Do we know enough about the safety of quat disinfectants?

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At first glance, the 10-days-old mouse embryo looked like any other. A closer look into the microscope, though, told Terry Hrubec something was wrong. The embryo's neural tube—what would become the mouse's brain and spinal cord—had a tiny gap. It had not closed fully.

In brief

With the COVID-19 pandemic raging around the globe, many people are depending on disinfectants, hand sanitizers, and sanitizing wipes to keep the novel coronavirus at bay. Such products often contain quaternary ammonium compounds, or quats. These workhorses of modern disinfection have been found in consumer products for decades. But in the last 10 years, scientists have linked quats to reproductive and developmental problems in animals, and found they can disrupt key cellular processes. So far there's no data linking the compounds to toxicity in humans, but some scientists say there's more to be done to fully assess quats' safety.

It was January 2009, and Hrubec, a reproductive and developmental toxicologist at Virginia Tech and Edward Via College of Osteopathic Medicine, was researching how to prevent such neural tube defects. She expected to see them in embryos from her test group of pregnant mice exposed to drugs known to cause the defects. This embryo, however, came from her control group of healthy, unexposed mice. In all, Hrubec would find neural tube defects in 10% of the control embryos.

So, Hrubec repeated the experiment to make sure she hadn't mixed up her mice or embryos. But she got the same results. Puzzled, she approached the laboratory staff who took care of the animals.

No, the mice hadn't come from anywhere new. No, their diet hadn't changed. Yes, their food and bedding were pathogen-free.

Finally, Hrubec asked the supervisor of the animal facility at Virginia Tech if they had made any changes at all recently.

It turned out there was one: a new disinfectant. To prevent disease among the animals, the animal care staff would typically foam the floors, walls, and racks weekly, and mop the floors daily. The staff had been using a chlorine-based disinfectant. But sometime during the fall of 2008, the staff switched to one containing a mixture of alkyldimethylbenzylammonium chloride (ADBAC) and didecyldimethylammonium chloride (DDAC). These compounds, which belong to a family of molecules called quaternary ammoniums, or quats, have long appeared in consumer products.

Through further research, Hrubec has linked ADBAC and DDAC exposure to developmental and fertility problems in mice. Separately, other scientists have found quats can exert negative effects on cellular processes. Now, as COVID-19 grips the globe, researchers are worried not only about the novel coronavirus SARS-CoV-2, but also the disinfectants used to destroy it on surfaces.

Hospital workers, school janitors, public transit operators, and homeowners have begun disinfecting and sanitizing buildings to protect against the deadly virus. Half of the disinfectants the US Environmental Protection Agency suggests could be effective against SARS-CoV-2 contain a quat, often as the sole active ingredient.

Consumer quats

Quats are used in thousands of consumer products across the world. Here are examples of where three quat compounds can be found and what role they play in each.

Credit: C&EN/Shutterstock

“I’m definitely very concerned,” says Libin Xu, a biochemist at the University of Washington. The public, he adds, may know that quats kill bacteria and viruses. But through his research, Xu has found that quats can also disrupt key cellular pathways.

So far, researchers have not reported data suggesting systemic toxicity in humans. But, at least one regulatory agency in the US wants to know more about the compounds. The California Department of Public Health on July 17 issued COVID-19 guidance for schools, which specified avoiding disinfectant products that contain asthma-causing chemicals, including quats. In a statement to C&EN, the Department says, “We proactively recommend the use of disinfectants that do not contain [quats] or other harmful chemicals, whenever possible.”

As we deploy quat disinfectants against the coronavirus, what risk could the chemicals pose?

A powerful family

Quats are a broad class of several hundred individual compounds that contain at least one positively charged nitrogen atom, typically connected to four alkyl or benzyl groups. Their biocidal activity was discovered in 1935 by Nobel Prize–winning German pathologist Gerhard Domagk. Domagk discovered that if at least one of the groups borne by the cationic nitrogen was a long-chained alkyl group, the compound could kill microbes. The molecule’s positive charge helps it adhere to the negatively-charged surfaces of bacteria and viruses. Once attached to bacteria, the long alkyl chain inserts into the microbes’ lipid membranes, bursting this outer layer and causing cellular contents to leak out. In viruses, quats disrupt their protein and lipid structures.

Soon after this discovery, doctors recognized that ADBAC with a 12-carbon alkyl chain was useful for sterilizing hands and instruments prior to surgery, and the compound hit the market as Zephirol. By 1940, quats were being used to disinfect utensils in restaurants and milking equipment at dairy farms.

Credit: *Birth Defects Res.*

A normal mouse embryo (left) and one that developed a neural tube defect (right, arrow) from mothers exposed to quat disinfectants. These 10-days-old embryos are typically 1-2 mm wide.

