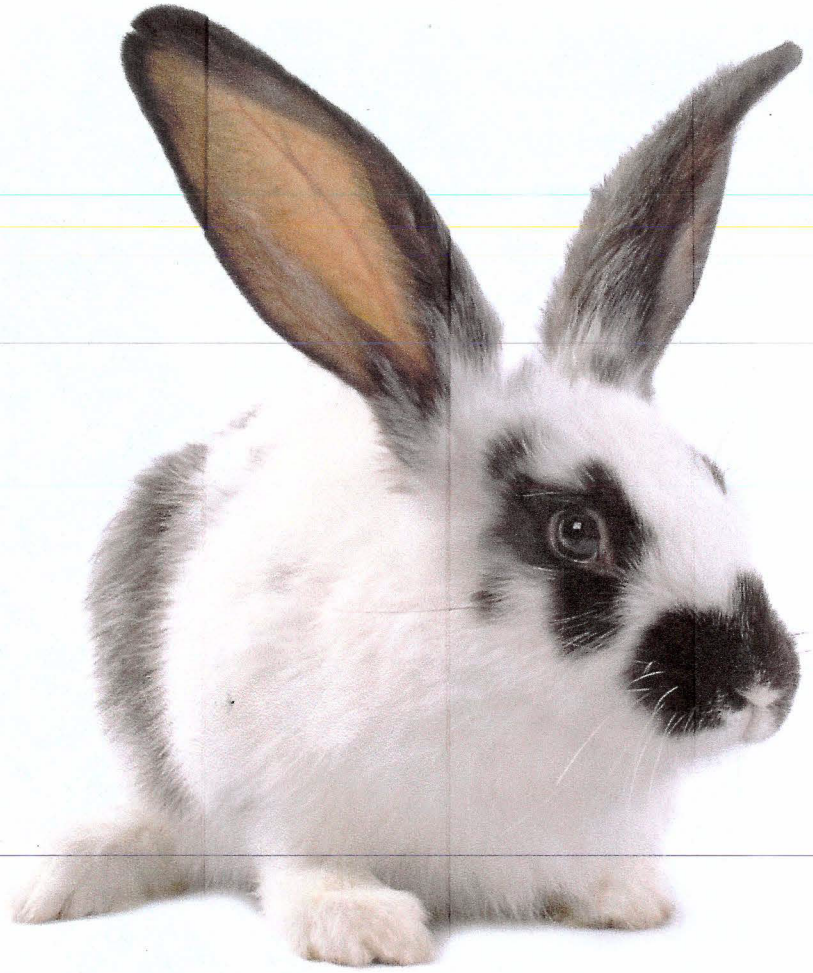


Nicola Beynon
Head of Campaigns
Humane Society International – Australia
[redacted]

