



The big test for bisphenol A

After years of wrangling over the chemical's toxicity, researchers are charting a new way forwards. **Brendan Borrell** investigates how the debate has reshaped environmental-health studies.

In her 25 years of research, Gail Prins, a reproductive physiologist at the University of Illinois in Chicago, had got used to doing science her way. But when her experiments started to question the safety of bisphenol A (BPA), a chemical found in thousands of consumer products from food-can linings to baby bottles, she found her work under a new level of scrutiny. The experience was unnerving, she says. "I feel I do solid science." Even federal evaluators in the United States agreed that her work was suitable for informing decisions about BPA's safety — at least at first.

A study published in early 2006, for example, helped explain how early exposure to BPA could increase rats' susceptibility to prostate cancer¹. The work complemented a growing body of research suggesting that the chemical posed several developmental and cancer risks (see 'Hazard warning'). That December, when a panel on reproductive health drafted a report on BPA for the US National Toxicology Program (NTP) it determined that Prins's study "makes important contributions and is suitable for the evaluation process".

But the following year, the NTP's final report discounted Prins's study. The chemical industry had stepped in to make its views heard.

BPA grosses some US\$6 billion a year for the five companies that produce it in the United States. Steven Hentges, who works on BPA for the American Chemistry Council, the industry trade group, wrote a 93-page letter to the NTP panel on 2 February 2007, detailing what he perceived as flaws in a slew of studies coming out of academic laboratories.

Prins's study came under attack for injecting the chemical under the rats' skin, rather than giving it orally, as humans would generally be exposed. Hentges found flaws in 60 of the roughly 80 studies that the panel found to be "adequate", "useful" or "suitable" for evaluating BPA's reproductive and developmental effects. Following Hentges's critique, the percentage of non-industry-funded studies deemed adequate for informing policy dropped from 70% to 30%. Most of those that remained found the chemical to be safe.

Three years on, the debate over BPA's potential for harm is unresolved. Canada and Denmark have banned the chemical's use in baby bottles, toys and other products for infants. And manufacturers and retailers worldwide have begun to limit its use in

response to mounting consumer concerns.

For researchers, though, the issue exposes a growing gulf between basic research and the regimented world of toxicity testing. "They are very different systems that serve different purposes," says Richard Denison, a chemical-risk analyst in the Washington DC office of the Environmental Defense Fund, a non-governmental organization. Scientists such as Prins were among the first to highlight concerns about ubiquitous environmental toxins, but because they were not specifically aiming to test toxicity, they were easy targets for industry. Consequently, the dispute over BPA has had an unexpected outcome: it is shaping the way studies will be funded and conducted in academic labs.

To bridge the divide, the US National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina, is bringing researchers together to create greater collaboration and a more rigorous, integrated body of research on BPA that can compete on an equal footing with the industry-sponsored studies. Endocrinologist Frederick vom Saal of the University of

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Missouri-Columbia, a pioneer in the study of BPA and one of those funded by the new NIEHS programme, calls it the “BPA master experiment”. The new approach is already being adopted for other types of toxicity investigation. “If we didn’t start doing business a little differently,” says Jerry Heindel, the BPA programme manager at the NIEHS, “we might not have the answers we need.”

Toxic science

The gap between the methods used in industry and those of basic researchers can be traced back to 1993, when vom Saal and his colleagues showed how organisms can be exquisitely sensitive to tiny amounts of hormone-like chemicals during development. These chemicals bind to the same receptors as hormones such as oestrogen and thus mimic their effect, potentially disrupting development. Vom Saal and his colleagues christened them ‘endocrine disruptors’². It was no secret that BPA was a potential endocrine disruptor. Scientists explored its effects on fertility in the 1930s, because of its similarities to oestrogen. It was abandoned for more powerful chemicals and ultimately found a use in making shatterproof polycarbonate plastic and epoxy resins used to coat metals. The FDA approved its use under food-additive regulations in the early 1960s.

In the late 1990s and early 2000s, researchers began to amass evidence that BPA leaching from such products was acting as an endocrine disruptor, even if the mechanism remained unclear. Ana Soto, a cell biologist at Tufts University School of Medicine in Boston, found that low doses of BPA alter the development of mammary glands in mice and could lead to cancer³. Other researchers found a link in mice between BPA and hyperactivity and a heightened sensitivity to illegal drugs. In Prins’s work, BPA had an effect on rat prostates similar to that of an injection of oestradiol, the body’s main oestrogen.

