CERM

Consortium for Environmental Risk Management

Hazard Assessment in the Era of Big Data

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Green Toxicology @ Johns Hopkins Bloomberg School of Public Health

Changing The Paradigm

- Regulatory approaches to chemical hazard have been focused on "black box" animal assays
- Protects against hazard but only very slowly - example asbestos, BPA
- Provides little mechanistic information to guide molecular design
- Assumes humans are 70 kg rats
- Impossible to cope with the numbers of new chemicals being produced

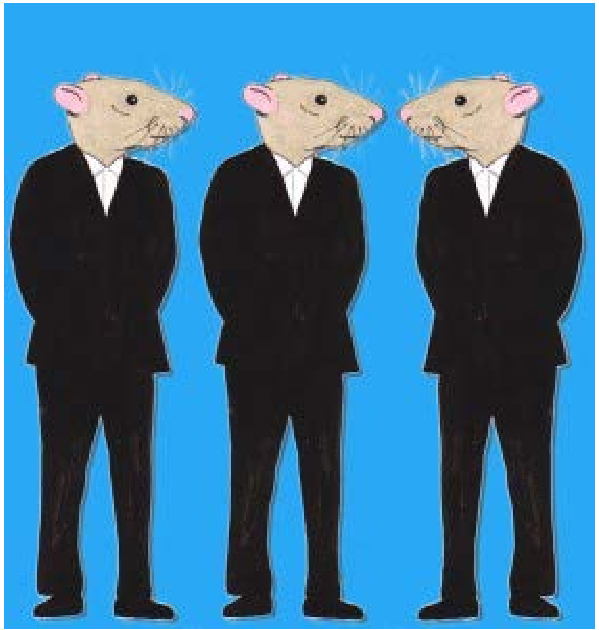

Image from: http://antidote-europe.org

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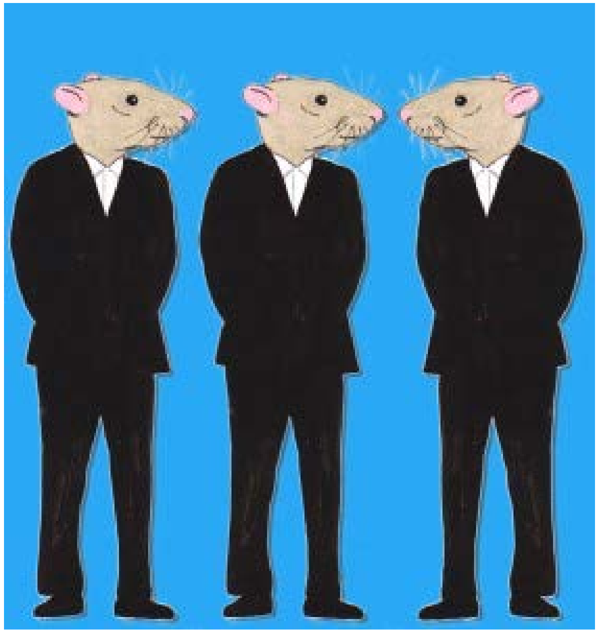
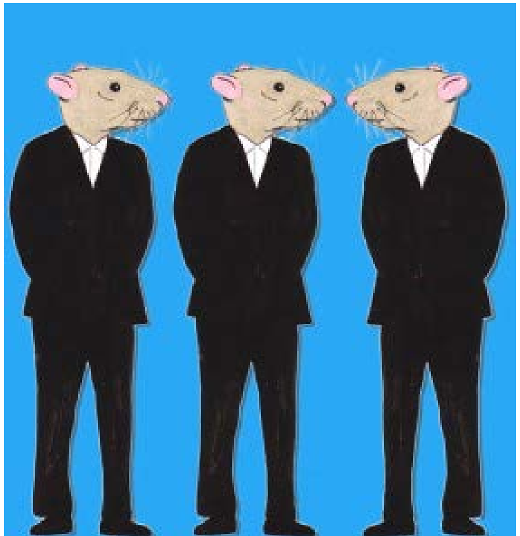


You mean it's 2019 and they are still using methods from the early 20th century?

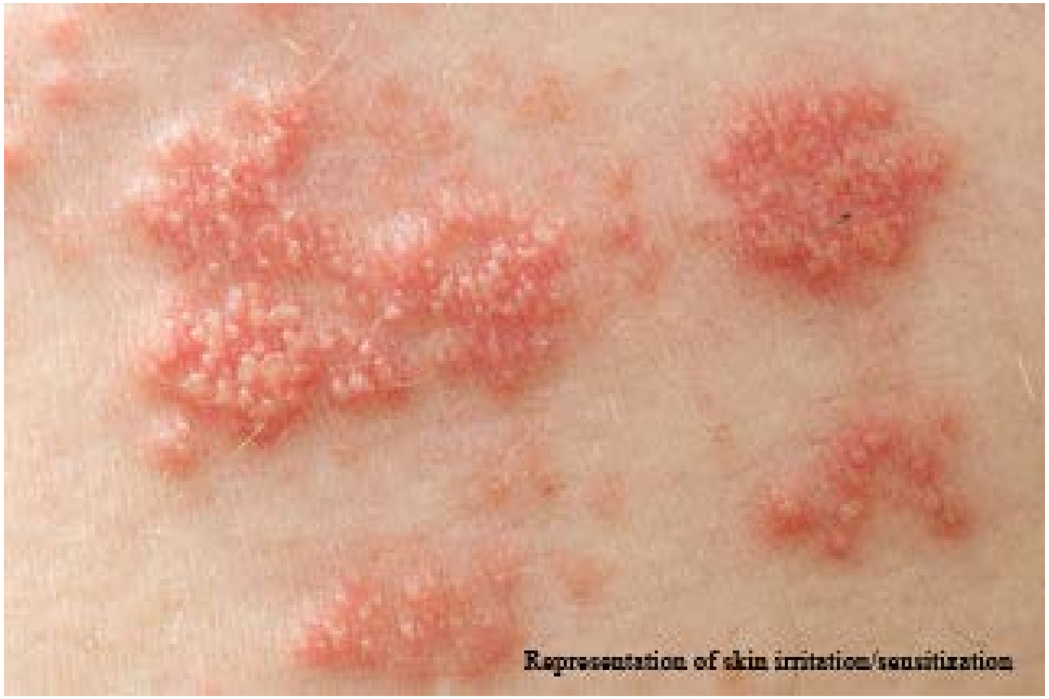
Image from: http://antidote-europe.org

- In vitro testing
  - In vitro tests act as drop-in replacements for animal tests – BCOP for Draize tests
    - < 5 per cent of tests in ECHA
  - In vitro tests for mechanism - Ames test for mutagenicity
- In silico predictions
  - Several models exist that are widely used – Toxtree, SimCyp (FDA) to predict metabolism, but mostly for screening purposes
- Read-across/QSAR
  - 80 per cent of ECHA dossiers use read-across for at least one end point and approximately 20 per cent of endpoints
  - 1 per cent use QSAR