HUMANE SOCIETY INTERNATIONAL

#BeCrueltyFree

Driving the World Toward Better Science

Celebrating Animals

HUMANE SOCIETY INTERNATIONAL

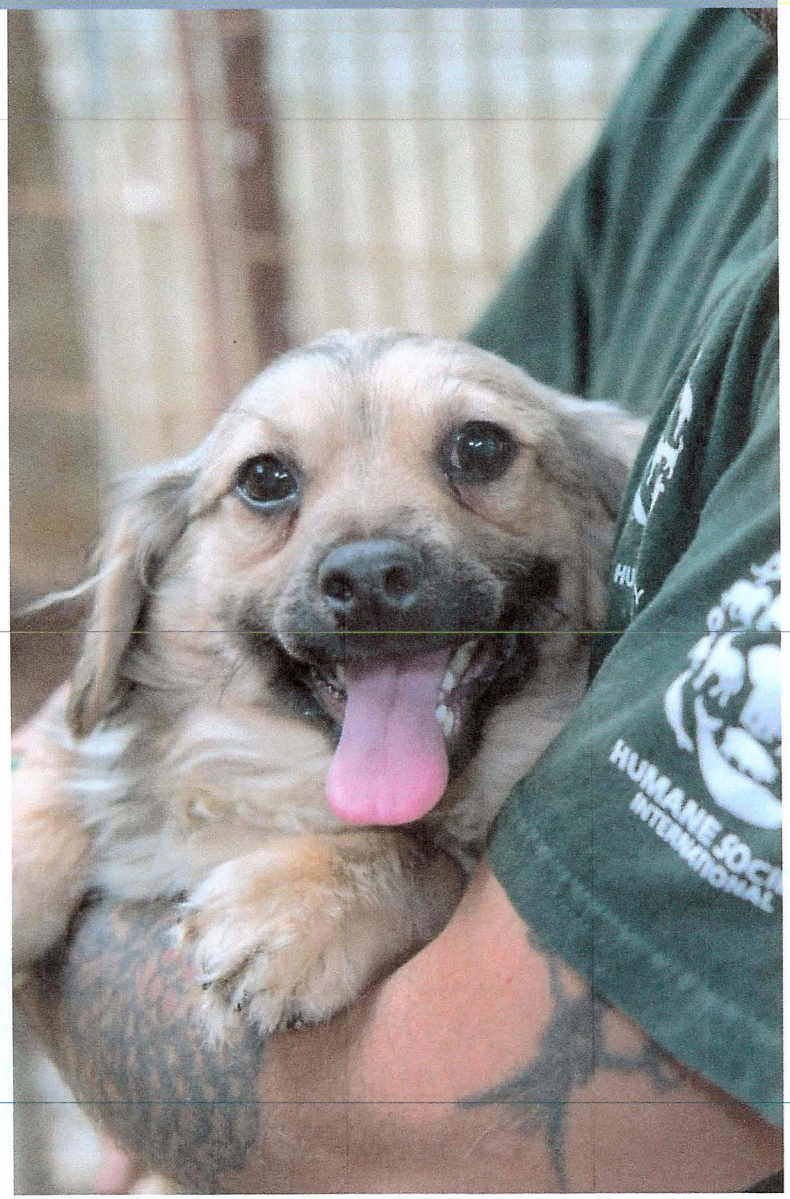
THE HUMANE SOCIETY OF THE UNITED STATES

About HUMANE SOCIETY INTERNATIONAL

