

## The science of alternatives: 25 years and tomorrow

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### Abstract

The 3Rs of alternatives started to be recognized at the end of the 60s with the establishment of FRAME and then experienced explosive growth in the 80s. The last 25 years have been remarkable, with the establishment of centers and programs dedicated to the 3Rs all over the world. This World Congress, being held in Japan, marks a milestone in the global reach of the 3Rs. Like all endeavors, what one learns at the beginning may not hold true as a field develops and matures – initial truths may not be current realities - the theme of this presentation.

When William Russell and Rex Burch published "The Principles of Humane Experimental Technique" in 1959, the concept of replacement was that a non-whole animal assay would replace an animal test. The initial truth, a one for one replacement, has been superseded by a new reality – the necessity for batteries of tests and strategies for in vitro testing approaches. From method development to validation, the simple initial truths have been replaced by complex current realities.

In 25 years, we have experienced, in the words of Thomas Kuhn, a scientific revolution. The presentation will look at the last 25 years of initial truths and will explore our current realities and our successes. In 1959, the goals laid out by Bill Russell & Rex Burch were far reaching almost beyond comprehension and a distant, very distant dream. That dream is our current reality.

**Keywords:** 3Rs, alternatives, humane science

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### Special lecture

When I was invited to present this lecture, the organizers asked me to focus on the last 25 years—coincident with the formation of CAAT. I looked at the invitation as an honor and an opportunity—both to look back at the history and to contrast past events with current activities. It also provided a chance to look at a few future issues.

I will start with a timeline of significant events. This is a selective history of those events I recognized as important. Clearly, these are not the only significant events, but to me they are representative. I am certain that I have missed some important milestones; this is not intentional.

The focus of this presentation is characterized by two phrases: Initial Truths and Current Realities. I chose this focus to illustrate how what we initially think of as truth gets modified and changes as we collect new information. The initial truths were appropriate at the time they were identified. But data, experience and events produce different truths, and like all of science, truth and understandings change as new input informs our thinking.

### Timeline:

I start my timeline with the introduction of the rodent into laboratory science. At the turn of the 20<sup>th</sup> Century, Professor E.V. McCollum, of the Johns Hopkins School of Hygiene & Public Health (nka Johns Hopkins Bloomberg School of Public Health) first used the rat in nutritional research. Fifty years later, Henry Foster of Charles River Laboratories provided a more uniform and standardized rat. Being interested in the 3Rs, we can look at the number of research animals used in the middle 1970s and recognize that over the subsequent 30 years the numbers fell by almost 50%. As in much of science, the activity preceded the ethical discussion.

In the 1950s, the Universities Federation for Animal Welfare (UFAW), contracted with Bill Russell and Rex Burch to write *The Principles of Humane Experimental Technique*. This classic text, which argues that the most humane science is the best science, became the basis of animal welfare laws worldwide.

In the 1960s, two events occurred, one in the UK and the other in the USA, that set the stage for our discussions at this World Congress. In the UK,

FRAME (Fund for the Replacement of Animals in Medical Experiments) was founded, and in the USA, the Animal Welfare Act was passed. It is noteworthy that, in the USA, the letters to Congress regarding the Animal Welfare Act were more numerous than all other correspondence to Congress—including correspondence regarding the escalating US involvement in the Vietnam War.

Peter Singer's book *Animal Liberation*, written in the 1970s, established laboratory animal welfare as a societal concern and served as the call to action for a most influential animal activist—Henry Spira. Henry's accomplishments were the result of dedication and honesty, and he almost single-handedly raised public awareness about animal well-being in the United States.

In the 1980s, Professor Sagawara started the Japanese Society for Alternatives to Animal Experiments (JSAAE). In 1981, as a result of Henry Spira's activities against the animal testing conducted by manufacturers of personal care products, the Johns Hopkins Center for Alternatives to Animal Testing (CAAT) was established.

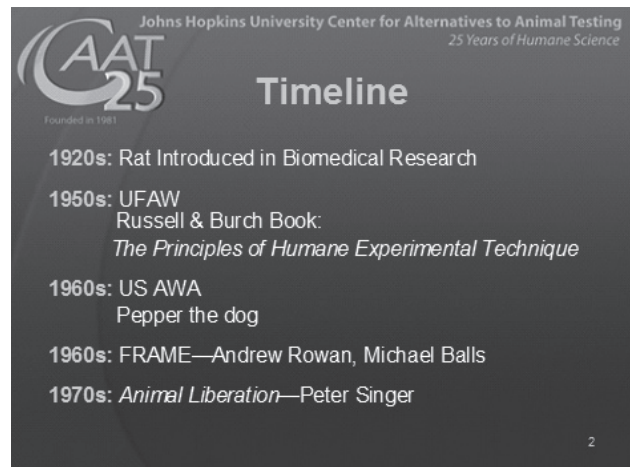
The 1990s brought a proliferation of national alternatives centers throughout Europe, and in 1993 the first World Congress on Alternatives and Animal Use in the Life Sciences was held in Baltimore, Maryland, USA. This was followed by World Congresses in Utrecht, The Netherlands (1996), Bologna, Italy (1999), New Orleans, Louisiana, USA (2002), Berlin, Germany (2005) and Tokyo, Japan (2007).