Case study: Skin Sensitization

Skin sensitization is an allergic response upon contact to a substance

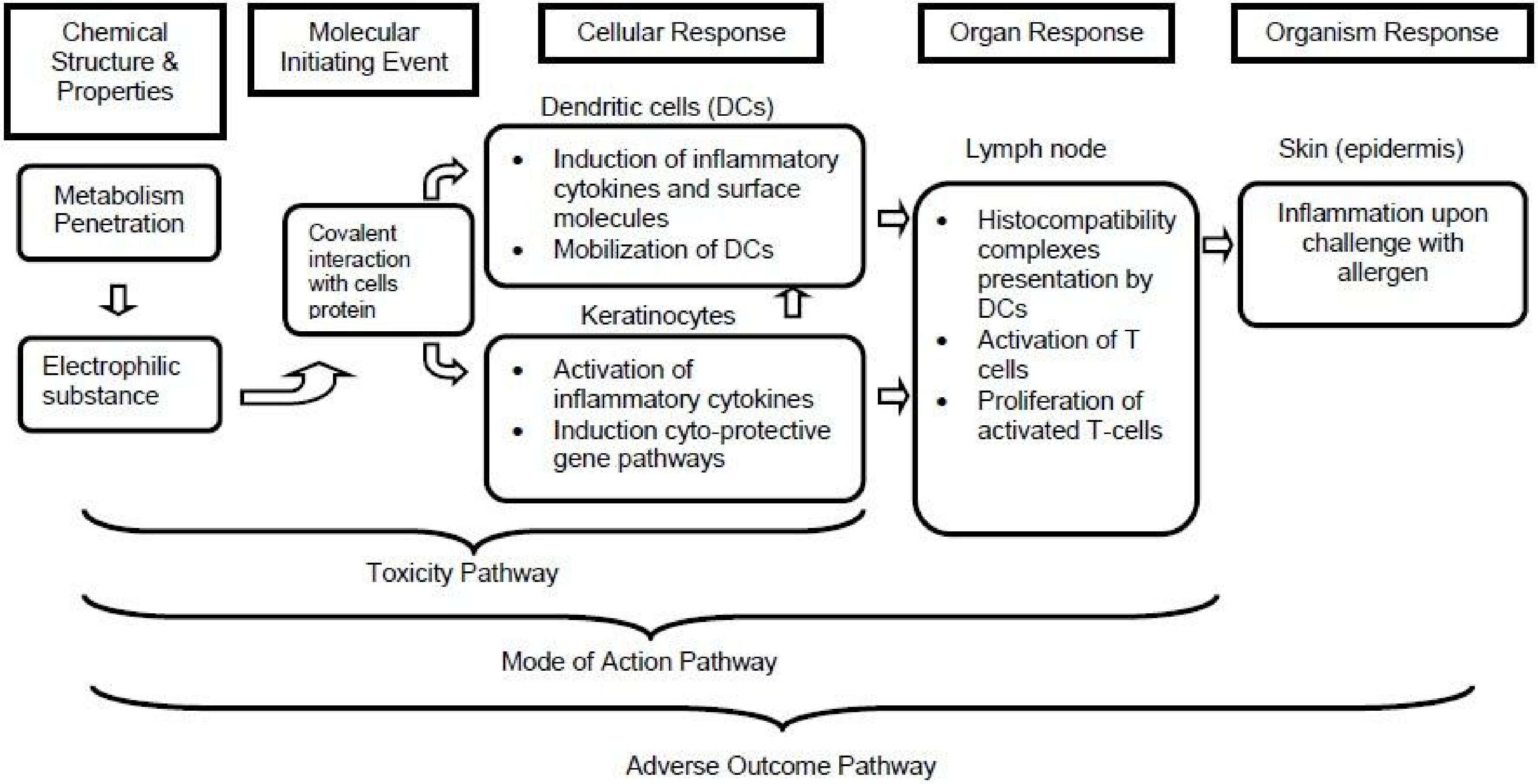


Representation of skin irritation/sensitization

What is sensitization and why is it important?

- 15-20% of the general population suffers from contact allergy (Thyssen et al., 2007) - prevalence may be increasing
- US Bureau of Labor Statistics (BLS) data shows that occupational skin diseases currently account for 10-15% of all occupational illness
- Occupational contact dermatitis is particularly prevalent in the personal services industry, with an estimated prevalence of 1.2% percent, e.g., in the beauty/ haircare industry (Warshaw et al., 2012),
- High prevalences in the petrochemical, rubber, plastic, metal and automotive industries (McDonald et al., 2006).
- Many chemicals used in occupational settings have NOT been tested for skin sensitization potential - prior to TSCA 21, only one in eight premarketing notifications to EPA is submitted with any toxicological data.
- However, EPA has of late been very proactive about sensitization

Skin sensitization: Mechanism


Chemical Structure & Properties
Molecular Initiating Event
Cellular Response
Organ Response
Organism Response
Metabolism Penetration
Electrophilic substance
Covalent interaction with cells protein
Dendritic cells (DCs)
• Induction of inflammatory cytokines and surface molecules
• Mobilization of DCs
Keratinocytes
• Activation of inflammatory cytokines
• Induction cyto-protective gene pathways
Lymph node
• Histocompatibility complexes presentation by DCs
• Activation of T cells
• Proliferation of activated T-cells
Skin (epidermis)
Inflammation upon challenge with allergen
Toxicity Pathway
Mode of Action Pathway
Adverse Outcome Pathway

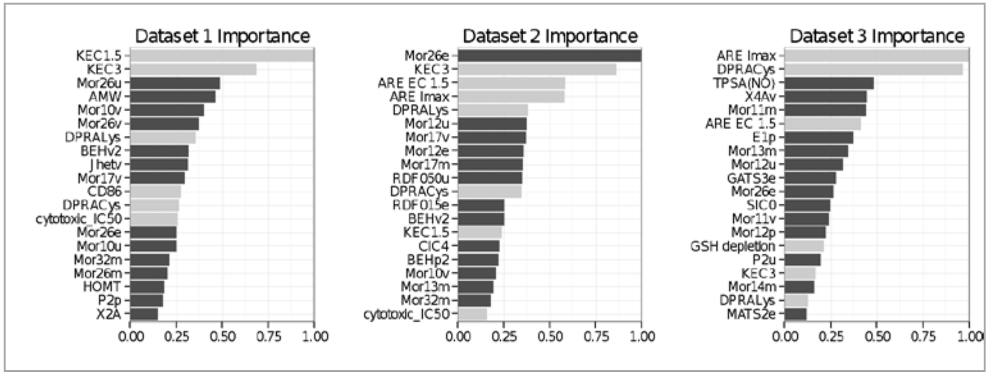
OECD. (2012). The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins Part 1: Scientific Evidence. [Series on Testing and Assessment No.168 ENV/JM/MONO(2012)10/PART1

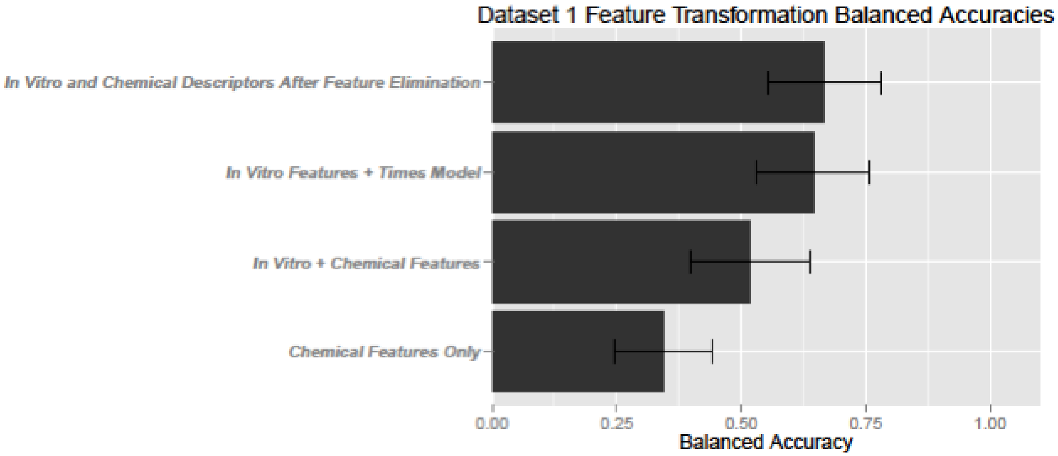
Integrated Testing Strategy

- Use machine learning to combine multiple sources of information at each point of the AOP
- 145 chemicals with LLNA testing (reference classification) and additional *in vitro* and *in chemico* tests
    - ARE induction
    - Cytotoxicity
    - Direct Peptide Reaction Assay

