Flawed Experimental Design Reveals the Need for Guidelines Requiring Appropriate Controls in Endocrine Disruption Research

Frederick S. vom Saal,† Benson T. Akingbemi,‡ Scott M. Belcher,§ David A. Crain,§ David Crews,¶ Linda C. Guidice,|| Patricia A. Hunt,||| Csaba Leranth,|||| John Peterson Myers,# Angel Nadal,** Nicholas Olea,†† Vasantha Padmanabhan, a Cheryl S. Rosenfeld, b Alan Schneyer, c Gilbert Schoenfelder, d Carlos Sonnenschein, e Ana M. Soto, e Richard W. Stahlhut, f Shanna H. Swan, g Laura N. Vandenberg, h Hong-Sheng Wang, i Cheryl S. Watson, j Wade V. Welschons, k and Robert T. Zoeller l

*Division of Biological Sciences, University of Missouri Columbia, Missouri 65211; †Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849; ‡Department of Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, Ohio 45267-0575; §Department of Biology, Maryville College, Maryville, Tennessee 37804-5907; ¶Section of Integrative Biology, University of Texas, Austin, Texas 78712; ‖Department of Obstetrics, Gynecology & Reproductive Sciences, University of California, San Francisco, San Francisco, California 94143-0132; ||School of Molecular Biosciences and Center for Reproductive Biology, Washington State University, Pullman, Washington 99164; |||Department of Obstetrics Gynecology & Reproductive Sciences, Yale University, New Haven, Connecticut 06520; #Environmental Health Sciences, Charlottesville, Virginia 22902; **Instituto de Bioingenieria and CIBERDEM, Universidad Miguel Hernandez de Elche Elche, Alicante, Spain; ††Hospital Clinico, University of Granada, 18071 Granada, Spain; †Department of Pediatrics, Obstetrics and Gynecology, and Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan 48109-0404; ‡‡Veterinary Biomedical Science, University of Missouri-Columbia, Columbia, Missouri 65211; †††Pioneer Valley Life Science Institute, University of Massachusetts-Amherst, Springfield, Massachusetts 01107; ††‡Institute of Pharmacology and Toxicology, Julius-Maximilians-Universität Würzburg, 97078 Wuerzberg-Germany; †‡Department of Anatomy and Cell Biology, Tufts University School of Medicine, Boston, Massachusetts 02111; † Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14624; †§Department of Obstetrics and Gynecology, Center for Reproductive Epidemiology, University of Rochester School of Medicine and Dentistry Rochester, New York 14624; †‡Department of Biology, Tufts University, Medford, Massachusetts 02155; †§Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267-0575; ††§Department of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston Texas 77555-0645; ††Department of Biomedical Sciences, University of Missouri-Columbia, Columbia, Missouri 65211; and †‖Biology Department, University of Massachusetts-Amherst, Amherst, Massachusetts 01003

1 To whom correspondence should be addressed at Division of Biological Sciences, 105 Lefevre Hall, University of Missouri, Columbia, MO 65211. Fax: (573) 884-5020. E-mail: vomsaalf@missouri.edu.

Received December 2, 2009; accepted December 7, 2009

A study published in Toxicological Sciences (Ryan et al., 2009) illustrates the importance of examining appropriate doses of both the positive control and the test chemical in research on endocrine-disrupting chemicals. For the three low doses of bisphenol A (BPA) that were fed to rats during pregnancy and lactation, there were no effects on female offspring (there were also no effects on male offspring from the same experiment; Howdeshell et al., 2008). A review of the results of the positive control doses makes it clear that the experiment cannot adequately assess the consequences of low-dose exposure to BPA because the animal model is insensitive to low doses of the positive control estrogen. Therefore, conclusions being drawn from this experiment about low-dose responses to any estrogen are invalid, including that of “no harm” from the low doses of BPA that were tested. However, the experiment is important because it highlights the need to apply basic principles of study design, long known and accepted in studies of hormones and hormonally active drugs, to toxicological studies of chemicals with hormonal activity.