A mechanism for BPA’s action was taking shape. For Prins’s control animals, injected with corn oil, the ageing process naturally silenced the expression of a gene linked to development of cancers. Animals injected with corn oil and BPA however, had the gene locked in the ‘on’ position and were more than twice as likely to develop pre-cancerous lesions.

Researchers have learned that the chemical is working on an epigenetic level — modifying gene expression, but not sequence, over a long period. Yet the academic goal of such work — to uncover and explore biological mechanisms — was quite different from those of guideline studies designed to evaluate chemical safety. Therein lies the room for contention.

Hentges’s letter found bones to pick with

the dozens of studies it attacked. Some used sample sizes that were too small; some had only looked at a single dose level; some had failed to carry studies through to the measurement of disease or dysfunction, stopping at surrogate endpoints. Some criticisms were method-specific. For example, Hentges said that an enzyme-based assay to detect concentrations of BPA in blood was not specific to BPA and can overestimate its levels. Academic researchers had also identified similar methodological problems in the published BPA literature, but they generally did not regard them as fatal flaws.

A common general criticism from Hentges, however, was that none of these studies were conducted according to Good Laboratory Practice (GLP), part of the testing guidelines developed by regulators around the world, outlining basic standards for equipment calibration and the storage of raw data. In general, when called on by federal regulators to test the safety of a substance, the chemical industry has relied on private labs such as RTI International in Research Triangle Park to carry out guideline studies using GLP. Academic researchers rarely conduct such studies, but in their deliberations about chemical safety, federal agencies are expected to examine all the evidence, GLP or not. Hentges says non-GLP studies should be given lesser weight. “There’s certainly some role for academic studies in generating hypotheses, but they don’t provide what regulators need to draw conclusions.” But compared to scientific protocols, which evolve

continuously, guideline standards advance in fits and starts, because adding new procedures requires a lengthy period of comment, revision and validation. The US Environmental Protection Agency (EPA) set limits for acceptable human exposure to BPA in the late 1980s. It set up a programme on endocrine disruption in 1998, but it took until October 2009 for methods to be sufficiently agreed on to request

a first round of tests, and some say tests are still inadequate.

“This was the most dysfunctional thing I’ve ever sat on,” says biologist Theo Colborn, who has been on the programme committee for its duration and runs the

Endocrine Disruption Exchange in Paonia, Colorado. In the early days, she says, the science of endocrine disruption wasn’t ready for standardized testing, but today, although the science is stronger, the tests EPA is requesting are inadequate. “A chemical like BPA could easily be missed in the assays they have selected,” she says.

Once the stakeholders agree on a procedure, it must be validated, which is, in a sense, a substitute for replication. Validation involves multiple contract laboratories performing the same procedure and coming back with a consistent result. But according to Thomas Zoeller, an endocrinologist at the University of Massachusetts, Amherst, well-established and reproducible scientific techniques may have trouble getting validated when contract laboratories can’t perform the procedures.

Inappropriate tools

At the EPA’s request, Zoeller reviewed the raw data from three contract labs asked to measure thyroid hormone levels, and found that they could not conduct radioimmuno assays that have been available for more than 40 years. “The problem is the assay is difficult,” says Rochelle Tyl, a toxicologist at RTI International, which was part of the validation process. “If experienced labs can’t run the assay how can we put it as a guideline?”

That’s exactly the point, Zoeller and other critics argue: contract labs may not be able to apply the appropriate tools. Academic scientists studying BPA are convinced that their techniques are better for understanding the dangers of oestrogen mimics. Prins replied to Hentges’s charges in her own comment to the NTP panel, one of a handful of researchers to do so. In her letter, she noted that “by selectively eliminating data collected from non-oral routes of administration, the committee has introduced a significant bias in the process.”

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Hazard warning

Bisphenol A exposure has been linked to developmental and reproductive issues in lab animals. Here are suspected health risks and the level of concern assigned to them by the US National Toxicology Program.