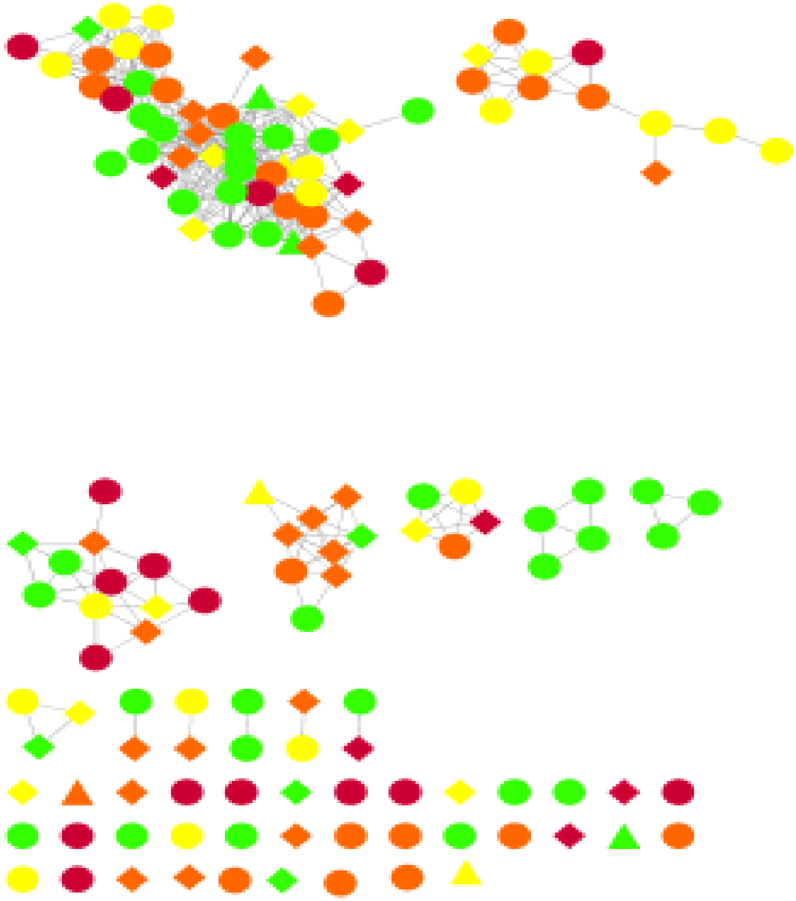
Integrated Testing Strategy

- Variable importance consistently included *in vitro* assays in the top
- More data is not always better; accuracy improved by <u>reducing</u> features
- Using in vitro and chemicals descriptors achieved 80 percent accuracy





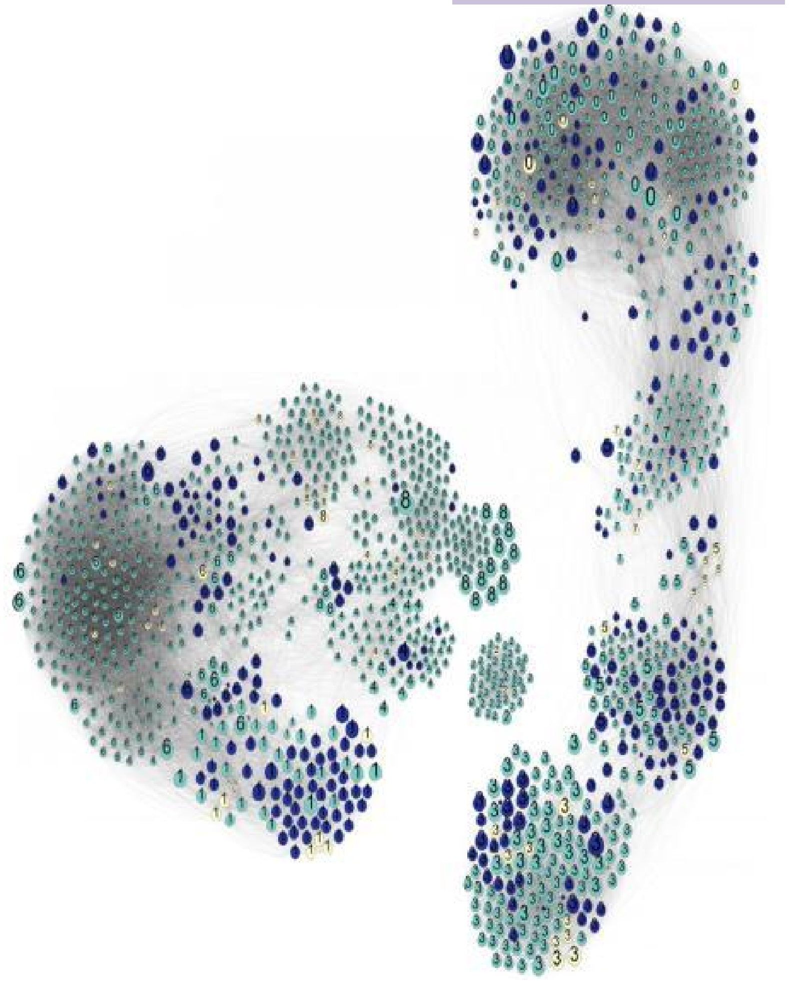
Chemical Similarity and Skin Sensitization



- Tanimoto distance was used to calculate chemical similarity
- Each chemical with more than 70 percent similarity was linked in a map
- Many chemicals that are structurally similar have different potential as sensitizers
- Many chemicals in the dataset have no similar neighbors

Red = Extreme
Orange = Strong
Yellow = Moderate
Green = Weak/Non

Chemical Similarity and Skin Sensitization



REACH Skin Sensitization

Skin sensitization: Simple classification by nearest neighbor

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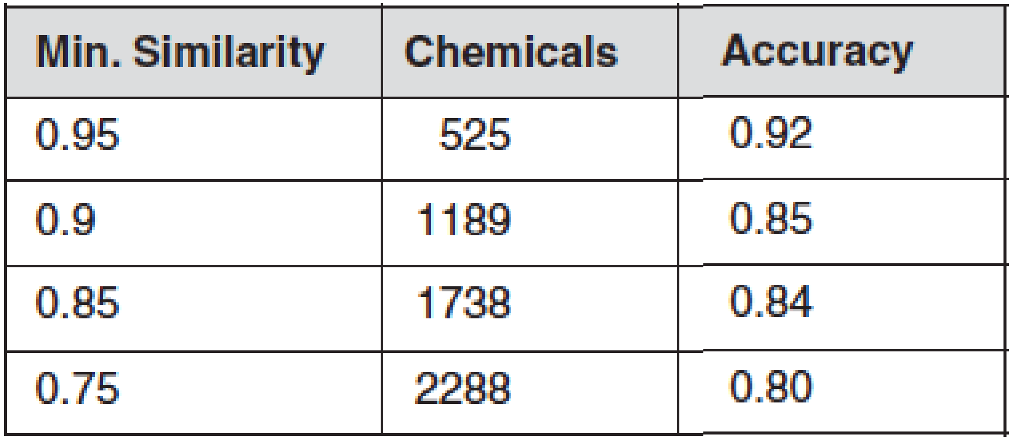
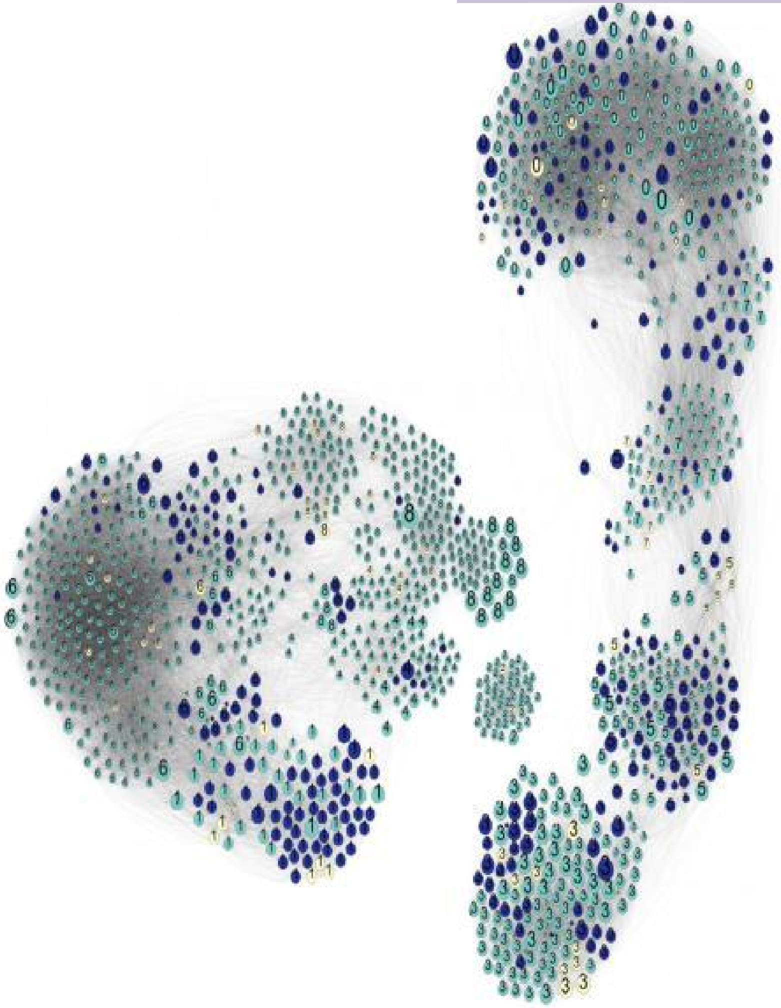


