PHOTO: TATIANA BULYONKOVA

The experimental rodent in the polycarbonate cage is the new canary in the coalmine. **Hannah Landecker** explores how environmental signals have slowly started to get clearer and louder.

WHEN THE CONTROL BECOMES THE EXPERIMENT

LABORATORY CREATURES AS ACCIDENTAL SENTINELS

Is there such thing as an accidental sentinel, warning of lurking insensible dangers that no one set out to detect? The experimental rodent of twentieth century life science, developed to display nothing but the workings of its inner biology, but becoming instead an accidental indicator of environmental change, might be an instructive example. It was just such a rodent that helped raise the alarm over BPA, now an acronym that floats through the consciousness of the Western consumer today. The story of how BPA or bisphenol-A, an organic compound used to make certain plastics that is also an endocrine disruptor that mimics the action of estrogen in the body, came to the attention of the plastic-bottle-sipping public has been detailed in a number of places. In most accounts, the chemical compounds or the scientist-investigators who study its health effects are the main characters (Vogel 2012). Here the story is retold with a focus on the control animal – in this case a laboratory mouse.

The story goes as follows: In 1998, biologist Patricia Hunt was studying the biology of infertility by using mouse strains that have unusually high numbers of chromosomal abnormalities resulting from aberrant meiosis. Meiosis is the kind of cell division that leads to germ cells – to sperm cells in male organisms and egg cells in females. Hunt's research was motivated by problems of human reproduction; the wrong number of chromosomes in eggs – aneuploidy—is a leading cause of miscarriage, congenital birth defects and mental retardation in humans. As is standard in experiments, Hunt also used "control" animals, supposedly normal mice, as a comparison – such animals are often referred to as "wild-type" if they do not carry the mutant genotype being tested although there is nothing wild about them. Hunt's control animals went through all the same interventions and experiences as the mutants used to study the chromosomal abnormalities.

The main function of the control animal is to pick up biological outcomes that might result from extraneous elements of the experimental protocol, rather than arising from the experimental intervention being tested or the mutation under study in the experimental animal. One week, out of the blue, Hunt found, "the control numbers were just completely bonkers" in more than one of her experiments; about 40% of the normal female mice suddenly began to suffer from failures of chromosomal alignment during meiosis (quoted in Gross 2007; see also Hunt et al. 2003). Upon a systematic review of the handling of the mice over the previous weeks, Hunt and her coworkers determined that the plastic cages in which the animals lived, and their water bottles, had been washed using a high pH detergent and sterilized at high heat. This caused the polycarbonate plastic to leach small amounts of chemicals, including bisphenol-A, which the animals ingest-

ed in their drinking water and absorbed through their skin.

Testing BPA by itself, Hunt then determined that the substance could be used to intentionally induce higher rates of these chromosomal abnormalities in the eggs of normal mice—compared of course to non-BPA-exposed controls (Hunt et al. 2003, 2009). In this way, the control subject of one experiment became the test animal in the next. Further research has raised the question of whether there is any such a thing as a non-BPA-exposed organism today: A recent review of the literature on BPA's potential role in obesity concludes that, "all human fetuses that have been examined have measurable blood levels of BPA, and mean or median levels found in humans are higher than levels found in fetal and neonatal mice in response to maternal doses that increase postnatal growth" (vom Saal et al. 2012).

Laboratory organisms began living in plastic instead of glass or metal enclosures about the same time Western consumer society was also making the switch in the middle of the twentieth century. However, awareness that something in the plastic could have negative effects on reproduction and health emerged much later, and was made possible in part by laboratory accidents such as Hunt's starting in the 1990s. In another lab, estrogen-sensitive cancer cells cultured in plastic dishes suddenly began to act as though they were being dosed with estrogen after a biological supply company changed the plastic composition of the bottles storing the nutrient medium used to culture the cells. Biologist Ana Soto and her collaborators determined that the substance feeding the cells' estrogen-mediated response was nonylphenol, another endocrine-disrupting estrogen mimic used in making polymers and detergents (Vandenberg et al. 2010).

The general category of "endocrine-disruptor" emerged as an issue of concern and debate during the 1990s. The book *Our Stolen Future* called attention to the effects of hormone mimics in industrial and waste effluent on wildlife, showing how the resulting abnormalities of sex development and reproductive damage were threatening exposed fish, amphibian and bird populations (Colborn et al. 1996). This context made it more likely that experimenters such as Hunt and Soto would be attuned to vacillations of the control numbers caused by industrially-derived substances. In addition, the larger research interest in endocrine disruptors gave them a body of science to contribute to when the control animal exited the periphery of the experiment and became the finding.