Now, as we move into the 2000s, we see a significant new commercial development: the initiation of CRO's dedicated to in vitro approaches. Alternatives have become mainstream. And that stream is gaining momentum. In November 2006, preceding the CAAT 25<sup>th</sup> anniversary symposium, representatives of 17 national 3R centers from around the globe came together for the first-ever such meeting. The second meeting of 3Rs centers—with

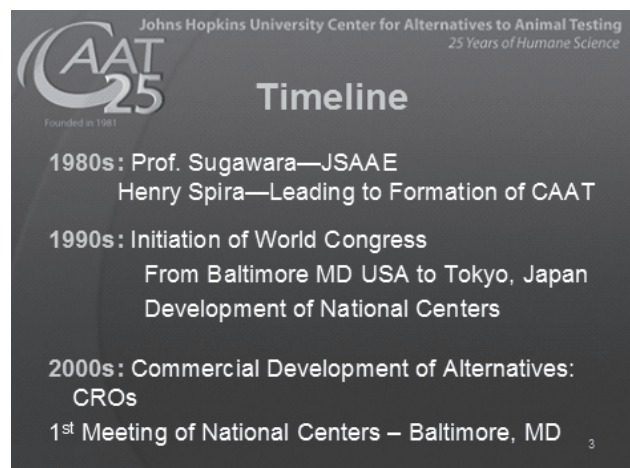


Slide 1

30 attendees representing 20 organizations—took place just before the opening of the World Congress in Tokyo. (Slide 1, Slide 2, Slide 3, Slide 4,)



Slide 2



Slide 3



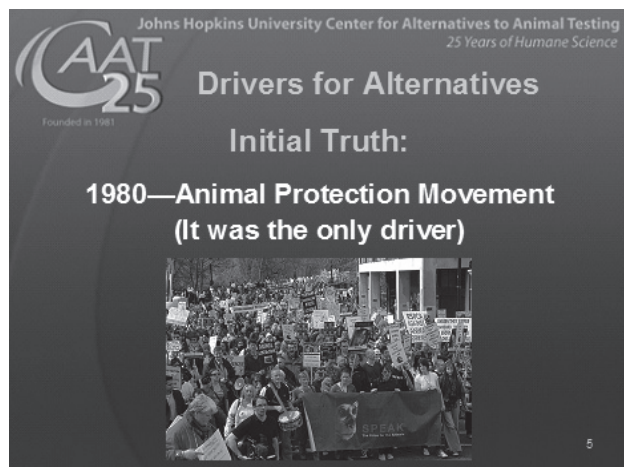
Slide 4

This timeline, from the "initial truth" of the rat as laboratory animal to the "current reality" of a worldwide network of national 3R alternative centers, spans just 100 years.

## Drivers for Alternatives

### Initial Truth

In the 1980s, the scientific community had begun using tissue culture methodologies, but this activity was still in its infancy. At the time, the animal protection movement was the only significant driver attempting to advance the alternatives movement. The academic use of in vitro methods was developing at its own pace. The animal protection movement activities greatly enhanced the acceptance and use of alternative activities in government and commercial activity. (Slide 5)



Slide 5

### Current Reality

The number of activities that now encourage (one could say force) the development of replacement alternatives are numerous. They include science, European Legislation (particularly the ban on marketing any cosmetic product if it or its ingredients have been tested in animals), nanotechnology (in vitro approaches are the only way to evaluate the safety of hazard of these products), and the animal protection movement. Societal demands in this regard are not restricted to the animal protection community, however, but are shared by a large portion of the general population. The public recognizes the "intrinsic value" of animals, above and beyond any utilitarian worth. (Slide 6, Slide 7)