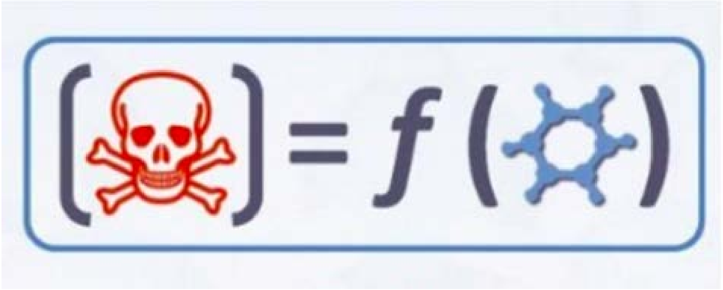
Min. Similarity	Chemicals	Accuracy
0.95	525	0.92
0.9	1189	0.85
0.85	1738	0.84
0.75	2288	0.80

Accuracies better than different animal guideline tests against each other

Chemical Similarity and Skin Sensitization

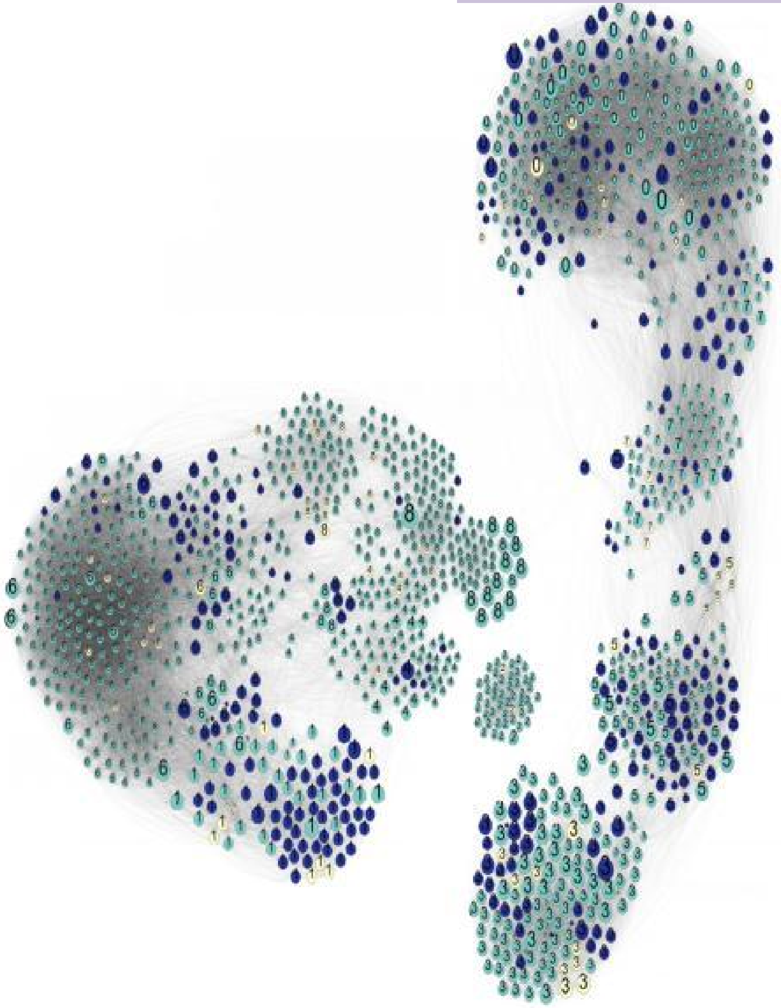
REACH Skin Sensitization

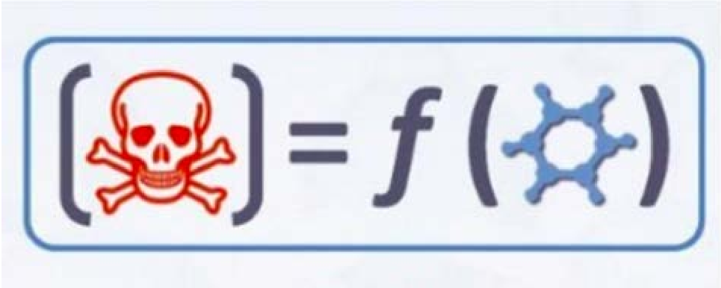


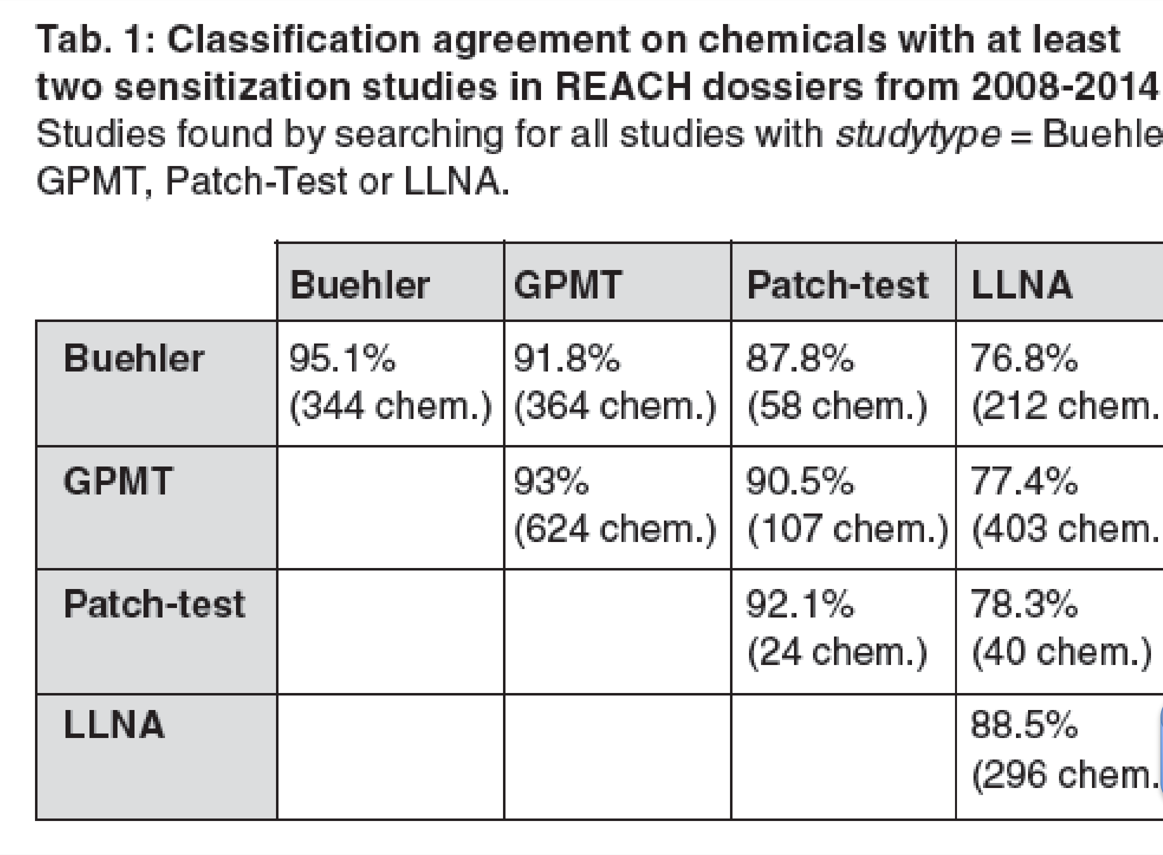


Chemical Similarity and Skin Sensitization

REACH Skin Sensitization

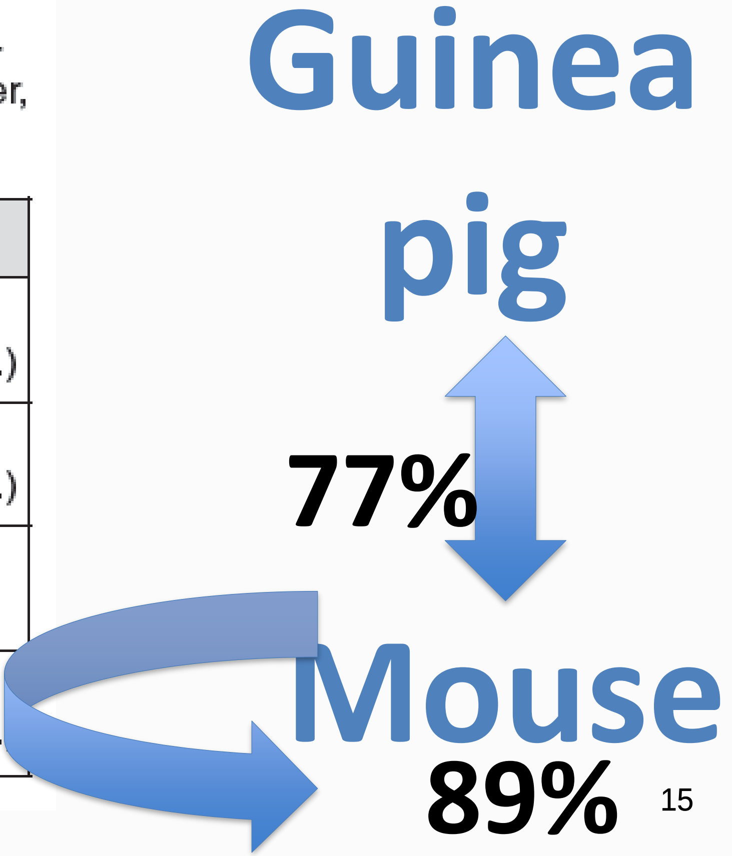






Tab. 1: Classification agreement on chemicals with at least two sensitization studies in REACH dossiers from 2008-2014
Studies found by searching for all studies with *studytype* = Buehler, GPMT, Patch-Test or LLNA.

	Buehler	GPMT	Patch-test	LLNA
Buehler	95.1% (344 chem.)	91.8% (364 chem.)	87.8% (58 chem.)	76.8% (212 chem.)
GPMT		93% (624 chem.)	90.5% (107 chem.)	77.4% (403 chem.)
Patch-test			92.1% (24 chem.)	78.3% (40 chem.)
LLNA				88.5% (296 chem.



Guinea pig

77%

Mouse

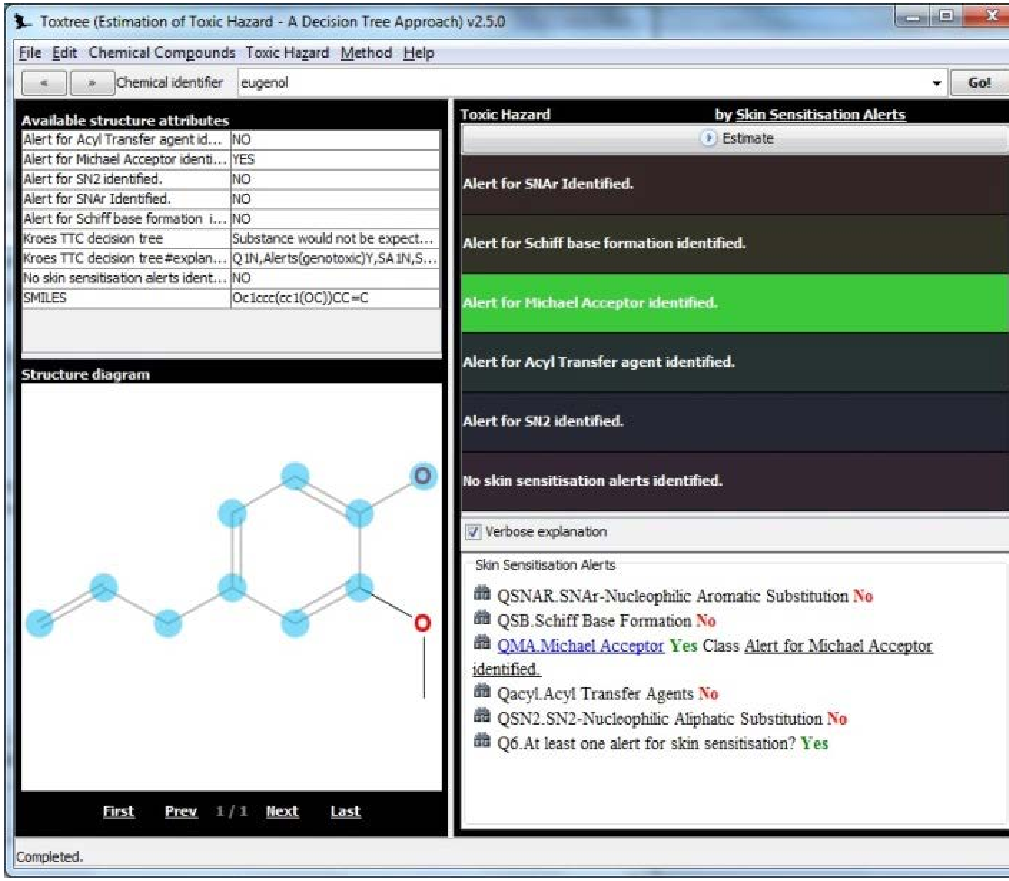
89%

SKIN SENSITIZATION MODELS

Model	Availability	Methodology	Results
PredSkin	Free	QSAR	Predicts whether chemical will be a skin sensitizer (Binary results for human prediction)
Toxtree	Free	Structural Alerts	Identifies structural alerts within target chemical
OECD QSAR Toolbox	Free	Read-Across/QSAR	Predicts whether chemical will be a skin sensitizer (Binary results)