The compounds' usefulness extends beyond disinfection. ADBAC's biocidal activity renders it an effective preservative for applications like wood treatments and eye drops. Another quat, 1-cetylpyridinium chloride (CPC), serves as a popular antiseptic active ingredient in mouthwashes, nasal sprays, and throat lozenges. Some in the family, like diquat and paraquat, are pesticides. Others are prized for their surfactant behavior in detergents and shampoos and in helping extract oil and gas from the earth. They're also added to dryer sheets and fabric softeners where their positive charge helps prevent static buildup in the laundry. Altogether, quats can be found in thousands of household products around the world, according to two public databases on consumer products. By one estimate, the market for quats was worth $963.7 million in 2019.

Related: Rise in quaternary ammonium compounds observed during coronavirus crisis

Quats entered the market in the early 20th century before the EPA began regulating the manufacture and sale of potentially harmful chemicals under the 1976 Toxic Substances Control Act. As a result, quats counted as existing chemicals on the market that could continue being included in consumer products without being evaluated for safety. However, the compounds have been extensively tested for safety, says Keith Hostetler, a toxicologist at Toxicology Regulatory Services, a consulting company under contract by groups representing quat manufacturers. The EPA is currently updating risk assessments for quats, which will be released for public comment in 2021.

We should be more concerned about controlling the growth of disease-causing organisms.

Keith Hostetler, a toxicologist at Toxicology Regulatory Services, on concerns about systemic quat toxicity

Accumulating evidence

After Hrubec learned Virginia Tech's animal facility switched to a quat disinfectant, she started looking for data on the compounds' toxicity. She didn't find any studies on quats' reproductive or developmental toxicity, but a Q&A in *Nature* revealed she wasn't the only one with work interrupted by the chemicals (*Nature* 2008, DOI: 10.1038/453964a).

The article detailed the experience of Patricia Hunt, who earned the nickname the "accidental toxicologist" for her earlier work linking bisphenol A to mouse embryo developmental problems. In 2005, Hunt had just moved from Case Western Reserve University School of Medicine, where the animal facilities did not use quat disinfectants, to Washington State University, where quats were used. Hunt noticed that fertility among her mice dropped noticeably after the move, and then it plummeted further when, following a pinworm outbreak, the facility was extensively fogged and cleaned with a quat-based disinfectant.

After reaching out to Hunt, Hrubec checked her own mice breeding records and realized that fertility had also subtly declined since the disinfectant swap at the Virginia Tech facilities. While Hunt had stopped investigating quat toxicity, Hrubec decided to probe further to understand what risks these prevalent chemicals could pose. "If it's causing a problem in mice, there's a good chance it could cause a problem in humans," she says.

Hrubec collected breeding data from mice and rats fed controlled doses of ADBAC and DDAC. In 2014, Hrubec published her data along with Hunt's: animals receiving the quats produced fewer pups than those that didn't (*Reprod. Toxicol.* 2014, DOI: 10.1016/j.reprotox.2014.07.071).

Later, Hrubec moved her control mice to a different facility that didn't use quat disinfectants, which eliminated the appearance of the neural tube defects. She followed up on the defects and found that they showed up even when only the fathers were exposed to quats and the mothers hadn't been (*Birth Defects Res.* 2017, DOI: 10.1002/bdr2.1064).

In a second study on fertility, Hrubec compared undosed male mice living in the facility disinfected with quats with those living in the quat-free facility (*Reprod. Toxicol.* 2016 DOI: 10.1016/j.reprotox.2015.10.006). This experiment mimics the kind of everyday exposure people might experience if they are in buildings cleaned with quat-containing products, Hrubec says. Male mice living in the quat-using building had about a 25% lower sperm count and 10% lower sperm motility.

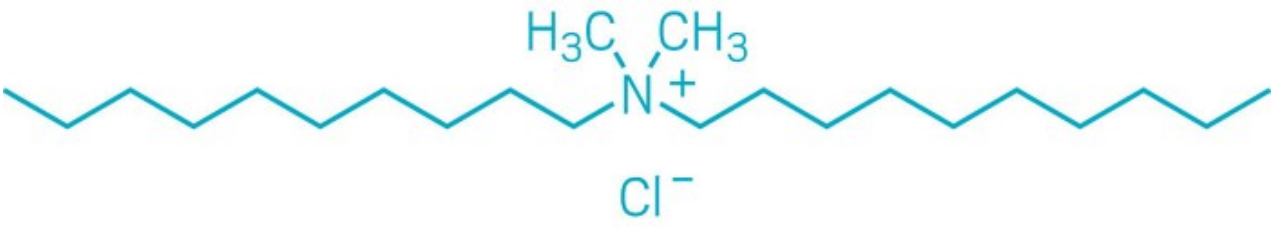
Hostetler has criticized Hrubec's studies for feeding mice doses of quats that far exceeded what humans are exposed to, and for using an ambient form of exposure, which wasn't quantified, in the latter experiments. He also points out that the EPA has assessed ADBAC and DDAC's reproductive and developmental safety based on six industry-supplied studies on rats and rabbits conducted between 1989 to 1992. In those studies, no negative effects on animal fetuses were found at doses that weren't already toxic to the mothers.

