Study calls into doubt previous BPA research

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(Medical Xpress)—Yellow coat color mice in Cheryl Rosenfeld's lab are not fortunate sons and daughters.Conventional knowledge says these mice will likely live fatter, more diseased lives than their black, brown and mottled (tiger-striped) siblings.

But recent data from her team at the University of Missouri calls into doubt influential studies on exposure to BPA and genistein, both estrogen-like chemicals known to influence obesity and disease in mice. Her research couldn't replicate the results of previous studies by another research group.

Proceedings of the National Academy of Sciences (*PNAS*) published Rosenfeld's work Dec. 24.

"Our original goal wasn't to prove or disprove previous research, but when our results started to show discrepancies compared to the prior studies, we decided to pursue it further, leading to experiments that spanned over three years," said Rosenfeld, an associate professor of biomedical sciences with MU's Bond Life Sciences Center. "Everyone has been using this study as the foundation for their own research, so we knew we had to go above and beyond with more numbers, more detailed analysis on determining whether developmental exposure to BPA and/or genistein effects offspring coat color and corresponding risk for later adult diseases."

Previous studies showed pregnant mice fed BPA turned on the agouti gene in their babies, causing more yellow in their fur and more disease in their bodies. This gene typically regulates hair color, but BPA can activate it in other tissues. One study – published by PNAS in 2007 – also claimed mice fed genistein, a soy-based estrogen mimic, counteracted the bad effects of BPA.

Rosenfeld found no increase in percentage of yellow mice with BPA or a counteracting effect from genistein. But she did see a rise in the number of brown and yellow agouti mice compared to black, non-agouti siblings, when mothers were fed a BPA/genistein combination and when fed as a synthetic hormone used in birth control, called ethinyl estradiol. In essence, the agouti gene might confer a selective advantage in certain uterine environments, but it also comes with the risk of adult-onset metabolic diseases.

All around us

BPA is abundant.

Used since the 1960s to make hard plastics, dental sealants, store receipts, and food cans, it accumulates in our environment and makes its

way into the bodies of humans and animals alike.

The Centers for Disease Control documented widespread human exposure in 2004, finding BPA in the urine of 93 percent of people tested. The Food and Drug Administration expressed concerns about BPA in a 2010, later banning its use baby bottles in 2012 for potential developmental risks.

Manufacturers produced more than 8 billion tons of BPA in 2008, according to market analysis from the Investor Environmental Health Network.

Epigenetic changes

BPA and other chemicals that act like estrogen cause concern, in part, because of potential to impact fetuses. That damage comes from what the chemicals can make a baby's DNA do before it's born.

Epigenetics, literally meaning "above the genome," control whether an individual strand of DNA has an impact on a particular tissue.

Think of it as the instructions to a manual to build a car. Epigenetics tell you what segments of that manual to read when building each part.

In an animal or human, reading the wrong sections of that manual mean unnecessary or harmful processes get activated in the wrong tissues.

"Even though the DNA isn't changed at all, changing the factors associated with the DNA can change gene expression," Rosenfeld said. "One of those epigenetic changes is DNA methylation, and adding methyl groups to your DNA essentially silences it. It's kind of like turning a light switch off."

In the case of BPA in mice, no methyl groups mean the agouti gene gets turned on, and it is read in fat tissue and organs, like the pancreas, instead of just in hair cells. This leads to more obesity and diabetes.

Above and beyond

For Rosenfeld's lab, above and beyond meant three years of research. From 2009 to 2012, they bred black, homozygous female mice to brown or yellow heterozygous males with an Avy gene. This guaranteed an even split of black to brown and yellow mice in an ideal situation. When litters of pups came, they analyzed 2,824 offspring from 426 litters, more than 10 times the size of the original study. They tracked weight during development and blood glucose levels. Rosenfeld's team also scrutinized the hair color of the mice, using densometric analysis to classify them into six different color ranges. To ensure their results, researchers analyzed the genotype of their males to make sure the desired 50/50 split of black pups to brown and yellow was possible.

Rosenfeld went further, collaborating with Mark Ellersieck – an experienced MU biostatistician – to analyze their mountain of data. He noticed a problem in the original statistics.

"The problem with the original study is they did Chi squared statistics, a yes or no sort of analysis," Rosenfeld said. "Since there's a whole continuum of coat colors it's not a yes or no sort of answer, and Mark used several other more appropriate approaches to analyze the data."

Ellersieck's analysis took into account the spectrum of mouse colors and analyzed each litter as a unit instead of lumping them into one large group or considering each individual pup as the unit, as was done previously. This created a more accurate outcome.

Thrifty genes

Rosenfeld wonder why pregnant micebirthed more agouti pups prone to obesity compared to non-agouti black offspring when on the BPA/genistein combination or ethinyl estradiol.

One answer may be a so-called "thrifty genotype" that would give some offspring an advantage in lean times.

"If you had a gene that allowed you to procure as much nutrients from the mom as possible you could grow and develop faster than siblings that don't have that gene," Rosenfeld said. "They could possibly be thought of as gluttonous, but during starvation times the offspring that expressed this gene would be at an advantage."

A pending grant proposal seeks to focus on studying this candidate "thrifty" gene.

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