Danish QSAR Database	Free	Battery algorithm based on three individual QSAR models[#]	Predicts whether chemical will be a skin sensitizer (Binary results for human prediction)
CAESAR **(VEGA)**	Free	QSAR	Predicts whether chemical will be a skin sensitizer (Binary results)
UL's REACHAcross™	Commercial	QSAR/ Read-Across	Predicts whether chemical will be a skin sensitizer (Binary results)
TIMES-SS	Commercial	QSAR/ Skin Metabolism Simulation	Predicts whether chemical will be a skin sensitizer (Results provided in potency scale: strong sensitizer, weak sensitizer, non-sensitizer)

Toxtree: Structural Alerts

- Advantages:
  - Any chemical can be analyzed for the key features
  - Provides a possible mechanism
- Disadvantages:
  - “Skin sensitization alert” or “Skin sensitization reactivity domain”?
- High amount of false positives
  - -approx. 30 percent of chemicals are Michaels acceptors



Available structure attributes

Alert for Acyl Transfer agent id...	NO
Alert for Michael Acceptor identi...	YES
Alert for SN2 identified.	NO
Alert for SNAr Identified.	NO
Alert for Schiff base formation i...	NO
Kroes TTC decision tree	Substance would not be expect...
Kroes TTC decision tree#explan...	Q1N,Alerts(genotoxic)Y,SA1N,S...
No skin sensitisation alerts ident...	NO
SMILES	Oc1ccc(cc1(OC))CC=C

Toxic Hazard by Skin Sensitisation Alerts

Alert for SNAr Identified.