Slide 6

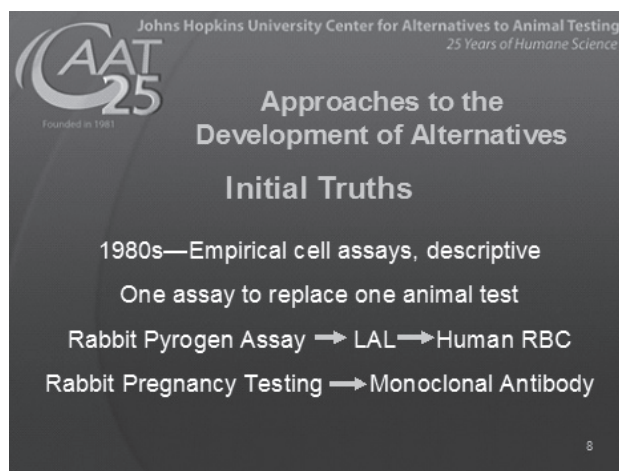


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## Approaches to the Development of Alternatives

### Initial Truths

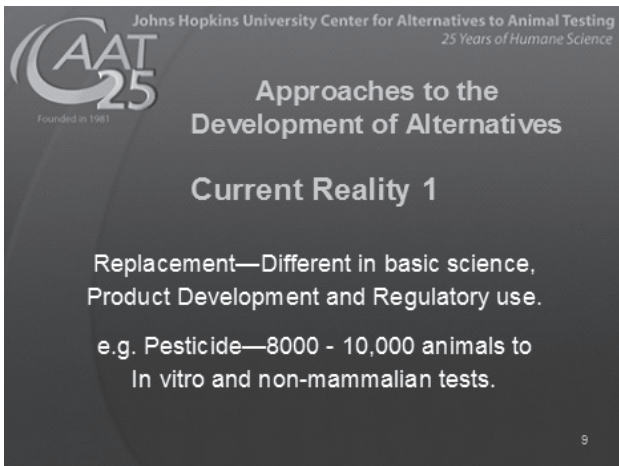
In the 1980s, in vitro assays were based on empirical cell assays, and they are best described as descriptive. It was thought that, in each case, an in vitro assay would replace an animal test, and the data confirmed this approach. The rabbit pyrogen test was replaced with the Limulus test (which since has been replaced by a human RBC test), and pregnancy testing using rabbits was replaced by a test-tube monoclonal antibody test. One test replaces one animal. (Slide 8)



Slide 8

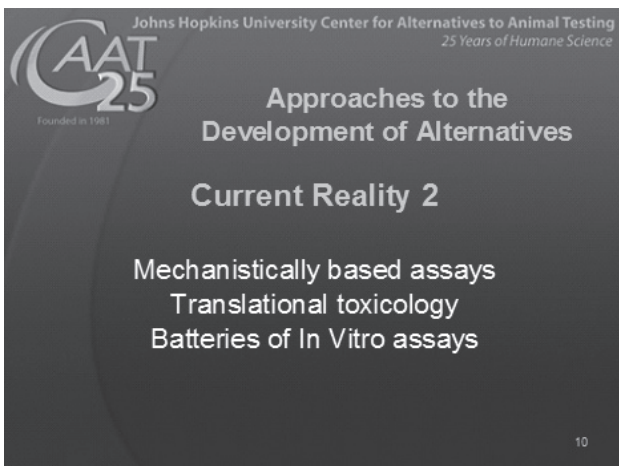
### Current Reality

The current reality is that replacement and refinement alternatives are used and thought about differently in academia, government and industry. In the academic community, in vitro methods are used extensively. They are not thought of as alternatives but rather as the way it should be done—as best practice. In industry, in vitro methods are widely used in product development and safety evaluations. In the crop protection industry, pesticides are evaluated with in vitro and short-term assays to make business decisions regarding whether a compound should be marketed. The animal tests are then done to meet the regulatory requirements. (Slide 9)



Slide 9

Most importantly, we now recognize that the next phase of replacement alternatives involves mechanistically-based assays, translational toxicology (i.e., the use of in vitro data to predict human health consequences), and the need for batteries of in vitro assays to determine the safety/hazard potential of a compound and to do risk assessment. (Slide 10)



Slide 10

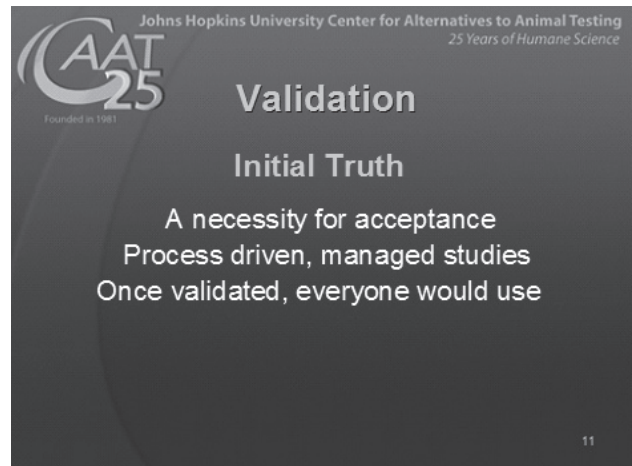
In 2007, the US National Academy of Sciences, at the request of the US Environmental Protection Agency (EPA), wrote what promises to be the roadmap for the future of toxicology: *Toxicity Testing in the Twenty-first Century*. This publication presents a wonderfully clear and persuasive case for the use of human cells in culture as the next stage of toxicity evaluation. (When I read the report for the first time, I wanted to retitle it: "Be more like CAAT.")