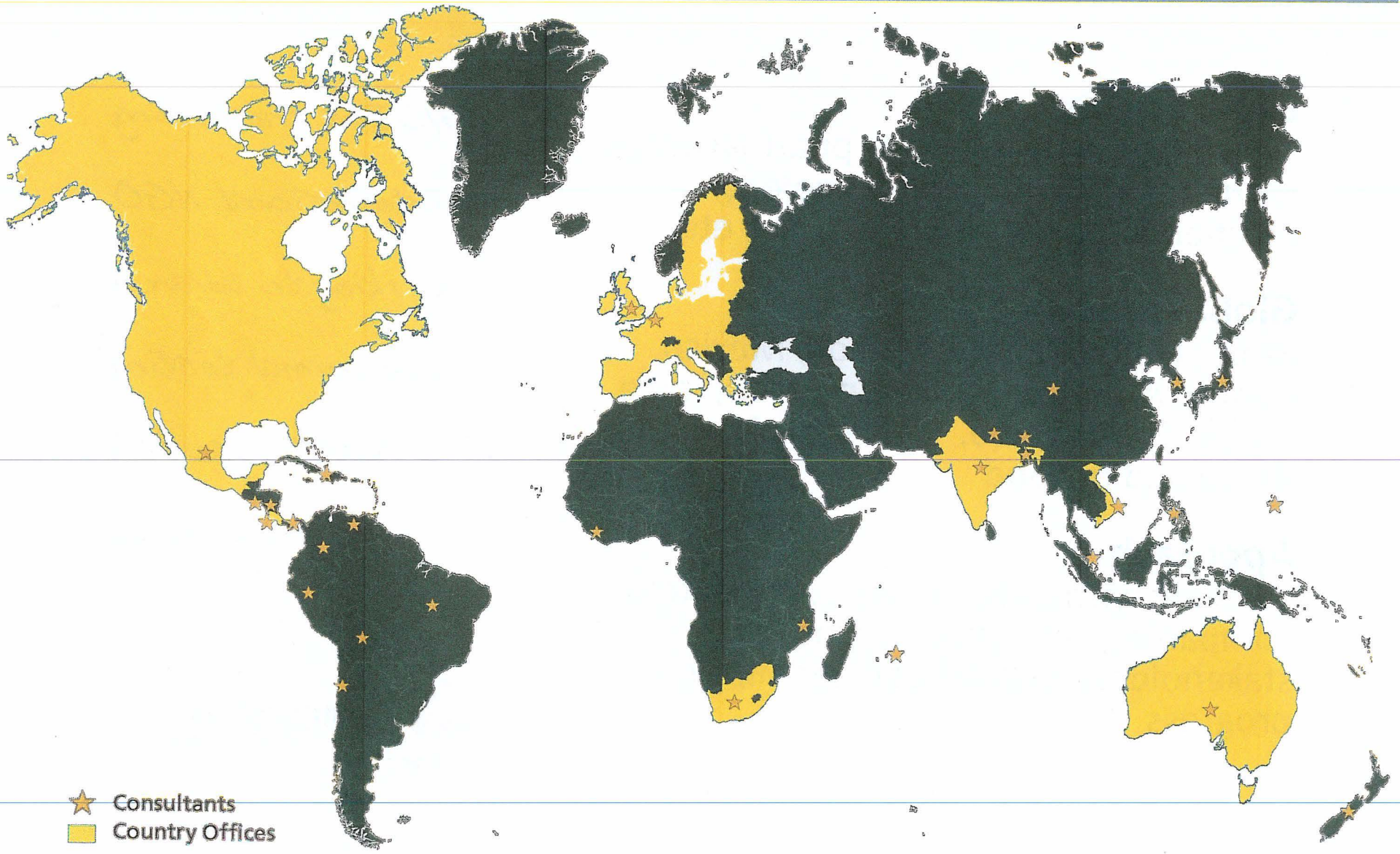
- **Our mission**
  Humane Society International is a global animal protection organization working to help all animals—including animals in laboratories, animals on farms, companion animals and wildlife.

