Researchers identify how PCBs may alter in utero, neonatal brain development

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In three new studies — including one appearing online today in the *Public Library of Science - Biology* (*PLoS - Biology*) — UC Davis researchers provide compelling evidence of how low levels of polychlorinated biphenyls (PCBs) alter the way brain cells develop.

The findings could explain at last — some 30 years after the toxic chemicals were banned in the United States — the associations between exposure of the developing nervous system to PCBs and behavioral deficits in children.

"We've never really understood the mechanism by which PCBs produce neurobehavioral problems in children," said Isaac N. Pessah, professor of molecular biosciences, director of the UC Davis Center for Children's Environmental Health and co-author of all three studies.

"With these studies we have now shown — from the whole animal level to the molecular level — how PCBs alter the development and excitability of brain cells. And that could explain why PCBs are associated with higher rates of neurodevelopmental and behavioral disorders," said Pessah, who is also a researcher with the UC Davis M.I.N.D. Institute.

Together, the studies — published within one month of each other — make a compelling case for the mechanism behind PCBs' harmful effects on human neurological development.

- In a groundbreaking animal study appearing online in late March in *Environmental Health Perspectives*, Pessah and his colleagues found that low-level, in utero and neonatal exposure to PCBs altered the development of brain cells in rats.

- A second study in Toxicology and Applied Pharmacology, also appearing online in March, showed which PCBs affected brain-cell circuits in the hippocampus, a region of the brain known to be impaired in several complex neurodevelopmental disorders including autism.

- The third study, which appears online today in *PLoS - Biology*, describes in detail the molecular target of the PCBs, the calcium channels known as ryanodine receptors, and shows that PCBs lock these calcium channels in the open position, which likely contributes to the over-excitations on neural circuits observed in the two other studies.

PCBs were used in a wide variety of products including transformers and capacitors and other electronic components, pesticides and flame retardants, from the early to late 20th century. Their production was banned in the 1970s due to the high toxicity of most PCBs. They do not break down in the environment and accumulate in animals' bodies. Exposure occurs when chemicals dumped into the environment years ago are released into the air or leach into groundwater and contaminate fish that people eat.

"Not only will this help us deal with current exposures," Pessah said, "but we can also identify similar compounds that have come on line since PCBs were banned and make better decisions about which ones we restrict and which new ones we allow to come to market."

PCBs have been implicated in epidemiological studies as an

environmental cause of diverse neurodevelopmental disorders, including ADHD, learning disabilities, sensory deficits, developmental delays and mental retardation

"There is a large body of scientific literature in humans that points the finger at PCBs, linking them to neurodevelopmental problems we see in kids," said Pamela Lein, lead author of the Environmental Health Perspectives animal study and a UC Davis associate professor of molecular biosciences.

"The problem is that it has been difficult to establish a cause-and-effect relationship from the human epidemiological literature without a known mechanism," Lein said. " Now that we have a plausible biological mechanism that could account for neurodevelopmental deficits, we can use the information for diagnosis and for developing potential treatments for PCB exposure."

Environmental Health Sciences study

The study published in *Environmental Health Sciences* shows that exposure to PCBs in utero and through mothers' breast milk alters a characteristic of neuronal development called dendritic plasticity in young rats. Dendrites are the small, branch-like projections on a neuron that receive signals from other cells in the body. The shape of dendrites changes in response to signaling activity — the phenomenon known as dendritic plasticity. Lein performed the exposure and behavioral studies with colleagues while a researcher at the Johns Hopkins University.

In the study, researchers tried to mimic the low levels of PCB exposure that human children might experience. Experimental rats were fed PCB-laced cookies, while control rats ate normal cookies. Then, when rat pups were weaned from their mothers, they were trained in a water maze to test their ability to use visual cues to learn the location of a platform

hidden under the surface of the water. The test has been used in other studies to stimulate dendritic growth, which makes it ideal for measuring effects of toxicants on dendritic plasticity.

The researchers looked at the pattern of dendritic growth in trained and untrained animals from both the control and experimental groups. They found that PCB exposure accelerated dendritic growth in the untrained experimental animals when compared to untrained controls. The trained PCB-exposed animals, however, took longer to learn the water maze and showed reversal of dendritic growth in response to water-maze training. This was in contrast to controls, which showed learning responses and increases in dendritic growth, as predicted by other published studies.

"This tells us that PCBs are altering dendritic growth and plasticity," Lein said.

