# Tox21: Transforming Environmental Health

Tox21 is a unique collaboration between several federal agencies to research and test chemicals in a new way.

## Who are the federal partners involved in Tox21?

Four government agencies participate in Tox21.

Three of the four agencies are part of the U.S. Department of Health and Human Services, including:

- National Institute of Environmental Health Sciences (NIEHS) / National Toxicology Program (NTP), National Institutes of Health (NIH)
- National Center for Advancing Translational Sciences (NCATS)/NIH Chemical Genomics Center (NCGC), National Institutes of Health (NIH)
- U.S. Food and Drug Administration (FDA)

The U.S. Environmental Protection Agency (EPA), Office of Research and Development, National Center for Computational Toxicology, is a founding partner in Tox21.

"We are working with our federal partners, as part of Tox21, to accomplish a paradigm shift in toxicological testing that will make it less expensive and time-consuming than our current methods, and hopefully provide better public health protection for humans."

– NIEHS/NTP Director Linda S. Birnbaum, Ph.D.

Each agency brings its own unique expertise, resources, and tools to work together to:

- Identify and/or develop new testing strategies, such as *in vitro* approaches that generate data using human cells
- Greatly expand the number of chemicals that are tested
- Reduce the time, effort, and costs associated with testing
- Minimize the number of laboratory animals used

No single organization could succeed in this endeavor by itself.

## What is the goal of Tox21?

To develop more efficient and less time-consuming approaches to predict how chemicals may affect human health. Initially, the main focus of Tox21 is to help prioritize chemicals for more extensive testing using traditional methods. Ultimately, it is hoped that Tox21 will develop strategies that can be used directly by regulatory agencies to regulate chemicals and reduce our current reliance on animal testing for toxicological assessments.

## How did Tox21 come about?

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

The NRC released its report in 2007, "Toxicity Testing in the 21st Century: A Vision and a Strategy."[1] The report called for a new approach to toxicity testing that would rely less on animal studies and focus more on *in vitro* methods to evaluate the effects that chemicals can have on biological processes using cells, cell lines, or cellular components.

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Printed on recycled paper October 2012

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A memorandum of understanding (MOU), which builds on the expertise of NIEHS/NTP, NHGRI/NCGC, and EPA, was released on February 14, 2008, to address key recommendations in the NRC report. This MOU was accompanied by a perspective piece in the journal Science[2] that presented the federal government's response to the NRC recommendations. In 2010, FDA joined the MOU.

### Why do we need to change how we do things?

Identifying which chemicals might be hazardous to human health has traditionally relied heavily on testing in laboratory animals. Although this approach has taught us much about the potential of chemicals to cause adverse effects in humans, animal testing is generally slow, expensive, and the resulting data must be extrapolated from animals to humans.

### How is Tox21 different from past technology-based testing efforts?

Several factors make Tox21 different from previous efforts. For one, the level of collaboration among different federal agencies is perhaps unprecedented in the field of toxicology research.

Also, Tox21 has a focus not only on what effect chemicals can have on health, but also how they do it. That is, identifying toxicity pathways that, when compromised, may lead to an adverse effect or disease in humans.

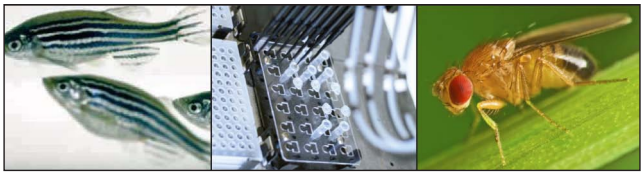
A biological pathway is a series of biochemical steps in a cell that leads to a certain product or a change in a cell. Toxicity or disease pathways refer to those pathways that, when sufficiently perturbed, are expected to result in an adverse health effect. An example of this might be exposure to a chemical resulting in the formation of a tumor.

The idea behind Tox21 is that scientists will be able to determine the potential for human harm from chemicals, based on how and to what extent they interact with various toxicity pathways.

There are about 1,100 known cellular pathways in humans. Scientists are working to identify and map as many of these pathways as possible that may contribute to toxicity.

### 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 risk to humans.



### How will this paradigm shift in toxicology testing be accomplished?

This can be accomplished through the use of appropriate biochemical- and cell-based assays, assays involving 3-dimensional models of different human tissues and organs, and assays using lower but complex organisms, such as worms and fish, rather than using traditional laboratory animals to examine compounds for potential toxicity. The extensive data generated by these approaches will be analyzed and interpreted using high-level computational methods.

### How long do you think it will take for this shift to occur and transform toxicology?

As indicated in the NRC report, transforming toxicity testing completely will likely take ten to twenty years to accomplish. However, some improvements to toxicity testing will occur much sooner. One example is the use of *in vitro* methods to compare the potential risk of chemicals to act as endocrine disruptors. Another is the measurement of activation of cellular stress pathways to rank the relative general toxicity of substances. Tox21 will continue to collect and evaluate data and take advantage of new science opportunities as they present themselves.

### Will *in vitro* tests ever completely replace the use of laboratory animals in toxicology?

That is a goal, but considering the complexity of living organisms and disease processes, there might be some classes of diseases, for example, neurological, that will be very difficult to completely mimic *in vitro* or by using more primitive organisms. Therefore, it might not be possible to completely eliminate the use of laboratory animals. However, we do expect to reduce reliance on laboratory animals by using a variety of *in vitro* tests that have been demonstrated to be relevant and reliable.