- **Our approach**
  HSI seeks out innovative and scientifically sound approaches to animal protection and relies on a network of on-staff and external experts to make the case for policy change to improve the lives of animals and people.

- **Our reach**
  HSI's programs are active in more than **50 countries** on nearly every continent.



HSI's global presence



Research & Toxicology Department

- **Expert team**
  Toxicology, ecotoxicology, pharmacology, regulatory science, endocrinology, biochemistry, neuroscience, law, etc.
- **Global presence**
  Brazil, United States, Canada, Mexico, Central America, European Union, India, Japan, South Korea, Viet Nam, Australia, Africa and beyond
- **Approach**
  Working with policy makers, regulators, companies, scientists, and other stakeholders to build partnerships for progress

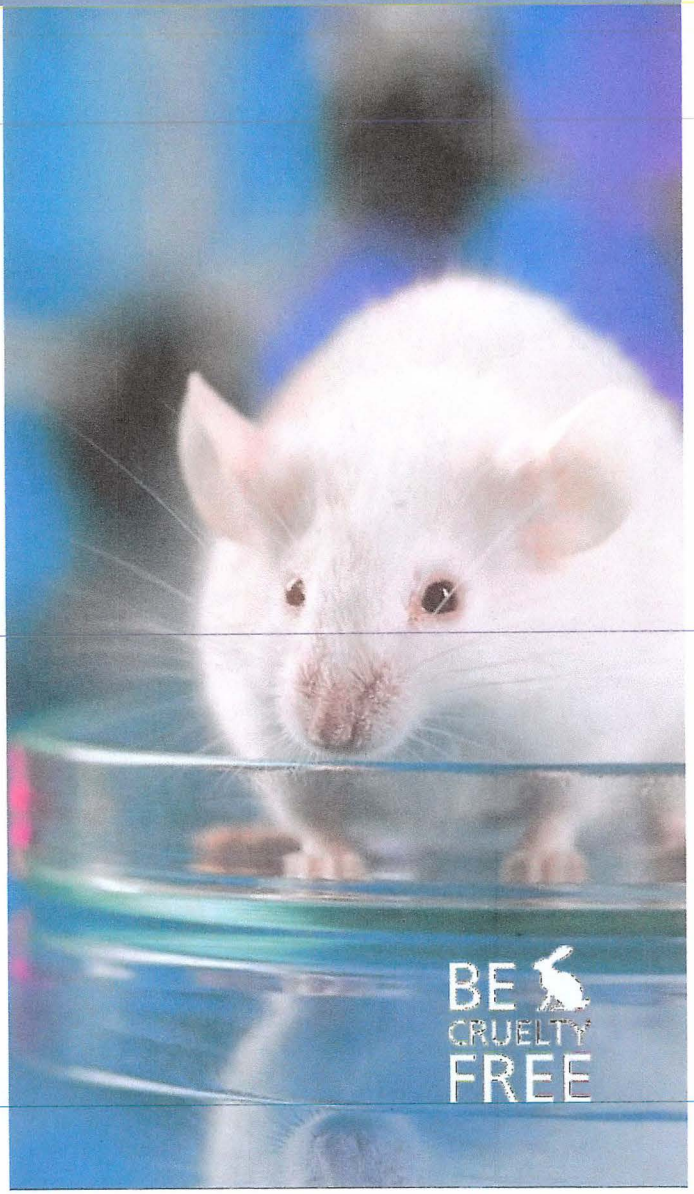
HSI is the leading international NGO working to advance non-animal testing & health research worldwide

