# Tox21: Chemical testing in the 21st century

The Toxicology in the 21st Century program, or Tox21, is a unique collaboration between several federal agencies to develop new ways to rapidly test whether chemicals may affect human health.

## What is the goal of Tox21?

Tox21 is using robotics and other testing approaches to more efficiently predict how chemicals may affect human health. Initially, Tox21 will determine which of the thousands of chemicals in use are most likely to harm human health, to prioritize chemicals for testing. Since traditional chemical testing using animals is expensive and time consuming, Tox21 is also developing cell-based tests and biochemical approaches, which measure chemical substances produced in living organisms. Ultimately, these new strategies will help to quickly evaluate thousands of chemicals and inform regulatory decisions about the safety of chemicals.

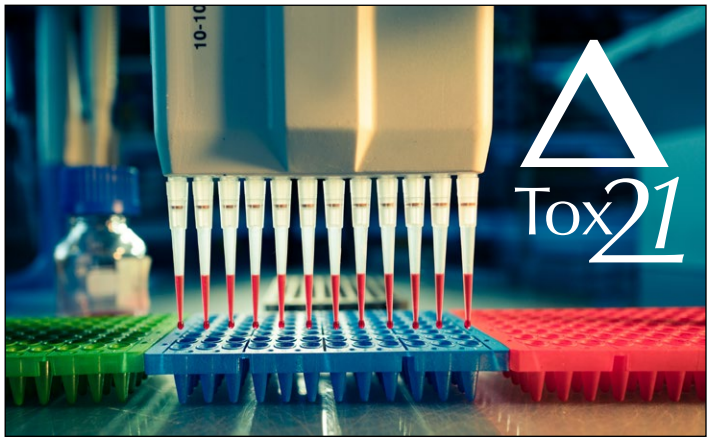
Tox21 aims to:

- Develop new testing methods that use human cells, called *in vitro* approaches
- Expand the number of chemicals that are tested
- Reduce the time, effort, and costs associated with testing
- Minimize the number of laboratory animals used

## Who are the federal partners involved in Tox21?

Four government agencies bring their unique expertise, resources, and tools to the Tox21 collaboration.

- National Institute of Environmental Health Sciences (NIEHS) / National Toxicology Program (NTP), National Institutes of Health (NIH), U.S. Department of Health and Human Services
- National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH)
- U.S. Food and Drug Administration (FDA), U.S. Department of Health and Human Services
- National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency (EPA)




## How did Tox21 come about?

In 2005, EPA, with support from NIEHS/NTP, asked the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan to accomplish it. NRC is the nation's leading organization known for providing independent expert advice on matters of science, technology, and medicine.

NRC's 2007 report, "Toxicity Testing in the 21st Century: A Vision and a Strategy," recommended relying less on animal studies and focusing more on *in vitro* methods to evaluate the effects that chemicals can have on biological processes, or processes that occur in living organisms.[1]

In 2008, NIEHS/NTP, the NIH Chemical Genomics Center, which became part of NCATS, and EPA issued a memorandum of understanding (MOU) stating how they would collaborate to address key NRC recommendations. This MOU was accompanied by a perspective piece in the journal Science that presented the federal government's response to the NRC recommendations.[2] FDA joined the MOU in 2010. In 2015, Tox21 collaborators renewed their commitment through a third MOU.

## Why do we need to change how we test chemicals?

Identifying chemicals that may be hazardous to human health has traditionally relied on animal testing. This approach has taught us much about the potential for chemicals to harm humans, but animal testing is generally slow, expensive, and the results don't always translate easily from animals to humans.

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March 2017

### How many chemicals need to be tested?

There are tens of thousands of chemicals in the world that we know very little about. Only a small number of chemicals have been assessed adequately for potential toxicity to humans.

### How is Tox21 different from past approaches?

Several factors make Tox21 different from previous methods for testing chemical toxicity. The new approach aims to identify biological pathways that, when compromised, may lead to an adverse effect or disease in humans. A biological pathway is a series of biochemical steps in a cell that lead to the creation of a new molecular product or change in the cell.

Toxicity or disease pathways are biological pathways that may result in an adverse health effect when sufficiently disturbed or compromised. An example of this might be exposure to a chemical that results in the formation of a tumor.

Scientists are working to identify and map as many of these pathways as they can. They will then use Tox21 approaches to test whether chemicals interact with various important pathways, in order to predict their toxicity.