### What do you mean by high throughput assays?

High throughput assays are rapid, automated experiments that can test many thousands of chemicals at the same time, for many different responses, over a wide range of concentrations, to determine how the chemicals affect cellular functions that are linked to disease.

### Where did the idea of using robots for toxicity testing come from?

Robotic technology has been used successfully by the pharmaceutical industry for decades.

### How does the Tox21 robot system work?

Thousands of chemicals are tested at the same time across 15 different concentrations on a 3-inch by 5-inch plastic tray with 1,536 tiny wells, each less than .04 inches across. To conduct what is referred to as a cell-based assay, 1,000-2,000 cells are added to each well and the plates are stored in a constant temperature incubator for a few hours to allow the cells to adjust to their new environment. For some cells, this means attaching to the well bottom.


Next, the robot arm moves the plate from the incubator to a pin-tool device, where chemicals dissolved in a solvent are transferred into each well. There is a different 1,536-well plate for each concentration. The total number of plates in each run depends on the number of chemicals and the number of concentrations being tested.

After chemicals are added, the plate is placed back in an incubator for a period of time, ranging from a couple of hours to a couple of days. At the end of that time, the robot arm transfers the appropriate plates to a multi-well dispenser, which adds a reaction solution. Next, the plates are moved to the appropriate reader instrument, which measures how the cells respond. The resulting data are used to determine which chemicals caused a positive response in the target of interest.

Working with the same number of 96-well, rather than 1,536-well, plates, it would likely take a person eight hours a day, five days a week, for 12 years to do what the new robot system can do in about three days.

*(See time-lapsed video of the robot being installed courtesy of NHGRI at http://www.genome.gov/27543670#al-2.)*

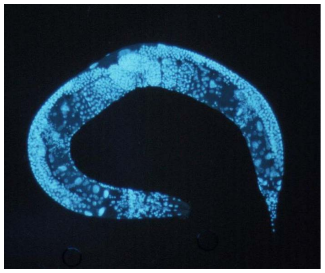
### What has been accomplished since the agreement was signed in 2008?

The agencies began collaborations even before the agreement was signed. For example, in 2005, NTP provided an initial set of *in vitro* assays and a 1,408 environmental compound library to the NCGC to determine if the concept of testing these chemicals in robot systems, used previously only for drug discovery, would work and prove meaningful for environmental chemicals as well.

Subsequently, the EPA provided more *in vitro* assays and a second, similar-sized compound library. In Tox21 Phase I, approximately 2,800 compounds were screened across more than 50 biochemical- and cell-based assays, measuring a variety of endpoints considered useful for evaluating the potential *in vivo* toxicity of a compound.

During the same time frame, the EPA's ToxCast™ Phase I program screened 320 chemicals, primarily pesticides, for potential toxicity in more than 500 *in vitro* and alternative animal tests. In ToxCast™ Phase II, another 700 chemicals are being screened for activity in an expanded set of *in vitro* and alternative animal assays. The chemicals tested are found in industrial and consumer products, are used as food additives, and include drugs provided by the pharmaceutical industry that never made it to the market.

Chemicals in ToxCast™ are also being screened for activity in the high throughput assays at the NCGC and in alternative animals assays at the NIEHS/NTP that use *Caenorhabditis elegans (C. elegans)*, a roundworm.



Because many cellular pathways are conserved across species, it is likely that the responses in *C. elegans* will be applicable to understanding similar processes in higher organisms, including humans.

"This dedicated robot marks a scientific milestone for the project and provides an important technological advance for the field."

– NTP Associate Director John Bucher, Ph.D.

## A Dedicated Robot for Tox21

After completing Phase I, which demonstrated that a computerized robotic facility like the ones used by the drug development field could be used successfully to screen environmental chemicals, a robot specifically dedicated to Tox 21 was purchased, with funds supplied by the NIEHS/NTP, and installed at the NCGC facility in Maryland in March 2011.

The robotics facility at the NCGC will be used to screen a Tox21 library of more than 10,000 chemicals. Initially, the assays used will focus on targets involved in endocrine disruption, for example, the ability of chemicals to interact with the estrogen receptor, and on stress response pathways. The results of these studies, when combined with the data obtained in ToxCast,™ will be used to develop schemes for ranking the compounds by activity and for predicting adverse health outcomes. These approaches may be used to help make regulatory decisions.

*Federal partners from NIEHS/NTP, NCGC, EPA and FDA at 2011 robot ribbon-cutting (Photo courtesy of NCGC)*

The compound libraries used in Tox21 and ToxCast™ include chemicals for which there is a great deal of animal and, in some cases, human toxicological data. Such data are critical for evaluating the relevance of *in vitro* assays for prioritizing chemicals for more extensive toxicological testing and for developing predictive models of adverse health outcomes.

### 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.

***Caenorhabditis elegans* or *C. elegans*:** A free-living, transparent nematode (roundworm), about 1 millimeter in length, which lives in temperate soil environments.

**Cellular 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 ultimately 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.

***In vivo:*** Biological or chemical work conducted in living animals.

**Toxicity or disease pathway:** Cellular pathway in the body that, when sufficiently perturbed, is expected to result in an adverse health effect.

[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.