HUMANE SOCIETY INTERNATIONAL

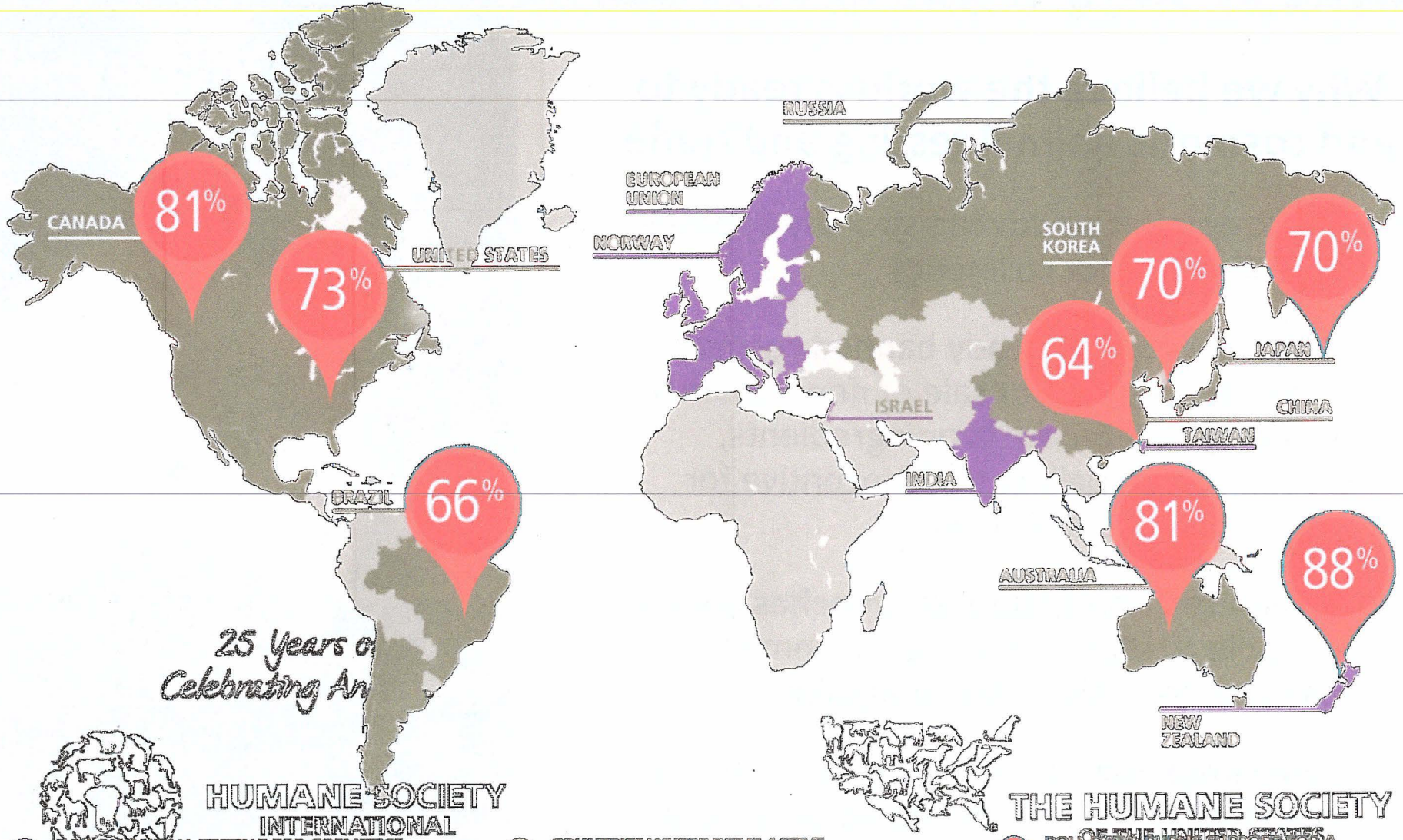
Global #BeCrueltyFree Campaign

Why we believe the world is ready to end cosmetic animal testing and trade

- **Public opinion** worldwide strongly opposes animal testing for cosmetics
- **37 markets** have already banned cosmetic animal testing and/or sale of newly animal-tested beauty products or ingredients, creating a compelling trade incentive for other countries to follow
- **Recognized non-animal approaches** are now available to replace the most commonly assessed cosmetic safety endpoints
- **>600 companies** have already adopted the "responsible innovation" model and made the switch to cruelty-free