The story that has unfolded from here is one in which the effects of endocrine disruptors such as BPA are tested directly, at "environmentally-relevant" levels – that is, the kind of exposures humans living with plastics in contemporary industrialized societies might experience—on animals, particularly as they develop *in utero*. The findings have been racking up: Female mice and rats exposed to low doses prenatally grow into adults that suffer breast cancers and reproductive abnormalities at higher rates than unexposed animals; BPA easily crosses the placenta in humans and other animals, and affects placental cells themselves in terms of gene expression; BPA causes epigenetic changes in developing tissues that in turn shift patterns of gene expression potential in the adult animals, causing changes such as increased adiposity (Vandenberg et al. 2009; vom Saal et al. 2012).

These findings remain contested in terms of their translation into regulatory action, in part because chemical toxicity testing traditionally has not looked at low dose effects in developing organisms, but at high dose toxicity in adults. In other words, looking for long-term disturbances to reproductive biology due to low exposures during development is a very different test than looking for cancers, poisoning, or heart attacks in adult animals exposed to high amounts of a substance. However, the rapidly expanding field of environmental epigenetics has underscored the legitimacy of such a lifespan perspective. It provides both a logic and a technical means for measuring environmental harm that lodges in the body—not as a lesion, mutation in DNA, or a toxic effect immediately visible as a birth defect or a poisoning—but rather as a shift in gene regulation and gene expression with important long-term, rather than short-term, health effects. Environmental epigenetics, with its focus on the setting or re-setting of the molecules controlling gene expression in cells, provides a powerful framework for transforming environmental harm into epigenetic harm (Landecker 2011).

What is the role of the experimental animal, and in particular the control experimental animal as an accidental sentinel, in physically registering the environmentally-relevant dose and making visible epigenetic harm? The control animal is a check or comparison, and appears in experimentation because "a discoverable fact is a difference or a relation, and a discovered datum has significance only as it is related to a frame of reference, to a relatum" (Boring 1954). The relatum—the control—is injected with saline solution where the test animal gets the real deal; the control animal is cut open even if no surgical change is then effected, while the test animal has something ablated or manipulated. It is fed the same things, handled the same way, and housed in the same conditions as the test animal. It experiences the experiment, but is supposed to weather it all and remain that against which the experimental intervention may be measured. It is the ground against which difference may be achieved; it is supposed to keep the experiment honest.

In the attention to control numbers that "go bonkers," the frame of reference comes to the fore, instead of receding into the background of necessary but banal experimental procedure. The control reveals "environment" where there is presumed to be none of consequence. The experimental rodent in a polycarbonate cage eating chow pellets accidentally revealed material harm in the environment in the very place the "environment" is most suppressed. The environment of environmentalism – rivers and lakes, air and earth and trees – could not be further from these experimental spaces built on the premise of control. No one set out to look for these warning signs of impending environmental danger, in fact, no one thought to look for these particular environmental harms, in part because the cage, the water bottle, the culture medium, the food, were the background to the organismal biology in the foreground and had no presence as "the environment"—until these abnormalities were evident.

This story may look like one of contingency, out of which many scientific discoveries have arisen. Just think penicillin, and Pasteur's declaration that "chance favors the prepared mind." But look one level deeper: The rodent in the polycarbonate cage shows the contingency in this story to be fundamentally structured by human industrial activity. The "accident" of the accidental sentinel is not that of fate or chance, but one of attention. Thanks both to the widespread use of the model organism in biological science in the twentieth century and the ensuing in-

terest of historians of science in the role of model organisms in generating twentieth century life science, we know a great deal about the production of experimental rodents in terms of their selection, genetic manipulation, and breeding (Rader 2004). We know next-to-nothing – because we have thought next-to-nothing – about how they have been fed and housed.

The production of mice with certain gene knockouts or transgenic additions was and still is central to many areas of genetic research. The intense refinement of experimental rodents as an instrument of genetics – an attempt to purify them by holding everything still while just changing one component of the animals' genome – has paradoxically brought the background of such experiments into view. And what we see there is this: While these animals might have been bred to be model organisms of particular human diseases or ailments – such as infertility – they or their "wild-type" counterparts raised under identical conditions have become unintentional models of the sedentary "ad libitum," plastic-infused, nutritionally synthetic lives of contemporary North American human animals.