**Validation**

**Initial Truths**

In the 80s, the concept of validation was recognized as necessary for acceptance of an alternative method, and a process had to be created that would codify validation. The concept was simple— once a test was validated, everyone would use it. However,

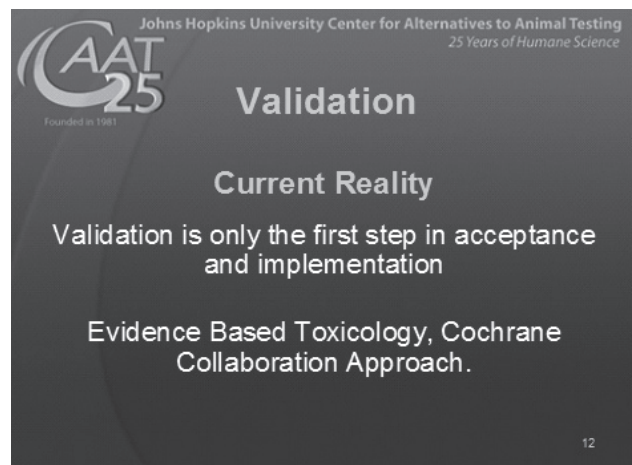
to paraphrase the *Baltimore Sun* reporter and commentator H.L. Mencken, for every problem there is a simple solution, and it is always wrong. (Slide 11)



Slide 11

**Current Reality**

The current reality recognizes that validation is only the first step in acceptance and implementation; the path from validation to acceptance and implementation remains long and arduous. One approach to speeding up the process is to draw heavily upon clinical medicine and apply the concepts of evidence-based medicine to toxicity testing. Some of us are beginning to look at the Cochrane Collaborative and to explore an evidence-based toxicology approach as a possible solution to the validation problem. I suspect that as the process develops, it will evolve into yet another current reality. (Slide 12)



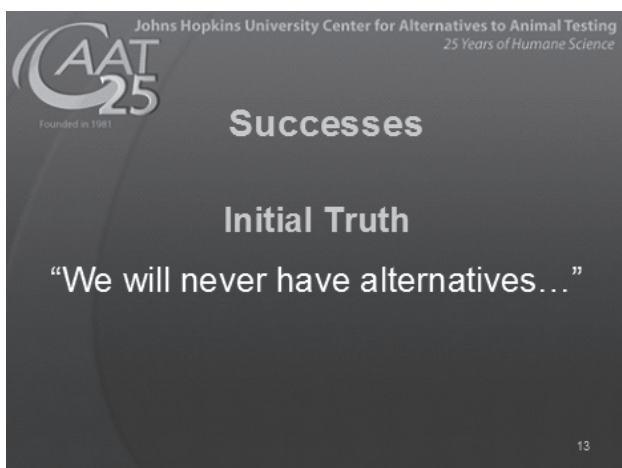
Slide 12

**Success**

**Initial Truths**

In the early 80s, I frequently heard the statement, "we will never have alternatives!" Occasionally, scientists still ask me, why don't you change CAAT's name from alternative to complementary? Most of this is either a misunderstanding of the 3Rs or

an unfounded hostility toward what they believe alternatives mean. (Slide 13)

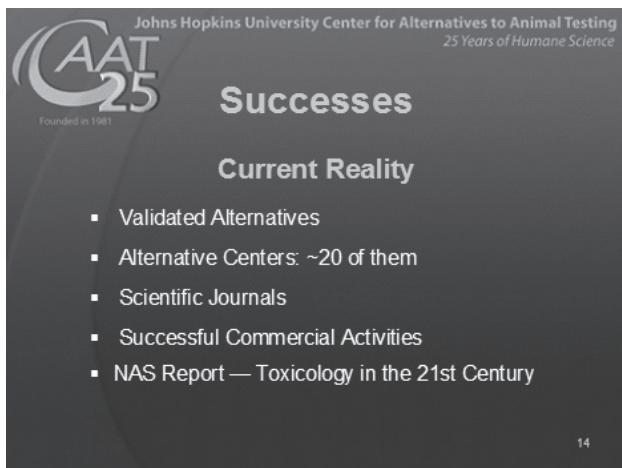


Slide 13

Current Reality

In the last 25, years we have identified and validated many alternative methods, established approximately twenty 3R centers around the world—each with different areas of focus but all very enthusiastic about the future, launched scientific journals devoted to in vitro methods and initiated numerous commercial ventures that offer in vitro and other services to companies developing systems for in vitro analysis. These commercial activities are quite remarkable.