- Changes in brain and behaviour** — some concern for fetal and infant exposure
- Prostate cancer** — some concern for fetal and infant exposure
- Breast cancer** — minimal concern for fetal and infant exposure
- Early onset of puberty** — minimal concern for fetal and infant exposure
- Miscarriage and birth defects** — negligible concern for exposure to pregnant women
- Effects on fertility** — negligible concern for low exposure; minimal concern for high occupational exposure
- Diabetes and obesity** — insufficient evidence

BPA circulating in the bloodstream of her rats, estimated to be about 10 parts per billion and consistent with amounts generally found in humans. Such calculations aren't even required in guideline studies. "Most academic scientists have quality-control standards that are above GLP standards," says Prins, who does run a GLP-compliant clinical lab. But getting certified is expensive, time-consuming and generally unnecessary for research goals.

Zoeller, vom Saal, and 34 other researchers published a commentary in *Environmental Health Perspectives* last year, arguing that regulatory agencies should give no greater weight to GLP-guideline studies than rigorous, replicated peer-reviewed research³. Zoeller admits it is a tough balance. "Regulatory agencies shouldn't have to run around every time some academic lab finds something, somewhere with some esoteric technology," he says. "What we have to figure out is how to invest modern science in regulatory toxicology and make it work."

A new direction

In 2008, in an attempt to close the holes poked in academic research on BPA, the NIEHS made plans to direct US\$30 million to BPA research in 2010 and 2011, including \$14 million in stimulus funds. In the past, the agency would typically put out a request for grant applications in a general area, fund researchers in different areas, and let them go off and conduct their research. This time, the NIEHS was going to do things differently. "We may fund the best research by the best investigators, but doing that doesn't guarantee we'll fill all the data gaps that need to be filled," says Heindel.

So last October, more than 40 scientists working on BPA, including Prins, arrived at the agency's campus in Research Triangle Park. The meeting alerted researchers to technical issues in working with BPA, and also allowed them to reach a consensus on certain study protocols. To counter concerns about the assays used to detect BPA levels in blood, Antonia Calafat, an analytical chemist at the Centers for Disease Control and Prevention in Atlanta, will do the lion's share of chemical analysis using mass spectrometry, calibrated with a BPA isotope not found in the environment. At the meeting, Calafat also advised researchers to include 'blanks' of distilled BPA-free water to prove that experiments were free from contamination, and to test food to ensure it contains minimal levels of plant-based oestrogens. Researchers also discussed the use of positive controls, such as the hormone oestradiol,

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B. VOGELZANG

Gail Prins now delivers bisphenol A to mouse pups orally rather than injecting it under the skin.

to prove that experiments that fail to find a response to BPA actually work.

The NIEHS is also encouraging researchers to use consistent BPA doses across labs, to measure additional variables such as markers related to diabetes, and to exchange tissues. Soto, for example, studies female development, but she will raise male and female mice during her experiments. She will send the males' prostates to Prins in Chicago. In addition, the Food and Drug Administration's National Center for Toxicological Research in Jefferson, Arkansas, will run animal studies as a GLP backbone for the entire project, making tissues and animals available to external grantees. To help grantees take advantage of these collaborations, the NIEHS offered them supplements of up to \$50,000 per year.

Heindel says that the agency has already adopted the same approach for a cohort of grantees beginning studies of nanomaterial safety this spring. "Let's learn from the lessons of BPA and start developing collaborations and interactions to move the field right from the start," he says. Hentges says he is "encouraged that the research will be moving towards studies that will really be helpful to assess the safety of BPA".

Although many of the funded researchers were quick to praise the programme, it also

sometimes means that they will be doing things they don't necessarily agree with. For example, vom Saal takes issue with stopping the enzyme-based assay for BPA concentrations. The test costs several dollars, compared with the \$150 for the more sophisticated methods the NIEHS now requires. He says that enzyme-based assays are only problematic for researchers not familiar with their nuances. However, he is willing to adopt the more expensive technique if it means producing unassailable results. "If you are going to spend millions of dollars on new research, then it's best not to open it up to criticism," he says.

Prins, like vom Saal, disputes the relevance of administering the chemical orally as opposed to under the skin, but they all agreed in October to conduct some of their experiments by feeding BPA to animals, as the chemical industry has insisted. "I can't particularly say I like people telling me how to conduct my research," Prins says. "But it's very important that things are done consistently between investigators if we are going to move the field forward." ■

Brendan Borrell is a freelance writer in New York City

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