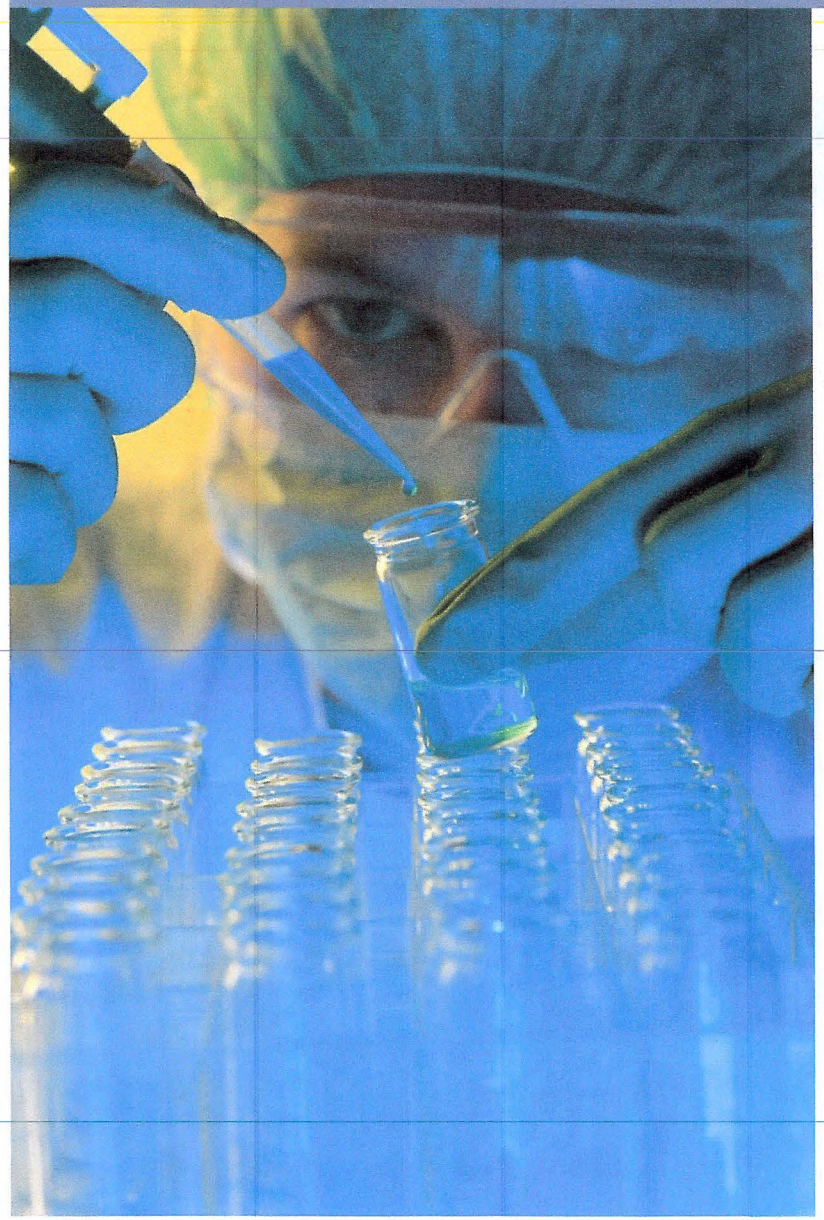


Public support for animal test ban



37 national testing/trade bans; 10+ others in development

'Responsible innovation'



1. Choose from among **thousands of existing ingredients** with established toxicity profiles and **history of safe use**
2. Evaluate new product formulations using modern **non-animal methods** (calculation, computer modeling, cell tests)
3. Avoid new-to-the-world chemical ingredients (subject to new animal testing under chemical laws)
4. Avoid ingredients with known health concerns that may be subject to further animal testing


>600 beauty brands produce safe new products without animal testing

Modern non-animal methods are **more predictive**, e.g. skin allergy

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Putting the parts together: Combining *in vitro* methods to test for skin sensitizing potentials

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ABSTRACT

Allergic contact dermatitis is a common skin disease and is elicited by repeated skin contact with an allergen. In the regulatory context, currently only data from animal experiments are acceptable to assess the skin sensitizing potential of substances. Animal welfare and EU Cosmetic Directive/Regulation call for the implementation of animal-free alternatives for safety assessments. The mechanisms that trigger skin sensitization are complex and various steps are involved. Therefore, a single *in vitro* method may not be able to accurately assess this endpoint. Non-animal methods are being developed and validated and can be used for testing strategies that ensure a reliable prediction of skin sensitization potentials. In this study, the predictivities of four *in vitro* assays, one *in chemico* and one *in silico* method addressing three different steps in the development of skin sensitization were assessed using 54 test substances of known sensitizing potential. The predictivity of single tests and combinations of these assays were compared. These data were used to develop an *in vitro* testing scheme and prediction model for the detection of skin sensitizers based on protein reactivity, activation of the Keap-1/Nrf2 signaling pathway and dendritic cell activation.

1. Introduction

As the interface between the environment and the body, the skin is continuously exposed to environmental insults, pathogens and xenobiotics. In particular, consumers and workers are often exposed to chemicals via cosmetic and household products or in industrial settings on a daily basis and to a significant degree. One of the adverse effects that can occur as a result of skin exposure to xenobiotics is contact sensitization, the clinical manifestation of which is allergic contact dermatitis (ACD). The principle objective of toxicological testing is to provide a basis for the assessment of hazards and to identify potential risks from use and handling of products, such as chemicals or cosmetic formulations, thus ensuring that adverse effects to human health do not occur. The evaluation of the sensitization potential of a substance has therefore been of central importance for hazard and risk assessments for decades. Currently, most toxicological endpoints in the regulatory context are assessed via animal testing. This is also the case for the sensitization potentials for which generally only the animal studies described in OECD 406 (guinea pig tests according to Buehler or Magnusson & Kligman) or OECD 429 and OECD 442 (murine local lymph node assays, LLNA) are accepted by the regulatory bodies.

The increasing emphasis on the ethics of animal testing has manifested itself in a regulatory context in the recent chemicals legislation on the registration, evaluation, authorization and restriction of chemicals (REACH (EU, 2006)) and even more so in