Alert for Schiff base formation identified.

Alert for Michael Acceptor identified.

Alert for Acyl Transfer agent identified.

Alert for SN2 identified.

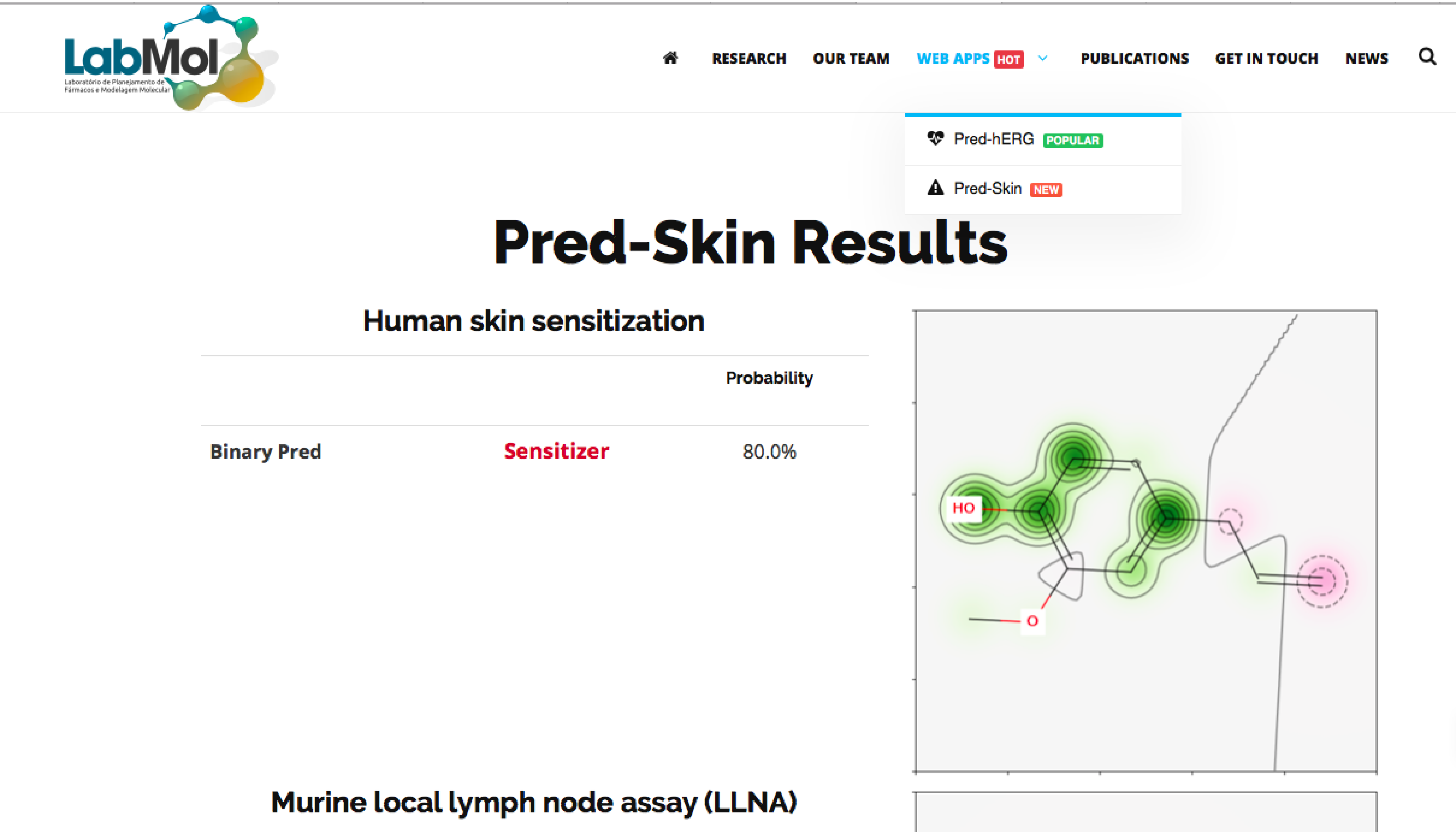
No skin sensitisation alerts identified.

☑ Verbose explanation

Skin Sensitisation Alerts

- QSNAR.SNAr-Nucleophilic Aromatic Substitution No
- QSB.Schiff Base Formation No
- QMA.Michael Acceptor Yes Class Alert for Michael Acceptor identified.
- Qacyl.Acyl Transfer Agents No
- QSN2.SN2-Nucleophilic Aliphatic Substitution No
- Q6.At least one alert for skin sensitisation? Yes

PredSkin



Pred-Skin Results

Human skin sensitization

		Probability
Binary Pred	Sensitizer	80.0%

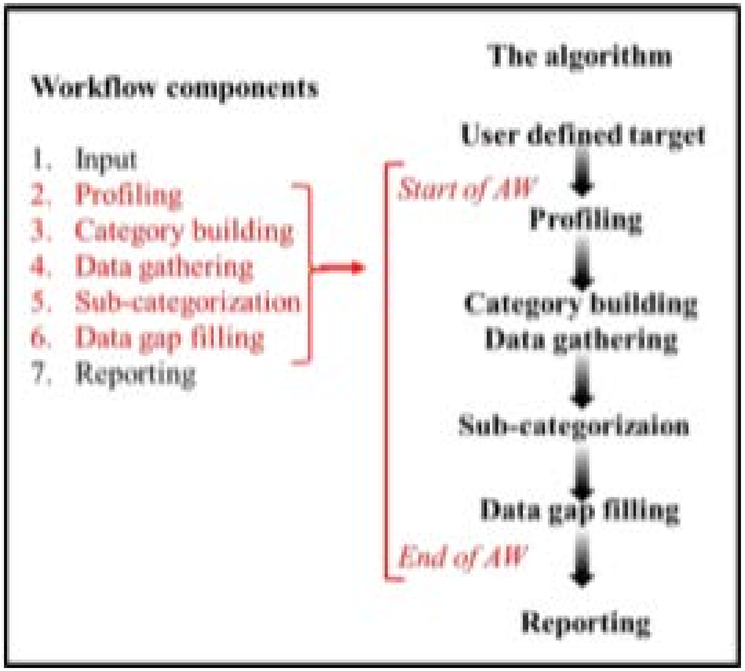
Murine local lymph node assay (LLNA)

PredSkin

- Advantages:
  - Simple to use
  - Based on human data, but also provides prediction for LLNA as well as DPRA
  - Includes probability estimate
  - Provides batch mode
- Disadvantages:
  - Highly sensitive, not as specific