As mentioned earlier, the National Academy of Sciences (NAS) recently published *Toxicology Testing in the Twenty-first Century: A Vision and a Strategy*. This publication focuses on biological pathways that can be studied in human cell cultures and examines how we can use in vitro data to predict human health consequences. I call this "translational toxicology." The concept of translation toxicology is being pursued at my institution in a unique pre/post doctoral program that will train students and fellows to become proficient at this task. (Slide 14)

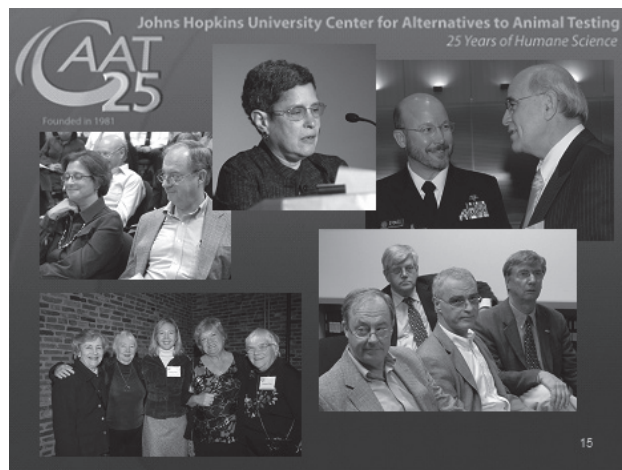


Slide 14

This World Congress marks yet another success. From Baltimore in 1993 to Tokyo in 2007 (with more than 1000 participants), with many steps in between, scientists and others have gathered to share knowledge, science, friendship, and a dedication to humaneness.

There are now academic departments and endowed professorships covering the area of science, society, and animals; all of them focus in some way on alternatives.

In 2006, at a meeting of the European Partnership for Alternative Approaches to Animal Testing (EPAA), Thomas Hartung presented a slide showing that there are thirteen REACH (alternative) projects involving more than 260 participants, 80 million Euro (120 million \$ USA), and 171 methods under evaluation—and this represents only one project among several. (Slide 15, Slide 16, Slide 17, Slide 18)



Slide 15

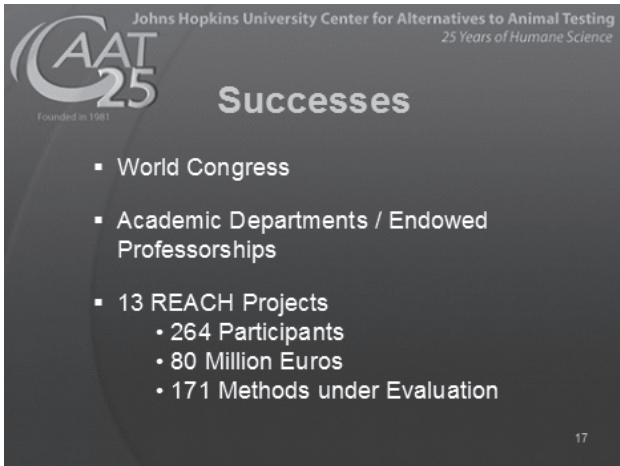


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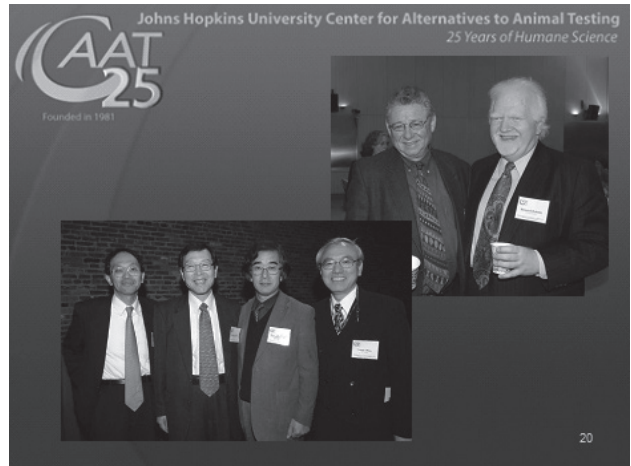
There are so, so many successes—and we have just begun.