Some of the tools scientists are using to predict toxicity include:

- Biochemical-based or cell-based assays, or tests, conducted using robotics
- Assays on 3-D models of different human tissues and organs
- Assays on organisms that are simpler than rats and mice but still complex, like worms and fish
- Computational methods to analyze and interpret the data generated, a process called predictive modeling

### How does the Tox21 robot system work?

A quickly moving robotic arm conducts rapid experiments called high-throughput assays. Thousands of chemicals are tested at the same time, at different concentrations, using 3-inch by 5-inch plastic trays, or plates, with 1,536 tiny wells less than 0.04 inches across. Each well holds miniscule volumes of liquid.

For cell-based assays, 1,000-2,000 cells are added to each well, and then the plates are stored in a constant temperature incubator. Next, the robotic arm moves the plate from the incubator to a pin-tool device, where chemicals are added. A different plate is used for each concentration of a chemical. The total number of plates depends on the number of chemicals and the number of concentrations being tested.


Watch the Tox21 robot in action on the Tox21 webpage.

After the chemicals are added, the robotic arm puts the plates back in an incubator for a set period of time, ranging from hours to days, to mimic chemical exposures. The robotic arm then transfers the appropriate plates to a multi-well dispenser, which adds a solution for a chemical reaction, and moves the plates to the appropriate instrument to measure how the cells respond. The results show which chemicals may have caused a change in the biological pathway of interest.

It takes the Tox21 robot system three days, using 1,536-well plates, to do approximately the same work as a person employed eight hours a day, five days a week, for 12 years, using standard 96-well plates.

### Will cell-based tests ever completely replace the use of laboratory animals in toxicity testing?

It might not be possible to completely eliminate the use of laboratory animals. However, scientists do expect to greatly reduce reliance on animal testing. It is likely that some complex human diseases and disease processes, such as neurological disorders, will be very difficult to mimic *in vitro* or by using organisms such as worms and fish.

### How long do you think it will take for this new way of testing to transform toxicology?

The NRC report predicts that a complete transformation of toxicity testing will likely take 10 to 20 years. However, some significant progress has already been made[3] and, other improvements are likely to occur much sooner than projected.

## What has been accomplished since 2008?

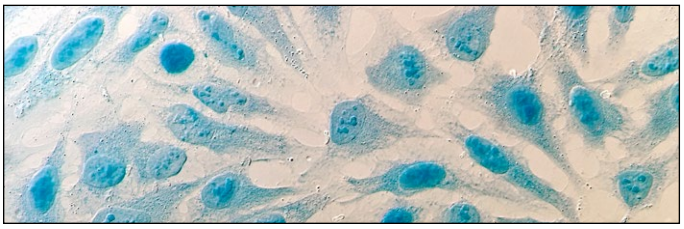
Scientists at NIEHS and cooperating federal agencies embarked on a three-phase plan.

### Phase I

The goal of Phase I was simple — to demonstrate that robotic testing could produce high-quality test results for thousands of chemicals, with the idea that this information might eventually reduce animal testing.

- Scientists at NCATS, NIEHS/NTP, and EPA began screening approximately 2,800 compounds in an advanced robotic system that is used in drug discovery screening, to determine if the system would also work for testing environmental chemicals.
- At the same time, EPA's ToxCast program screened 320 well-studied chemicals, primarily pesticides, using more than 500 high-throughput, *in vitro* laboratory tests. Scientists then compared *in vitro* test results with previous animal testing.

By 2010, scientists had established that high-throughput screening, using robotics, produced high quality data on environmental chemicals in a fraction of the time required for traditional animal testing. Given these findings, in March 2011, a robot specifically dedicated to Tox21 was purchased with funds supplied by NIEHS/NTP and EPA, and installed at the NCATS facility in Maryland.



### Phase II

During Phase II, the number of compounds tested and types of assays used were greatly increased.

- The Tox21 partners expanded the chemical library to more than 10,000 (10K) compounds, including industrial and consumer products, food additives, drugs, and chemical mixtures.
- Tox21 partners then screened the 10K chemical library using more than 42 assays, most of which tested immortal cancer cells, or cells able to divide without limit, and produced more than 65 million measurements.
- Scientists combined results from the Tox21 screening, and findings from EPA's ToxCast evaluation of approximately 1,800 chemicals, to develop an efficient 18-test battery that can identify compounds that act like estrogen or interfere with hormones like estrogen, called endocrine disruptors.
- EPA is using this battery, referred to as the ToxCast/Tox21 estrogen receptor model, to prioritize chemicals that need testing for potential endocrine disruption. This is the first time EPA proposed using robot-based results in place of animal testing.