The results are important because problems in dendritic growth and plasticity have previously been implicated in many neurodevelopmental disorders, including autism, schizophrenia and mental retardation, she said.

"Dendritic plasticity is important to how we process information and, when you perturb that, you interfere with complex behaviors like learning and memory," Lein said.

Pessah and his colleagues showed that brain tissue from untrained rats exposed to PCBs expressed higher levels of the ryanodine receptors.

"We think PCBs are increasing the activity of these calcium channels, which we know generate the signals needed for the extension and branching of dendrites," Pessah said.

He said he believes PCBs lead to overgrowth of dendrites and inhibition

of neuronal pruning that takes place during gestational development. Brain cells exposed to PCBs cannot then respond properly to learning.

Toxicology and Applied Pharamacology study

In the study appearing in *Toxicology and Applied* Pharmacology, Pessah and his colleagues examined the hippocampus, one region of the brain involved in water-maze learning. The researchers measured the excitability of neurons in hippocampal brain tissue of rats before and during exposure to two structurally different PCBs.

Neurons process and transmit information in the form of electrical signals. Their electrical excitability is due to the presence of voltage-sensitive ion channels that directly communicate with ryanodine receptors that reside inside the cell. When excitation is blocked, it is called inhibition. Normal information processing involves a complex balance between excitation and inhibition.

The researchers found that the two PCBs had different effects. The more potent, PCB95, enhanced the excitability of the brain cells. Researchers were able to decrease this effect by adding a chemical that dampens ryanodine signaling, again implicating the calcium channel as being the key to the disruptions caused by PCBs. The second compound, PCB170, first excited the circuitry, but then the signals returned to baseline because of enhanced inhibition.

These results are significant to the understanding of the potential impact of PCBs on human neurodevelopment, Pessah said.

"We think that in autism, for example, at-risk children have deficient inhibitory circuits. So, if you have a PCB that promotes the excitatory side of the circuit, they would be much more at risk of developing the disorder," he said. "In fact, we chemically blocked inhibitory circuts that

unmasked the purely excitotoxic properties of PCB170."

***PLoS - Biology* study**

In the collaborative study between researchers at Davis and Harvard that appears in *PLoS-Biology* today, researchers showed that PCBs dramatically stabilize the ryanodine receptor in the "on position," which could explain how PCBs are altering brain cell development (as seen in the first study) and altering their excitability (as seen in the second).

"We needed evidence that these compounds directly interact with what we believed to be the target of PCBs," Pessah said.

To that end, the researchers exposed purified ryanodine recptors to PCBs and used electron microscopy to generate extremely high-resolution images of this interaction.

"Our results show that PCB binds directly to ryanodine receptors and locks the channel in the open state, causing mayhem in calcium signaling," Pessah said. This, he added, would account for the effects seen in the first two studies.

"These channels are a target for PCBs, and they are contributing to brain cell dysfunction, even at the behavioral level."

Pessah said that, as early as 1995, he and his colleagues suspected ryanodine receptors were one of the principal targets of PCBs.

"In cellular studies, we couldn't find a way to block the effects of PCBs unless we blocked ryanodine receptors," he said.

Many studies used high doses of PCBs to find subtle or no changes from control. However, in the animal study, Lein actually used both high and

low doses. She found that the low-dose group showed more pronounced effects on dendritic growth in the weanling rats than the higher dose.

According to Pessah, the brain has ways of dealing with high levels of toxicity.

"We think that one of the major reasons we have not seen effects in previous studies is that at higher doses PCBs become toxic to cells and the brain has defense mechanisms to deal with disposing of these damaged cells," he said.

These processes, like programmed cell death, would not necessarily be triggered if a neuron's shape is altered rather than damaged, he added. Both Lein and Pessah agreed that the current PCB studies have broader implications for the future study and regulation of PCBs and other environmental toxicants.

Future PCB studies

"Future studies of PCBs and related compounds should be examined at lower doses more relevant to human exposures," Pessah said.

The researchers are planning to study PCB effects on mice that carry some of the same genetic variations of the ryanodine receptors that humans do.

"These studies are important if we are to determine if some people are more susceptible to PCB toxicity than others," Lein said.

The team also will look at PCBs' effects on other ares of the brain that control behavior as well as testing compounds with structures similar to those of PCBs.

"We believe other PCB-like compounds in use today are also capable of changing the structure of protein targets that are contributing to neurobiological problems in humans," Pessah said, "and we hope to identify those and help get them off the market."

Source: University of California - Davis