OECD QSAR Toolbox

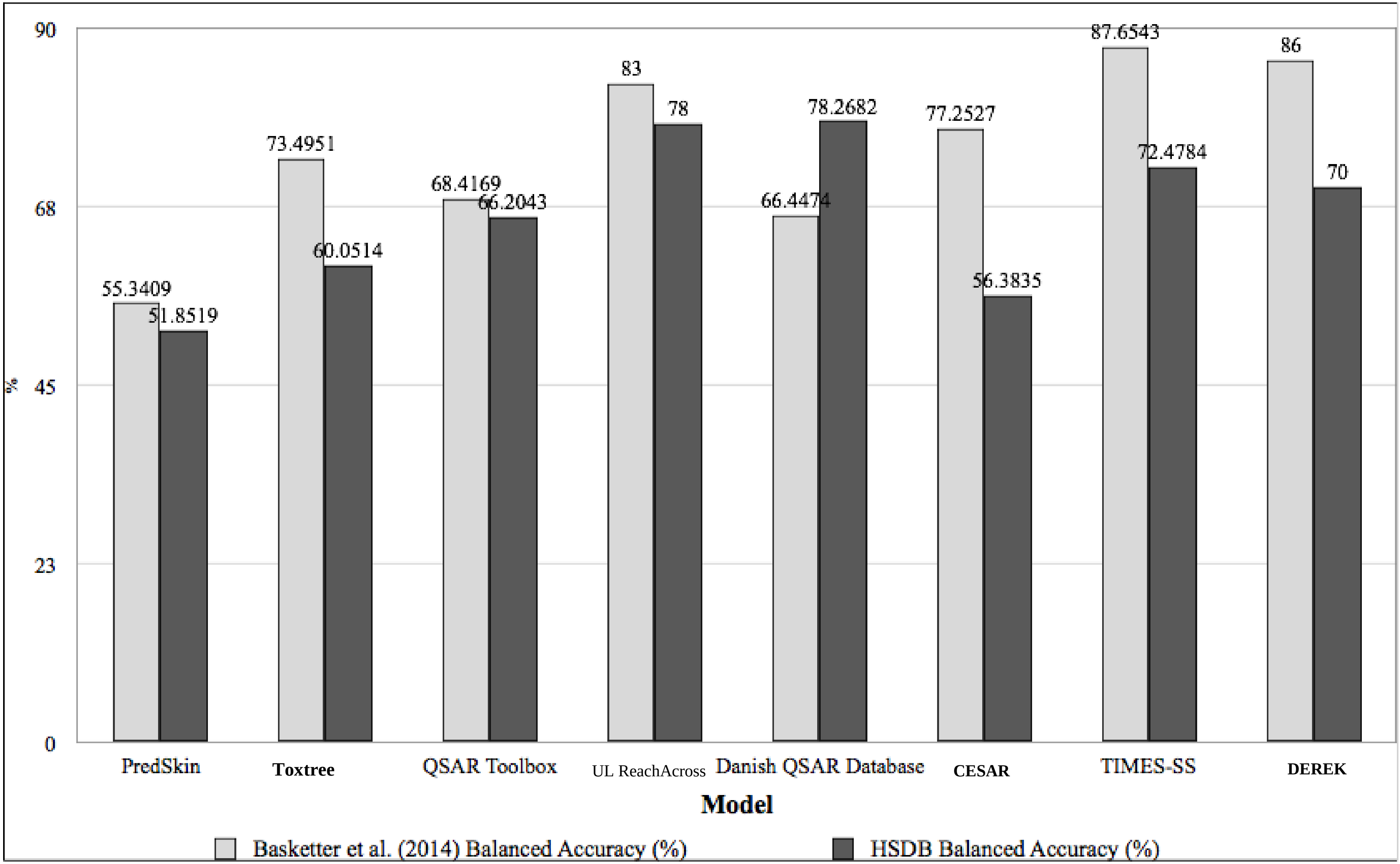
- QSAR Toolbox is very powerful, but there is a learning curve - Windows only
- Includes an automated workflow for data gap filling for skin sensitization
- OECD QSAR Toolbox has a large data set of analogs with LLNA and GPMT data



OECD QSAR Toolbox

- Advantages:
  - Highly accurate if you have similar analogs in the data set and an obvious chemical category
  - Includes potential metabolites
  - Batch mode
- Disadvantages:
  - Does not give a probability estimate
  - Minimal coverage

Model Accuracy

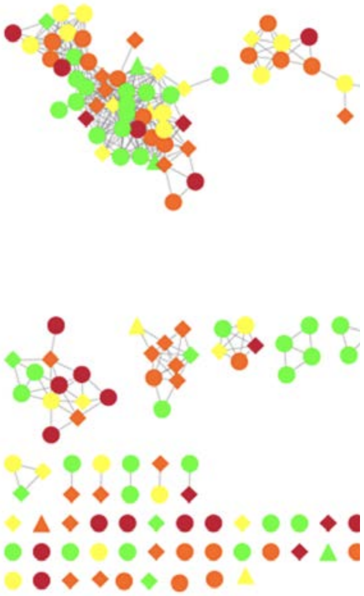


Which model should I use for a hazard assessment?

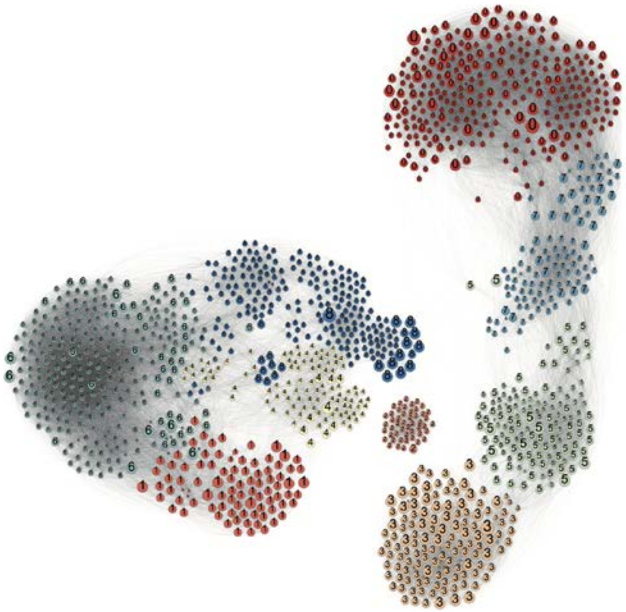
- You need to know about any *potential* alerts for skin sensitization from ToxTree but structural alerts are low information
- PredSkin can alert you to potential instances where human and LLNA data diverge
- If you there are adequate analogs, the OECD QSAR Toolbox will make a highly accurate prediction
- Accuracy is *highly dependent* on how similar your target chemical is to the data set

