

## How Much Longer Will Moratorium Last?

When will it end? That's what many influenza researchers want to know about the landmark self-imposed moratorium on certain experiments on the H5N1 avian influenza virus that they agreed to earlier this year. The short answer: Who knows?

Initially, the 39 researchers who announced the moratorium on 20 January said it would last just 60 days (*Science*, 27 January, p. 387). But in February, the loosely organized coalition agreed to an indefinite extension to give experts and the public more time to discuss and address concerns about the safety and wisdom of experiments that could alter H5N1 in ways that make the virus more dangerous to humans. (Other H5N1 research, such as the testing of newly detected strains, continued.)

Now, some of the moratorium's signers are eager for research to resume. But many say they are perplexed about how that decision will be reached and who will decide. "I wish I knew how it was going to be resolved," says virologist Robert Webster of St. Jude Children's Research Hospital in Memphis, Tennessee, a moratorium signer who was deeply involved in the flu papers controversy. "We haven't discussed this," says virologist Yi Guan of the University of Hong Kong.

Some key players, meanwhile, predict it will be months before the standstill ends. "We've still got a lot of homework to do ... and some boxes to check" before the moratorium should be lifted, believes Anthony Fauci, the head of the National Institute of Allergy and Infectious Diseases (NIAID), which funded the controversial studies by Ron Fouchier of Erasmus MC in

Rotterdam, the Netherlands, and Yoshihiro Kawaoka of the University of Wisconsin, Madison, and the University of Tokyo. Although Fauci isn't a signer of the moratorium, he played an influential role in encouraging Fouchier, Kawaoka, and other leading influenza researchers to organize it.

The researchers reluctantly agreed, driven in part by warnings that governments, reacting to public fears and media reports of "doomsday" viruses, might clamp down on the field if scientists didn't act on their own. Some dubbed the move "Asilomar 2," a reference to the historic 1975 agreement among recombinant DNA researchers that halted experiments in their emerging field until safety guidelines were established.

Before the current moratorium can end, several things have to happen, according to moratorium signers, Fauci, and others:

- The U.S. government must release for public comment a document that explains how universities and private laboratories can help federal funding agencies screen proposed research projects for "dual use research of concern" (DURC) that could be used for good or nefarious purposes. The goal of the new DURC screening program, which was announced in late March and covers 15 "high risk" pathogens including H5N1, is to spot problematic studies before they begin. The document—which is expected to run to nearly 30 pages and will be accompanied by a 100-page background—could be released "sometime this summer," Fauci says.

- Scientists and funders will need to agree on which lines of H5N1 research are—and are not—worth the risks. Particularly problematic, say Fauci and others, are "gain of function" studies, such as Kawaoka's and Fouchier's, in which researchers create mutant viruses that gain capabili-

ties to the punch. "Usually when you discover something important, somebody else is discovering it, too," Fouchier says. (He was right: Kawaoka, who says he didn't know about Fouchier's work, had submitted his manuscript less than 2 weeks earlier.) Now, Fouchier declines to discuss the results from the additional experiments, which are on hold as a result of the moratorium.

The published paper shows that the core set of five mutations includes the three that the team introduced themselves and two more that arose during passaging. And the resemblance to what Kawaoka found is "quite remarkable," says James Paulson,

a glyco biologist at the Scripps Research Institute in San Diego, California. Both teams found that two mutations at the receptor binding site—one of them identical in the two studies—are important; both discovered an additional mutation that makes hemagglutinin lose a sugar group, which apparently helps make room for the mammalian host cell receptor. Kawaoka also found a mutation in the hemagglutinin's stalk that improves the virus's stability and compensates for the other mutations, Paulson says. "It's tempting to think" that one of Fouchier's mutations plays a similar role—although it's not in the stalk but

in another area where three hemagglutinin molecules align to form a so-called trimer.

Cox says she's "surprised" that it didn't take more mutations. In a study with Paulson published online by *Virology* in November, her team also mutated the receptor-binding site, but they had to make several other changes—including slotting in a human-adapted version of another viral gene called *neuraminidase*—to get airborne transmission. That led the authors to conclude that the virus might require "extensive evolution" to become pandemic. Fouchier's paper upsets that reassuring notion.

Both papers will aid surveillance efforts because they suggest which genetic changes to look out for in H5N1, Peiris says. But they also point to a limitation: Several mutations can have the same effect on the virus. "It wouldn't be appropriate to focus just on these mutations," Peiris says. "The virus has different ways to go from A to B." More research is needed to discover how many ways, he says.

In a second paper published this week and co-authored by Kawaoka and Fouchier (p. 1541), a group led by mathematician Derek Smith of the University of Cambridge in the United Kingdom takes a stab at understanding the likelihood of the emergence of a pandemic H5N1 strain. The researchers first combed through surveillance databases to determine whether the mutations identified in the two controversial studies have already appeared in



**Nothing to sneeze at.** The study tested for airborne transmission of H5N1 by placing infected and uninfected ferrets in adjoining cages.