Experimental animals share, to a greater extent than is normally recognized, the habitat of their keepers – and this goes for much more than the plastic water bottles. Consider the finding that the control rodents kept under standard laboratory protocol used in biomedical research and preclinical drug testing in the United States are metabolically morbid: "Compared to those that are fed less, exercise more, and have a stimulating environment, animals maintained under the standard laboratory conditions are relatively overweight, insulin resistant, hypertensive, and are likely to experience premature death" (Martin et al. 2010). Elsewhere, attempts to test the effects of certain micronutrients or endocrine disruptors on developing or newborn mice have accidentally focused attention on the biological effects of standard synthetic or "natural" experimental animal diets (Waterland et al. 2006). Extensive interest in the role of "environmental enrichment" in affecting brain development and the making of new neurons in adults has brought attention to the "standard" environment of the control to which an "enriched" environment must be compared. Enrichment begs the question: Enriched compared to what? What lasting effects on the biology and behavior of generations of standard control rodents have resulted from living in a few square feet of bedding covered cage with continuous access to an overhead dispenser full of dry pellet food and no exercise wheel? Against what condition, what relatum, does one measure enrichment and its neurological effects?

It is impossible to say how many experiments have simply been discarded, discontinued, or judged to have "failed" when controls went awry, rather than interpreted as problems that signal something fundamentally wrong in the cage environment. For the control animal to have become an accidental sentinel, someone had to interpret the indication of danger, to see it as meaningful, and invert the experiment, bringing the control into the role of test subject in its own right. It is overreaching to conclude that control animals have somehow in themselves *caused* the reading and registering of environmentally relevant exposures and their potential epigenetic harms. However, as biomedical research looks increasingly to experimentally trace the developmental and epigenetic effects of environmental exposures, from nutrition to stress to pollution to exercise to visual stimulation, the standard cage environment of the twentieth century experimental rodent, which shares so much with the environment of the twentieth century industrialized human, is increasingly beginning to register as a measure and probe of environmental harm. If the canary in the coal mine was the warning figure for an age of the extraction of raw materials and the onset of industrialization, the experimental rodent in the polycarbonate cage may be seen as the warning figure for the early twenty-first century and the legacies of industrialization. ■

HANNAH LANDECKER *teaches in the Department of Sociology and the Institute for Society and Genetics at UCLA.*

REFERENCES

Boring, Edwin G. 1954. "The Nature and History of Experimental Control." *The American Journal of Psychology,* 67(4):573–589.

Colborn, Theo, Dianne Dumanoski, and John Peterson Myers. 1996. *Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival?* New York: Dutton.

Gross, Lisa. 2007. "The Toxic Origins of Disease." *PLoS Biol,* 5(7):e193. doi:10.1371/journal.pbio.0050193.

Hadlow, W.J., Edward Grimes, and George Jay Jr. 1955. "Stilbestrol Contaminated Feed and Reproductive Disturbances in Mice." *Science* 122(3171):643–644.

Hunt, Patricia, Martha Susiarjo, Carmen Rubio, and Terry Hassold. 2009. "The Bisphenol A Experience: A Primer for the Analysis of Environmental Effects on Mammalian Reproduction." *Biology of Reproduction,* 81: 807–813.

Hunt, Patricia, Kara Koehler, Martha Susiarjo, Craig Hodges, Arlene Ilagan, Robert Voigt, Sally Thomas, Brian Thomas, and Terry Hassold. 2003. "Bisphenol A Exposure Causes Meiotic Aneuploidy in the Female Mouse." *Current Biology,* 13(7):546–553.

Landecker, Hannah. 2011. "Food as Exposure: Nutritional Epigenetics and the New Metabolism." *BioSocieties,* 6:167–194.

Martin, Bronwen, Sunggoan Ji, Stuart Maudsley, and Mark Mattson. 2010. "'Control' laboratory rodents are metabolically morbid: Why it matters." *PNAS* 107(14):6127–6133.

Rader, Karen. 2004. *Making Mice: Standardizing Animals for American Biomedical Research.* Princeton: Princeton University Press.

Vandenberg, Laura, Maricel Maffini, Carlos Sonnenschein, Beverly Rubin, and Ana Soto.2010. "Bisphenol-A and the Great Divide: A Review of Controversies in the Field of Endocrine Disruption." *Endocrine Reviews* 30(1):75–95.

Vogel, Sarah.2012. *Is It Safe? BPA and the Struggle to Define the Safety of Chemicals.* Berkeley: California University Press.

vom Saal, Frederick, Susan Nagel, Benjamin Coe, Brittany Angle, and Julia Taylor. 2012. "The estrogenic endocrine disrupting chemical bisphenol A (BPA) and obesity." *Molecular and Cellular Endocrinology,* 354:74–84.

Waterland, Robert, Juan-Ru Lin, Charlotte Smith, and Randy Jirtle. 2006. "Post-weaning Diet Affects Genomic Imprinting at Insulin-like Growth Factor 2 (Igf2) Locus." *Human Molecular Genetics,* 15(5):705–716.