Jamie DeWitt, a toxicologist at East Carolina University, agrees that for a true assessment of human health risks from specific chemicals, it is crucial to use doses comparable to what humans are exposed to and modes of exposure that are relevant to humans' experiences. But that does not invalidate Hrubec's studies, she adds. "It's a lot of really good sleuthing," DeWitt says, noting that Hrubec and Hunt's experiences with quats mirror how problems with endocrine-disrupting chemicals were first identified. "I think these papers do a very good job of starting to identify the hazards associated with [quats]."

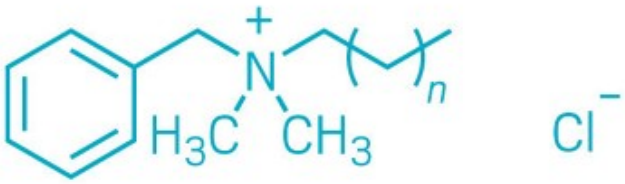
Gaining allies

After Hrubec published her first study on quat toxicity in 2014, two researchers reached out to her to collaborate. They had had their own run-ins with quats.

"All the problems that we've been working on together now for the last couple years, have been discovered independently and also by chance," Hrubec says.



Didecyldimethylammonium chloride (DDAC)



Alkyldimethylbenzylammonium chloride (ADBAC)

***n* = 6, 8, 10, 12, 14, 16**

1-Cetylpyridinium chloride (CPC)

Gino Cortopassi, a toxicologist at the University of California, Davis, had been studying diseases involving the mitochondria, the energy-producing component of cells. Mitochondrial dysfunction causes excess fatigue, and is a feature of aging and most chronic diseases. Beginning around 2010, Cortopassi and his team undertook a large high-throughput screening campaign looking for compounds that could boost mitochondrial function and serve as new therapeutic leads for such diseases. They put 1,600 compounds from MicroSource Discovery Systems' Pharmakon library through five cellular tests to see what effects the compounds would have on various indicators of mitochondrial health, like oxygen consumption and synthesis of adenosine triphosphate, a molecule that cells use to store energy.

Related: 2 common flavoring chemicals in e-cigarettes can damage lung cells

The researchers found 11 compounds that slowed both of those processes. Six of them were quats, among them ADBAC and CPC (*Environ. Health Perspect.* 2017, DOI: 10.1289/EHP1404). By 2015, when the group had finished collecting data, it became clear that quats were the most potent compounds at inhibiting mitochondrial function in the library.

It's a lot of really good sleuthing. I think these papers do a very good job of starting to identify the hazards associated with [quats].

Jamie DeWitt, a toxicologist at East Carolina University, on Hrubec and Hunt's research on quat toxicity

Meanwhile, the University of Washington's Xu stumbled upon a different mode of quat toxicity. Xu had been studying a disorder called Smith-Lemli-Opitz syndrome since he was a postdoctoral researcher. The rare disease is associated with significant neurological impairment and is caused by mutations in the gene coding for the enzyme DHCR7, which mediates the final reaction in cholesterol biosynthesis. Cholesterol is a key component of neurons' membranes and myelin sheaths, the insulating, protective wrappers around electrically active nerve cells.

Xu wanted to find out if nongenetic factors like environmental chemicals could play a role in Smith-Lemli-Opitz syndrome too. He performed a computational search for chemical candidates that could inhibit DHCR7 and potentially lead to conditions like the one he'd been studying.

Xu found that ADBAC was 72.9% structurally similar to a DHCR7 inhibitor called AY9944 (*Toxicol. Sci.* 2016, DOI: 10.1093/toxsci/kfw041). Next, Xu confirmed ADBAC's ability to inhibit DHCR7 by determining that the precursor to cholesterol, 7-dehydrocholesterol, accumulated in cells exposed to ADBAC. Because the brain manufactures the cholesterol it needs locally, Xu later checked if ADBAC consumed in food could travel there and suppress cholesterol biosynthesis. He laced the food of pregnant mice with deuterium-labeled ADBAC and found the compound in the neonatal mice's brain tissue, along with lowered total cholesterol levels (*Toxicol. Sci.* 2019, DOI: 10.1093/toxsci/kfz139).