**At a standstill.** Certain experiments with H5N1 are on hold until signers of a self-imposed moratorium, including Yoshihiro Kawaoka (*left*) and Ron Fouchier (*right*), agree to lift it.

ties—such as mammalian transmission—that naturally occurring versions do not have. A key step in this process could come in late July, when the heads of NIAID-funded influenza laboratories are scheduled to meet in New York City.

- Laboratory safety officials and scientists will need to “at least have a consensus on the level of biocontainment required” for H5N1 studies, says microbiologist Adolfo García-Sastre of Mount Sinai School of Medicine in New York City, a leader of the moratorium. Currently, most H5N1 studies occur in biosafety level 3 (BSL-3) laboratories, but some critics argue that they should be restricted to a small number of higher-containment BSL-4 laboratories.

In the meantime, many signers say the moratorium has already achieved its goal. “The voluntary action ... helped calm people’s concerns so that discussion could take place,” Kawaoka says. And it “provided the time to deal with these issues in some depth,” says Thomas Mettenleiter of the Friedrich Loeffler Institute in Griefswald-Insel Riems, Germany, even though “no universal ‘solution’ was found.”

—DAVID MALAKOFF

With reporting by Martin Enserink.



nature. They found that many H5N1 isolates are three—and in a few rare cases, just two—mutations away from Kawaoka’s quartet and four from Fouchier’s quintet.

They then developed a model of viral evolution to test whether existing viruses, once they happen to infect a mammalian host, might accumulate the missing mutations and become excreted in respiratory droplets, which could start a chain of transmission. The model takes into account a variety of factors, such as the duration of the infection and whether individual mutations by themselves benefit the virus. The conclusion, says first author Colin Russell of the University of Cambridge, is that a virus that is only three mutations away from the full set is “likely” to acquire them and end up in droplets. But the paper can’t put a number on that risk; there are too many unknowns.

“You can do a lot of fancy maths, but in the end the probability is hard to pin down,” Peiris says. Still, “it’s a model of how modeling should be done,” says Steven Wolinsky, who studies HIV evolution at Northwestern University in Chicago, Illinois. “They do a very nice job of explaining all the caveats.”

The study was presented both at a World Health Organization meeting about the papers in February and during the second NSABB review in March, and it helped convince a majority on the panel that H5N1’s risks were real enough to warrant publishing Fouchier’s

paper, says NSABB acting chair Paul Keim, a microbial geneticist at Northern Arizona University in Flagstaff.

Another factor that swayed the board to support publication, NSABB members say, is that the published version of Fouchier’s paper does a much better job of clarifying the lethality of his airborne virus than the first version they read. Fouchier says the draft he submitted to *Science* did not discuss whether his airborne viruses killed the ferrets they infected. But two of the three reviewers asked for additional experiments examining lethality, which Fouchier said took just a week to perform, “so we did them,” and added a line to the paper describing the outcome.

That language—combined with Fouchier’s remarks in interviews and sometimes hyperbolic press coverage—appeared to suggest that the airborne mutants were extremely lethal, which “greatly alarmed” many NSABB reviewers, says virologist Robert Webster of St. Jude Children’s Research Hospital in Memphis, Tennessee, an influenza expert who was asked to advise NSABB on the two papers. In fact, however, none of the ferrets had died from airborne transmission; six ferrets that had the virus squirted directly into their trachea all died. But that outcome is “not very relevant” for evaluating the virus’s risk, Fouchier notes, because that’s not how humans or animals typically contract flu. He says the manuscript

NSABB saw in its first review last year made clear that the different routes of infection led to different outcomes.

But Webster says the results were presented in a way that confused NSABB. He says that the experienced virologists involved in NSABB’s discussions—including himself—should have pushed harder to clarify those results and emphasize that lethality in ferrets does not necessarily predict lethality in humans. When the lethality data finally became clearer in the months after the NSABB’s initial recommendation, many members say they began to reconsider. In retrospect, Fouchier says, “we should have ignored [*Science*’s] reviewers’ request for lethality data,” given the confusion that ensued.

The publication of Fouchier’s paper isn’t likely to be the last word on such issues, however, especially as influenza researchers seek to restart similar studies now stalled by the moratorium. Many, however, are treading cautiously, eager to avoid replaying the drama of the last 8 months. Fouchier, for his part, says he’s “sick of all these discussions,” and he declined to release his first manuscript to reporters in order to help clarify how the story unfolded, a step Keim says he would support. “I want to move on,” Fouchier says. “Maybe in 5 or 10 years’ time, when someone writes a book about all of this.”

—MARTIN ENSERINK

With reporting by David Malakoff.