Science

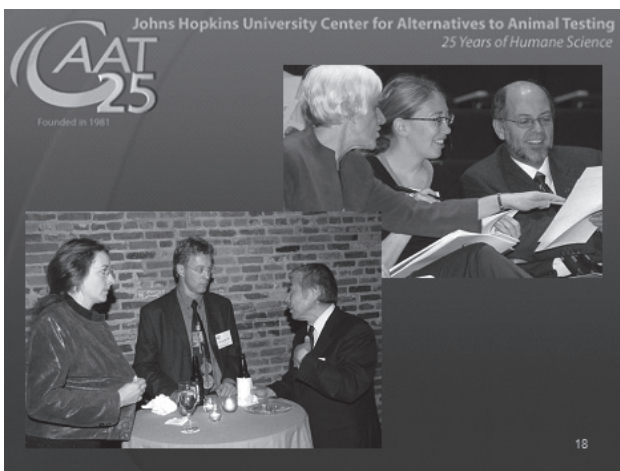
As this 6<sup>th</sup> World Congress is taking place in Japan, with more than 35 countries represented, I remember



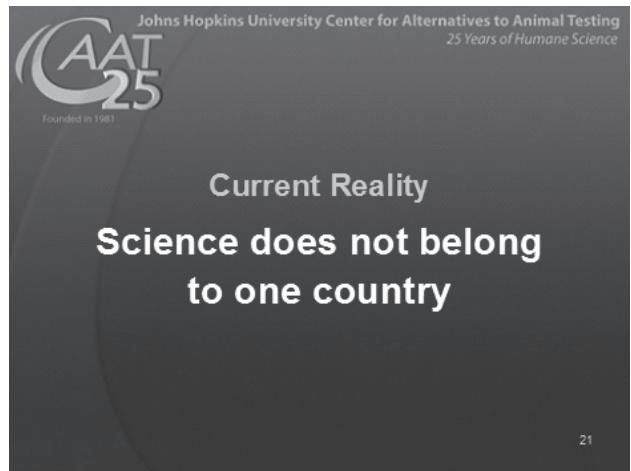
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Slide 20



Slide 18



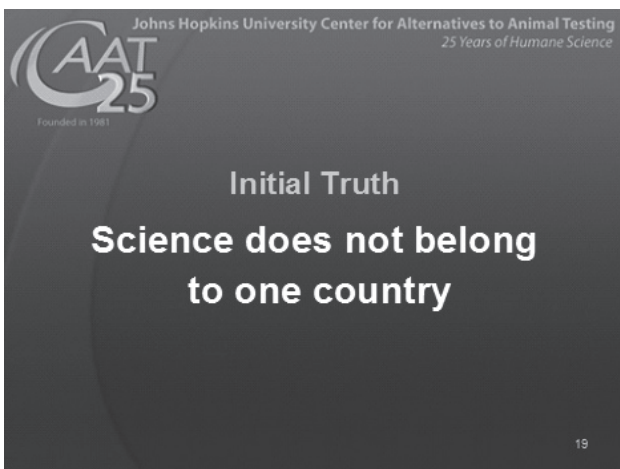
Slide 21

that, as a high school student (less than 15 years of age), I read a book that made the statement, "Science does not belong to one county." That statement captured my interest and influenced my decision to pursue science as my career path. The phrase "science does not belong to one county" is both initial truth and current reality. (Slide 19, Slide 20, Slide 21)

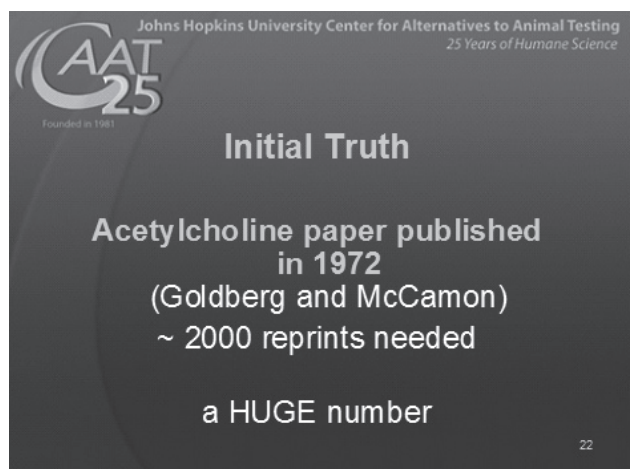
**To Whom We Speak**

**Initial Truth**

In 1972, I published a paper (A method for the determination of acetylcholine with R.E. McCaman) that was a major success. We received requests for 2000 copies—a huge number. All of the reprints went to scientists. (Slide 22)