Specifically, the study by Ryan et al. (2009) using long evans (LE) rats was designed to test low doses of BPA that had previously been reported to cause effects in mice. However, the authors did not establish the sensitivity of the LE rat to the positive control ethinylestradiol (EE) for the outcomes being examined prior to determining what doses of BPA to test in their study. The lowest maternal dose of EE reported to cause effects in LE rat offspring by these authors was between 5 and 50 μg/kg/day (Ryan et al., 2009) thus report no effects of EE in their animal model at doses higher than the maternal dose required to stimulate effects on hormones and hormonally active drugs, to toxicological studies of chemicals with hormonal activity.

Received December 2, 2009; accepted December 7, 2009

© The Author 2010. Published by Oxford University Press on behalf of the Society of Toxicology. All rights reserved. For permissions, please email: journals.permissions@oxfordjournals.org
properly use oral contraceptives (Thayer et al., 2001). One potential contributor to the low sensitivity to estrogen in this experiment is the use of polycarbonate cages made from BPA.

Numerous reports show that for the types of outcomes examined in LE rat offspring, maternal doses of BPA between 100- and 1000-fold greater than the effective EE dose would be required (Richter et al., 2007; Timms et al., 2005). Thus, the minimum dose of BPA predicted to produce an effect on rodent reproductive organs observed at 5 μg/kg/day EE would be 500 μg/kg/day BPA. However, the only doses of BPA tested in LE rats were 2, 20, and 200 μg/kg/day. The appropriate conclusion to be drawn from this experiment is that due to the relative insensitivity of the outcomes examined in LE rats to the positive control estrogen, effects previously observed in numerous studies in response to low doses of BPA in other more sensitive model animals were not observed.

By failing to establish the sensitivity of the animal model to the class of chemical being tested, the authors violated U.S. National Toxicology Program (NTP) recommendations for low-dose studies of endocrine disrupting chemicals. The NTP (2001) recommends that: “Because of clear species and strain differences in sensitivity, animal model selection should be based on responsiveness to endocrine-active agents of concern (i.e., responsive to positive controls), not on convenience and familiarity” (p. VII). This comment was prompted by a BPA study published in Toxicological Sciences that used an insensitive rat and did not include a positive control but concluded, as did Ryan et al. (2009), that BPA caused no harm at low doses (Tyl et al., 2002). It is unacceptable in any research with experimental animals to not include both a negative control and “appropriate” positive control doses if the conclusion reached is no harm due to low-dose exposure to BPA or any other endocrine disrupting chemical.

In summary, publishing studies that conclude no harm in response to low doses of endocrine disrupting chemicals, when the studies did not include a positive control (Tyl et al., 2002), included inappropriate doses of positive controls (Ryan et al., 2009; Tyl et al., 2008), or included positive controls that showed no effect (Cagen et al., 1999), is inappropriate in peer-reviewed journals (Myers et al., 2009a,b; vom Saal and Welshons, 2006). Such studies violate basic principles of study design. To avoid allowing flawed research to enter the peer-reviewed literature, we recommend adoption of the following criteria:

1. Determine the sensitivity of systems being examined in a model animal by establishing the dose-response relationship for an appropriate positive control prior to designing an experiment to test chemicals for activity via similar mechanisms; Ryan et al. (2009) would have examined higher doses of BPA if they had followed this approach.

2. Include appropriate doses of “concurrent” positive controls, such as EE, for estrogenic test chemicals.

The latter is required to determine the relative potency and efficacy of a test chemical such as BPA in the model system at the time the chemical is tested since there are a myriad of factors that can alter outcomes from one experiment to another. In particular, a response to only very high concurrent positive control doses (e.g., Ryan et al., 2009; Tyl et al., 2008) is not relevant for predicting receptor-mediated low-dose activity of hormonally active chemicals (Myers et al. 2009a,b). We urge adoption of these essential control standards for publication of research on endocrine-disrupting chemicals.

REFERENCES