The Tox21 partners are also pursuing additional cell-based experiments to further define and clarify the effects of chemical exposures.

### Phase III

As Phase II work continues, Phase III has already started and is producing results. Tox21 partners have identified approximately 2,800 genes in human cells and tissues that may respond to toxic chemicals, and are in the process of identifying similar gene sets in rats, mice, and zebrafish.

Using these sets of genes in a new test, Phase III will study changes in levels of ribonucleic acid (RNA) messages, called transcripts, which carry information from our DNA to make proteins necessary for cells to live. Tox21 scientists are measuring all the messenger RNAs within a cell, together called the transcriptome, in a low-cost, high-throughput manner. This may help shed light on how chemicals affect the function of our genes.

Phase III will also attempt to predict human disease by assessing the effects of chemicals on cells more like those in human organs, such as the liver, heart, or brain. Phase III will test chemicals in 3-D organ-like models, as well as organisms, like zebrafish, that have shared biological complexity with humans.

### Beyond Phase III

Ultimately, researchers hope to create reliable computational models to better predict whether a chemical might be toxic to humans. Scientists can already predict toxicity, if a chemical's structure is similar to that of a known toxicant. They hope to also predict toxicity when chemicals have different structures, but similar biological functions. With these computational models, chemical testing might be reduced significantly in the future.

## Engaging the broader scientific community in Tox21

Tox21 hosts workshops, webinars, and other events to engage the broader scientific community and ensure the research is valid, relevant, and applicable to policy decisions about chemical safety. In addition, all federally-funded, high-throughput screening data is publicly available.

**Tox21 Toolbox.** Data from all phases of Tox21 are being deposited into integrated public databases accessible through the Tox21 Toolbox. NIEHS/NTP has developed data visualization tools that provide quick access to Tox21 data or plots of results, with the ability to superimpose results from different compounds for easy comparison. Compounds can be compared based on their chemical structure or patterns of biological activity.

**PubChem.** The PubChem database is managed by the NIH National Center for Biotechnology Information. It allows access to large-scale screening data, including data from Tox21, along with supplemental information to assist the study of genes, cells, and biochemical pathways. The Tox21 Data Browser was developed by NCATS to provide access to chemical quality control information.

**Transform Tox Testing Challenge.** The two-stage Transform Tox Testing Challenge called on innovative thinkers to find ways to incorporate measures of cell metabolism, or energy usage, into high-throughput screening assays. The results should help researchers more accurately assess the effects of chemicals and


better protect human health. Ten winners from Stage 1 were announced in 2016 and invited to participate in Stage 2. Winners from Stage 2 will be announced by summer 2017. This challenge is supported by NIEHS/NTP, NCATS, and EPA.

**NIEHS-NCATS-UNC DREAM Toxicogenetics Challenge.** In 2013, NIEHS, NCATS, and the University of North Carolina at Chapel Hill challenged teams of researchers to build computer models that could predict whether 156 environmental pollutants and drugs might be toxic to cells. The objective was to better understand how a person's individual genetics may influence the cellular response to toxic effects from widely-used chemicals. In December 2013, the winners presented their models at NIEHS. A 2015 paper summarized the results, concluding that it may be possible to predict health risks from chemical compounds, but the accuracy of current models is still suboptimal.[4]

## Glossary

**Assay:** A procedure used by researchers to test or measure the activity of a chemical.

**Biochemical:** Pertaining to chemical substances and vital processes occurring in living organisms.

**Biological pathway:** Complex sequences of proteins and other molecules that, when activated, ultimately change some aspect of cell behavior. These pathways may alter cell behavior in an abnormal way, which can then lead to disease.

**High-throughput:** Automated assays capable of testing large numbers of chemicals in a short time frame.

***In vitro:*** Biological or chemical work conducted in culture dishes rather than in living animals.

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[1] Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council. 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: National Academies Press.

[2] Collins FS, Gray GM, Bucher JR. 2008. Transforming Environmental Health Protection. Science 319(5865):906-907.

[3] National Academies of Sciences, Engineering, and Medicine. 2017. Using 21st Century Science to Improve Risk-Related Evaluations. Washington, DC: National Academies Press.

[4] Eduati F, Mangravite LM, Wang T, Tang H, Bare JC, Huang R, Norman T, et al. 2015. Prediction of human population responses to toxic compounds by a collaborative competition. Nat Biotechnol 33:933–940.