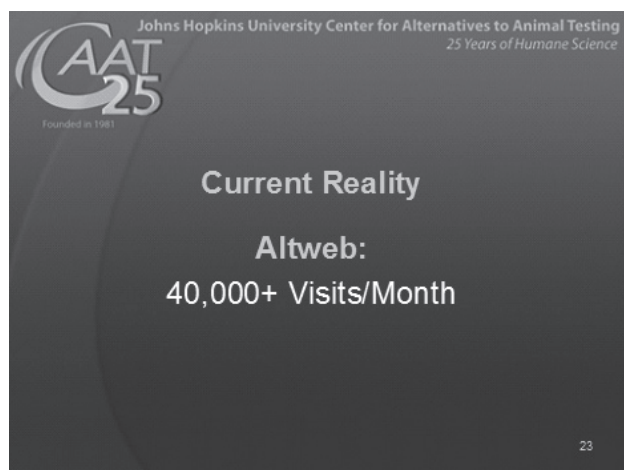
Slide 19



Slide 22

## Current Reality

Altweb, the Alternatives to Animal Testing Website, receives more than 40,000 visits per month—not only scientists, from both academia and industry, but also regulators, students, educators, animal welfare advocates, the general public, and more. CAAT manages this site on behalf of a 27-member international Project Team. (Slide 23)

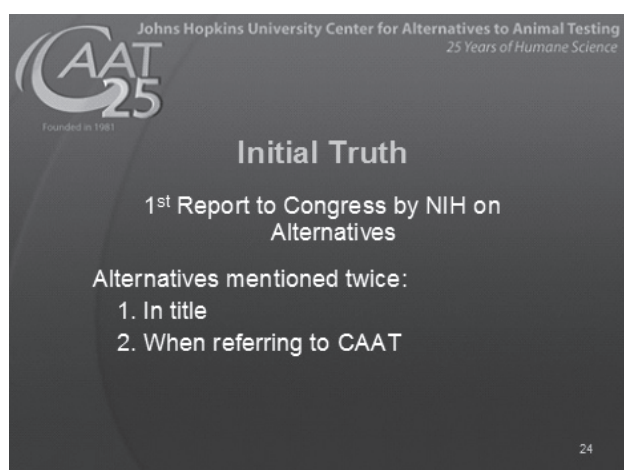


Slide 23

## Acceptance of Alternatives

### Initial Truth

The scientific community did not accept, and in some cases still does not accept, alternatives. An NIH report to Congress on alternatives, a requirement for NIH funding in the mid 1980s, mentioned alternatives only twice—once in the title and the second time when referring to the Johns Hopkins University Center for Alternatives to Animal Testing (CAAT). What was not said is more telling than what was said. (Slide 24)

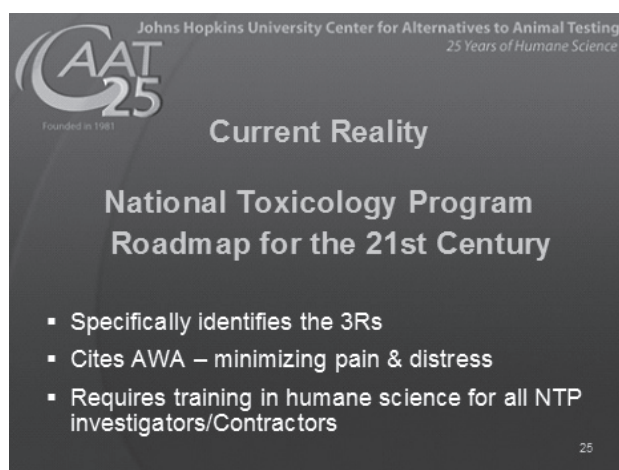


Slide 24

## Current Reality

Both the NAS Report, *Toxicology Testing in the Twenty-first Century* (mentioned earlier), and the National Toxicology Program (NTP) Roadmap for

the 21<sup>st</sup> Century, which specifically identifies the 3Rs and requires training in humane science for all NTP investigators and contractors, point to the enormous strides in the acceptance of alternative. (Slide 25)



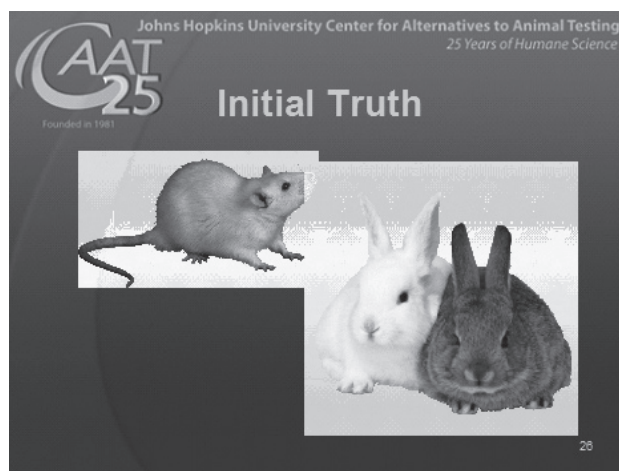
Slide 25

As a popular cigarette advertisement of several years ago says, "we've come a long way baby."

