME, Delgado JM, De la Cruz E, Aradillas-García C, Jiménez-Suárez V, et al. 2012. Effects of arsenic exposure during the pre- and postnatal development on the puberty of female offspring. Exp Toxicol Pathol 64(1–2):25–30, doi:10.1016/j.etp.2010.06.001.

Gandhi DN, Panchal GM, Patel KG. 2012. Developmental and neurobehavioural toxicity study of arsenic on rats following gestational exposure. Indian J Exp Biol 50(2):147–155, PMID:22670478.

Kozul-Horvath CD, Zandbergen F, Jackson BP, Enelow RI, Hamilton JW. 2012. Effects of low-dose drinking water arsenic on mouse fetal and postnatal growth and development. PloS One 7:e38249, doi:10.1371/journal.pone.0038249.

Markowski VP, Currie D, Reeve EA, Thompson D, Wise JP Sr. 2011. Tissue-specific and dose-related accumulation of arsenic in mouse offspring following maternal consumption of arsenic-contaminated water. Basic Clin Pharmacol Toxicol 108(5):326–332, doi:10.1111/j.1742-7843.2010.00660.x.

Markowski VP, Reeve EA, Onos K, Assadollahzadeh M, McKay N. 2012. Effects of prenatal exposure to sodium arsenite on motor and food-motivated behaviors from birth to adulthood in C57BL6/J mice. Neurotoxicol Teratol 34(2):221–231, doi:10.1016/j.ntt.2012.01.001.

Ramsey KA, Larcombe AN, Sly PD, Zosky GR. 2013. *In utero* exposure to low dose arsenic via drinking water impairs early life lung mechanics in mice. BMC Pharmacol Toxicol 14:13, doi:10.1186/2050-6511-14-13.

Reilly MP, Saca JC, Hamilton A, Solano RF, Rivera JR, Whitehouse-Innis W, et al. 2014. Prepubertal exposure to arsenic(III) suppresses circulating insulin-like growth factor-1 (IGF-1) delaying sexual maturation in female rats. Reprod Toxicol 44:41–49, doi:10.1016/j.reprotox.2013.09.005.

Tokar EJ, Diwan BA, Waalkes MP. 2010. Arsenic exposure *in utero* and nonepidermal proliferative reponse in adulthood in Tg.AC mice. Int J Toxicol 29(3):291–296, doi:10.1177/1091581810362804.

Tokar EJ, Diwan BA, Ward JM, Delker DA, Waalkes MP. 2011. Carcinogenic effects of "whole life" exposure to inorganic arsenic in CD1 mice. Toxicol Sci 119(1):73–83, doi:10.1093/toxsci/kfq315.

Waalkes MP, Liu J, Ward JM, Powell DA, Diwan BA. 2006. Urogenital carcinogenesis in female CD1 mice induced by *in utero* arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. Cancer Res 66(3):1337–1345, doi:10.1158/0008-5472.CAN-05-3530.

Waalkes MP, Ward JM, Diwan BA. 2004. Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. Carcinogenesis 25(1):133–141, doi:10.1093/carcin/bgg181.

Waalkes MP, JM Ward, J Liu, BA Diwan. 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol Appl Pharmacol 186(1):7–17, doi:10.1016/S0041-008X(02)00022-4.

Response to "Comment on 'Effects of *in Utero* Exposure to Arsenic during the Second Half of Gestation on Reproductive End Points and Metabolic Parameters in Female CD-1 Mice'"

http://dx.doi.org/10.1289/ehp.1511181
Refers to http://dx.doi.org/10.1289/ehp.1509703

In a letter in response to our paper, Williams and DeSesso questioned why a significant weight gain in arsenic (As)-exposed offspring was found in our study but not in others (Markowski et al. 2011; Markowski et al. 2012; Tokar et al. 2010; Waalkes et al. 2003; Waalkes et al. 2004; Waalkes et al. 2006). In addition, they point out that early onset of vaginal opening in response to gestational As exposure was not observed by Markowski et al. (2012) or Gandhi et al. (2012). As we mentioned in the Discussion section of our

paper, some of the results under our exposure scheme do not recapitulate those observed in other studies.

The main difference that could contribute to these discrepancies is that the offspring in our study were fostered to dams that were not exposed to As during gestation. Dams exposed gestationally to As are known to produce lower-quality milk, which can result in weight deficits in their pups (Kozul-Horvath et al. 2012). In contrast, the studies mentioned by Williams and DeSesso left offspring with their As-exposed mothers. It is therefore possible that the impacts of gestational As exposure on milk quality could offset the effects of As on offspring weight gain and vaginal opening.