Both Cortopassi and Xu's findings could help explain what Hrubec was observing in her mouse fertility studies. "Sperm are highly dependent on mitochondria" for generating energy to swim, Cortopassi's postdoc Sandipan Datta explains. He and Cortopassi think that quats could be contributing to the decline in fertility among Hrubec's male mice by disrupting mitochondrial function. Quats could also disrupt hormone signaling in the animals since all steroid hormones, like testosterone and estrogens, are derived from cholesterol.

These studies show that quats have the potential to perturb physiology at a cellular level, DeWitt says. Scientists, she says, now need to ask if these effects can change how organisms function.

Human exposure

So far, Hrubec, Xu, and Cortopassi have not linked what they have found in cells and animals to downstream health effects in humans.

As with Hrubec's studies, Hostetler dismisses Cortopassi and Xu's findings as irrelevant for human health, because they tested high doses of quats that humans aren't typically exposed to. Because quats are so effective against microorganisms, they are typically formulated at low concentrations, around 0.1% by mass in a solution, Hostetler says. The concentrations of quats within cells would not reach the levels used in Cortopassi's mitochondria studies, he argues. And animal studies conducted by industry didn't observe downstream health effects of inhibited cholesterol biosynthesis.

Instead, Hostetler says, people should be more concerned with local irritation effects. Among workers like janitors or nurses who routinely handle concentrated disinfectant solutions, the occupational health risks associated with using quats are well established, including dermal irritation, skin sensitization, and occupational asthma.

The key to minimizing these negative effects, Hostetler says, is to use quat disinfectants as directed. The compounds pose their biggest risks when they get on the skin, he points out, but only about 10% gets absorbed into the body. If they enter the bloodstream, they're "quickly cleared," he says. "Because they're not particularly well absorbed, they don't pose systemic toxicity risks." Quats are also nonvolatile due to their high molecular weight, making them unlikely to be inhaled, he adds. "We should be more concerned about controlling the growth of disease-causing organisms."

But some scientists have started to question these assumptions about quats, including how much can enter the body. Oliver Fiehn, a metabolomics researcher at the University of California, Davis, and his lab process 30,000 blood, tissue, and urine samples from human and animal studies every year. They analyze the samples for the full spectrum of chemicals present, supporting other laboratories that want to identify important metabolites or environmental chemicals. They have found 5–10 different quats in 10–20% of their samples. However, these samples all came from other laboratories, so Fiehn cannot rule out the possibility of laboratory contamination.

The widespread use of quats coupled with the observations that they showed up in human samples and findings of potential toxicity in animals and cells has pushed some researchers to scrutinize these chemicals further. On March 4, after hearing Hrubec and Xu present their research and Hostetler's counterarguments, a panel of nine scientists voted unanimously to

place quats in the Biomonitoring California program. The state of California established this program in 2006 to reduce Californians' exposure to harmful chemicals. Through this program, the California Department of Public Health can collect data on listed environmental chemicals, including measuring the chemicals' levels in residents, tracking sources of exposure, and investigating how exposure may vary across different groups of people. Chemicals in this program include lead, arsenic, phthalates, and per- and polyfluoroalkyl substances (PFAS).

Together, Hrubec, Cortopassi, and Xu have also begun a small biomonitoring pilot study to assess levels of quats in people and look for any associated impacts. "I think there is an urgent need to fill these gaps in knowledge," Xu says.

Hrubec recruited a small cohort of people from Blacksburg, Virginia, where the Virginia Tech campus is, and collected three tubes of blood from each participant. She mailed one tube to Cortopassi and another to Xu. Xu analyzed the blood for quat levels and cholesterol biomarkers. Cortopassi assessed mitochondrial function. And Hrubec looked for inflammation biomarkers. The researchers have found quats in 80% of the participants' blood. Those with more compounds present in their blood had stronger indications of lowered mitochondrial function and elevated inflammation biomarkers (medRxiv 2020, DOI: 10.1101/2020.07.15.20154963). The results are currently undergoing peer review.

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Meanwhile, one big research question remains: How might quats get into the human body? Cortopassi and Datta think that inhalation of droplets, when disinfectants are sprayed, could be the most important exposure route.

For Hrubec, Xu, and Cortopassi, COVID-19 presents a unique opportunity to study any pre- and post-pandemic effects on the quat levels in their Blacksburg cohort, and look for any associated impacts. But for now, all research with human participants has been suspended by the scientists' universities. "Right now, we can't monitor anything," Hrubec says. "But we do plan to start as soon as we're given the big green light to go ahead."

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