## The Biological Matrix of Research

### Initial Truth

Whole animals of many species were used, and regulatory requirements in some cases identified specific species. (Slide 26)



Slide 26

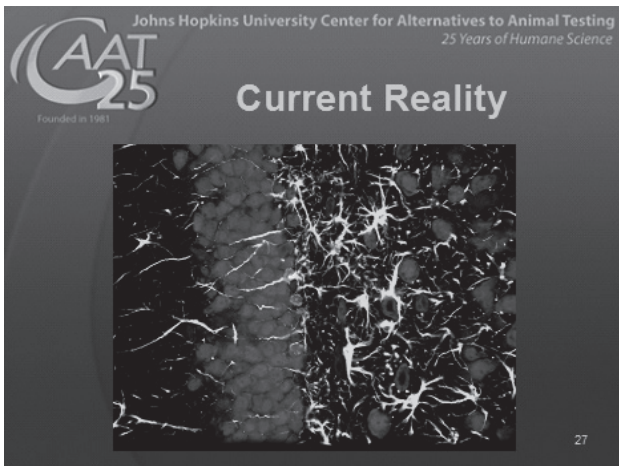
## Current Reality

More papers use in vitro approaches, animal use has decreased from its high by 50%, and fewer species are used in research. (Slide 27)

## Conclusions

### Current Reality

We have made incredible progress. Animal use is down, in vitro is the first approach, and the US Food and Drug Administration (FDA) requires in vitro metabolism studies. Truly incredible progress.



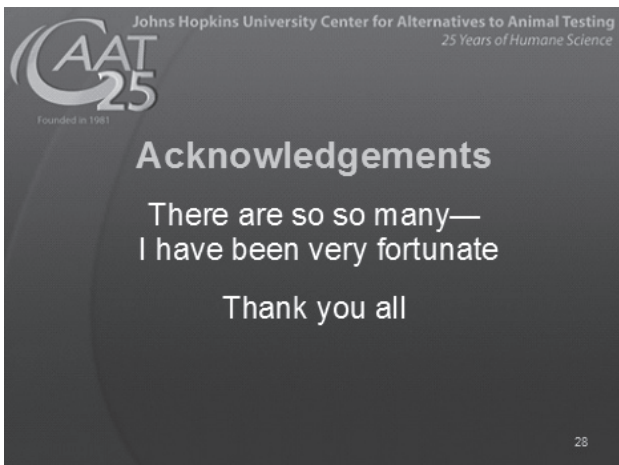
Slide 27

### Tomorrow

We have just begun; the best is yet to come.

### **Acknowledgements**

There are so, so many – I have been very fortunate.  
Thank you all. (Slide 28)



Slide 28

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6. Goldberg, A.M., McCaman, R.E., The Determination of Picomole Amounts of ACh., *Journal of Neurochemistry*, 20: 1, 1973.

Slide 1: (from left clockwise) Michael Balls, Andrew Rowan, Thomas Hartung, Herman Koeter, Bert van Zutphen

Slide 4: (from left) Henry Spira, Mitsuteru Masuda, Tohru Inoue, Hajime Kojima, Kenichi Nakazama, Yasuo Ohno

Slide 6: (from top clockwise) Vicki Robinson, Patric Amcoff, Horst Spielmann, Coenraad Hendriksen

Slide 15: (from top center clockwise) First photo: Marilyn Wind, 2<sup>nd</sup> photo: William Stokes, Herman Koeter, 3<sup>rd</sup> photo: (top left clockwise) Michael Balls, Horst Spielmann, Manfred Liebsch, Rodger Curren, 4<sup>th</sup> photo: (right to left) Joan Poling, Jean Larsen, Kristina Adams, Lee Krulisch, Jeannene Tylee, 5<sup>th</sup> photo: Joanne Zurlo, Rodger Curren

Slide 16: (left) Group from NC3Rs; (right top) Group from FRAME; (bottom right) Group from Netherlands Centre Alternatives to Animal Use (NCA)

Slide 18: (left bottom) Frankie Olds, Jan van der Valk, Tsutomu Kurosawa, (right top) Gillian Griffin, Allison Guy, Clement Gauthier (Canadian Council on Animal Care)

Slide 20: (top right from left) Alan Goldberg, Bernard Robaire (bottom left from left) Hajime Kojima, Makoto Hayashi, Noriho Tanaka, Yasuo Ohno