Regarding the lack of a dose response, our study was designed to examine the impact of two specific As doses: 10 ppb (the U.S. Environmental Protection Agency drinking water standard) and 42.5 ppm (tumor-inducing concentration). Dose–response experiments are usually performed to identify either the proper dose for further experiments or the mode of action of a particular chemical (linear, biphasic, or others). Neither of these two parameters were an end point of our study. We do not have an explanation for the different responses between the 10-ppb and 42.5-ppm treatment groups, and further studies are definitely required.

Williams and DeSesso further suggest that the control pups in our study may have been unusually small, such that our results reflect a statistical anomaly. However, data on CD-1 female weights (Lang and White 1996) indicate that the weight of our control mice at 25 weeks (approximately 34.4 g) falls in the normal range of approximately 31–42 g.

Williams and DeSesso also questioned whether an increase in body weight could contribute to the early onset of vaginal opening. This argument is indeed the focal point of our experiments, as we described in the Results and Discussion sections of the paper. Based on the analyses that examined the association between weight at weaning (postnatal day 21) and age at vaginal opening (Figure 2D of our paper), we observed that the 42.5-ppm treatment and control groups showed a positive association between weight at weaning and onset of vaginal opening. This association was not found in the 10-ppb treatment group. Although we did not have the weight records at the time of vaginal opening, we believe the population data in Figure 2D are sufficient for us to make a valid conclusion regarding the associations. The two studies mentioned by Williams and DeSesso (Markowski et al. 2012 using B6 mice; Gandhi et al. 2012 using rats) found no effect of *in utero* exposure to As on vaginal opening. Strain and species differences may contribute to these discrepancies.

In summary, in our Discussion section we fully recognize the differences between our results and those of other studies. We agree with Williams and DeSesso that the discrepancies could result from experimental conditions such as diet, strain, and species. Like all animal studies, our study provides observations on a particular strain of mouse under specific experimental conditions. The differences among studies only strengthen the point that more studies are needed to understand the mechanisms of action of As and how these different experimental conditions influence the outcomes.

The authors declare they have no actual or potential competing financial interests.

Karina F. Rodriguez, Erica K. Ungewitter, Yasmin Crespo-Mejias, Chang Liu, Barbara Nicol, Grace E. Kissling, and Humphrey Hung-Chang Yao

National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, North Carolina USA

Address correspondence to H.H-C. Yao, National Institute of Environmental Health Sciences, 111 T.W. Alexander Dr., Mail Drop C4-10, Research Triangle Park, NC 27709 USA. E-mail: humphrey.yao@nih.gov

References

Gandhi DN, Panchal GM, Patel KG. 2012. Developmental and neurobehavioural toxicity study of arsenic on rats following gestational exposure. Indian J Exp Biol 50(2):147–155, PMID: 22670478.

Kozul-Horvath CD, Zandbergen F, Jackson BP, Enelow RI, Hamilton JW. 2012. Effects of low-dose drinking water arsenic on mouse fetal and postnatal growth and development. PloS One 7:e38249, doi:10.1371/journal.pone.0038249.

Lang PL, White WJ. 1996. Growth, development, life span, and select lesion incidence in the aging CD-1 mouse. In: Pathobiology of the Aging Mouse, Vol. 1 (Mohr U, Dungworth DL, Capen CC, Carlton WW, Sundberg JP, Ward JM, eds.). Washington D.C.:ILSI, 27–44.

Markowski VP, Currie D, Reeve EA, Thompson D, Wise JP Sr. 2011. Tissue-specific and dose-related accumulation of arsenic in mouse offspring following maternal consumption of arsenic-contaminated water. Basic Clin Pharmacol Toxicol 108(5):326–332, doi:10.1111/j.1742-7843.2010.00660.x.

Markowski VP, Reeve EA, Onos K, Assadollahzadeh M, McKay N. 2012. Effects of prenatal exposure to sodium arsenite on motor and food-motivated behaviors from birth to adulthood in C57BL6/J mice. Neurotoxicol Teratol 34(2):221–231, doi:10.1016/j.ntt.2012.01.001.

Tokar EJ, Diwan BA, Waalkes MP. 2010. Arsenic exposure *in utero* and nonepidermal proliferative reponse in adulthood in Tg.AC mice. Int J Toxicol 29(3):291–296, doi:10.1177/1091581810362804.

Waalkes MP, Liu J, Ward JM, Powell DA, Diwan BA. 2006. Urogenital carcinogenesis in female CD1 mice induced by *in utero* arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. Cancer Res 66(3):1337–1345, doi:10.1158/0008-5472.CAN-05-3530.

Waalkes MP, Ward JM, Diwan BA. 2004. Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. Carcinogenesis 25(1):133–141, doi:10.1093/carcin/bgg181.

Waalkes MP, JM Ward, J Liu, BA Diwan. 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol Appl Pharmacol 186(1):7–17, doi:10.1016/S0041-008X(02)00022-4.