Abbreviations: ARE, antioxidant response element; AUC, area under the curve; CC, control cells; CV75, concentration reducing viability to 75%; DC, dendritic cells; DMSO, dimethyl sulfoxide; DNCB, 1-chloro-2,4-dinitrobenzene; DPRA, Direct Peptide Reactivity Assay; ECHA, European Chemicals Agency; ECVAM, European Centre for the Validation of Alternative Methods; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; h-CLAT, human Cell Line Activation Test; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Keap-1, Kelch-like ECH-associated protein 1; ITS, integrated testing strategy; LLNA, local lymph node assay; MCI/MI, mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; MFI, mean fluorescence intensity; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; mMUSST, modified myeloid U937 dendritic cell activation-based skin sensitization test; NADP1, nicotinamide adenine dinucleotide phosphate; Nrf2, nuclear factor (erythroid-derived 2)-like factor 2; OECD, Organisation for Economic Cooperation and Development; PBS, phosphate buffered saline; PI, propidium iodide; QSAR, quantitative structure-activity relationship; REACH, Registration Evaluation Authorisation and Restriction of Chemicals; RLU, relative luminescent unit; RP- HPLC, reverse phase high performance liquid chromatography; RT, room temperature; SDS, sodium dodecyl sulfate; T.C, treated cells; TNBS, 2,4,6-trinitrobenzene sulfonic acid.

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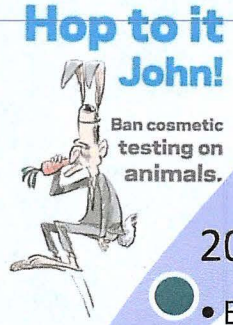
Compared to human		Accuracy
Animal test	**Mouse LLNA**	**84%**
Individual non-animal tests	DPRA	87%
	LuSens	82%
	MUSST	85%
	h-CLAT	78%
Combinations of non-animal tests (1 out of 2 is positive)	DPRA + LuSENS	85%
	DPRA + MUSST	81%
	DPRA + h-CLAT	83%
	LuSens + Musst	80%
	LuSens + h-CLAT	82%
Non-animal (2 out of 3) approach	**DPRA + LuSens + MUSST**	**94%**

5 years of cosmetics policy progress



2013

- EU – sales ban
- Israel – sales ban
- India – test ban
- Brazil – PLC 70/2014

2014

- India – import ban
- Brazil – São Paulo & Mato Grosso do Sul state test bans
- China – ends mandatory animal testing for domestic non-special use cosmetics.

2015

- New Zealand – test ban
- South Korea – sales ban (dependent on available alternative methods)
- Turkey – mandatory alternatives
- Brazil – Paraná & Amazonas state test bans

2016

- Taiwan – test ban
- Switzerland – test ban
- Brazil – Pará state test ban

2017

- Switzerland – sales ban
- Guatemala – test ban
- Brazil – Senate CCT unanimously endorses amendments to PLC 70/2014
- Australia – promises to implement dual test + sales ban

Supporting the transition to animal-free testing & assessment

- Concerns expressed by regulatory and scientific authorities about the transition to non-animal methods include technical issues, training & availability of infrastructures
- HSI offers scientific and technical support to implement these changes
- We work to ensure that the transition to non-animal technology is a success by promoting exchange of information, technology transfers, training and funding to validated alternative methods

HSI's commitment to support validation, acceptance, training & use worldwide

- Member of **ECVAM's Stakeholder Forum (ESTAF)** – validation of alternatives

- Member of the **International Council on Animal Protection at OECD (ICAPO)** – regulatory acceptance & global alignment

- Co-founder, top-tier sponsor and member of scientific program committee of the **World Congress on Alternatives and Animal Use in the Life Sciences** – research and dissemination

- Coordinator of **Human Toxicology Project Consortium**– training and dissemination

EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing

OECD

25 years of celebrating animals

HUMANE SOCIETY INTERNATIONAL

HUMAN TOXICOLOGY PROJECT CONSORTIUM
Advancing a New Paradigm for Assessing Chemical Safety

HSI's commitment to support validation, acceptance, training & use worldwide

China

- Collaboration with Guangzhou CHN-ALT Biotech Co. Ltd.
- Contributed to publication of academic textbook on alternative methods in Chinese.

South Korea

- National Assembly Forum on the Adoption of International Testing Guidelines

Japan

- Diet Conference on non-animal testing methods

Russia

- Training workshop in OECD approved in vitro methods.

IIVS staff and Russian regulators and delegates who participated in the June Workshop

To date nearly USD $500K invested in non-animal method training & support


25 Years of
Celebrating Animals
HUMANE SOCIETY
INTERNATIONAL
Thank you!