Future Directions: Big Data

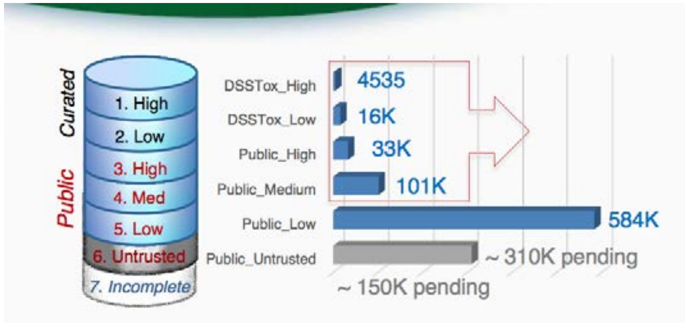
2015



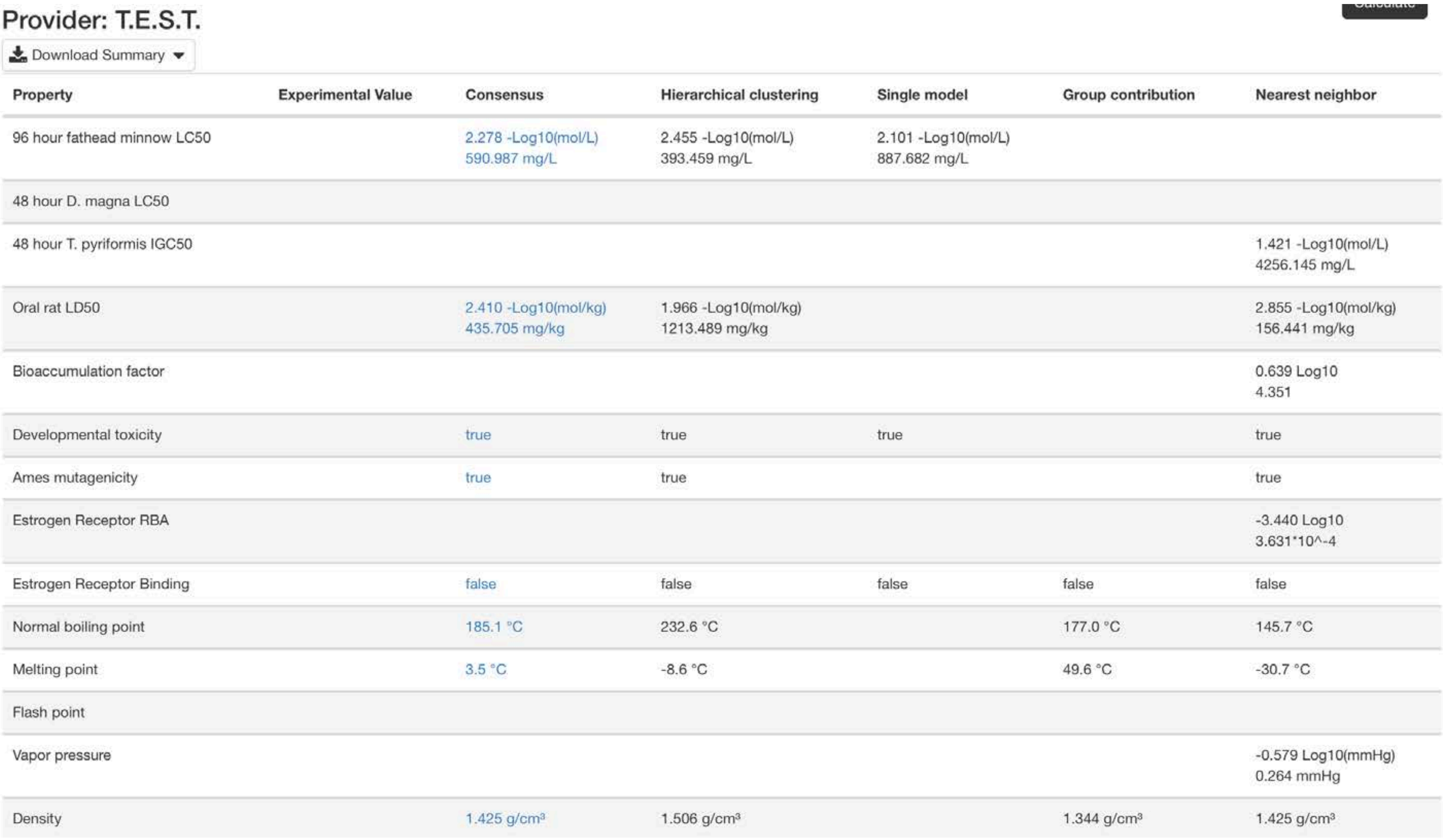
2016



2019



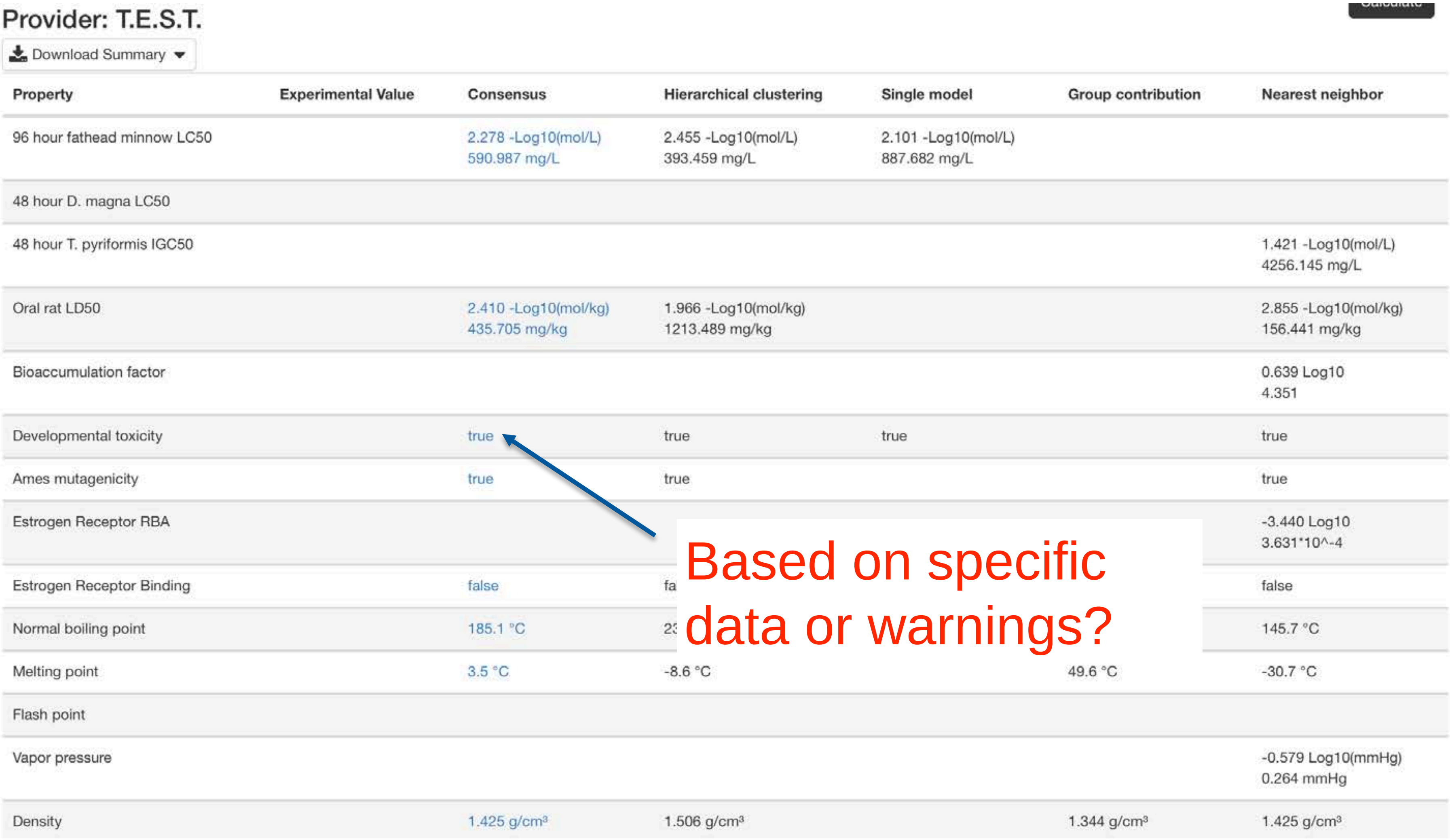
From: Progress in Using Big Data in Chemical Toxicity Research at the National Center for Computational Toxicology
Anthony Williams/EPA



Provider: T.E.S.T.

Property	Experimental Value	Consensus	Hierarchical clustering	Single model	Group contribution	Nearest neighbor
96 hour fathead minnow LC50		2.278 -Log10(mol/L) 590.987 mg/L	2.455 -Log10(mol/L) 393.459 mg/L	2.101 -Log10(mol/L) 887.682 mg/L		
48 hour D. magna LC50						
48 hour T. pyriformis IGC50						1.421 -Log10(mol/L) 4256.145 mg/L
Oral rat LD50		2.410 -Log10(mol/kg) 435.705 mg/kg	1.966 -Log10(mol/kg) 1213.489 mg/kg			2.855 -Log10(mol/kg) 156.441 mg/kg
Bioaccumulation factor						0.639 Log10 4.351
Developmental toxicity		true	true	true		true
Ames mutagenicity		true	true			true
Estrogen Receptor RBA						-3.440 Log10 3.631*10^-4
Estrogen Receptor Binding		false	false	false	false	false
Normal boiling point		185.1 °C	232.6 °C		177.0 °C	145.7 °C
Melting point		3.5 °C	-8.6 °C		49.6 °C	-30.7 °C
Flash point						
Vapor pressure						-0.579 Log10(mmHg) 0.264 mmHg
Density		1.425 g/cm³	1.506 g/cm³		1.344 g/cm³	1.425 g/cm³

Type to enter a caption.



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Density		1.425 g/cm^3	1.506 g/cm^3		1.344 g/cm^3	1.425 g/cm^3

Based on specific data or warnings?

Type to enter a caption.

Provider: T.E.S.T.

Download Summary ▾

Property	Experimental Value	Consensus	Hierarchical clustering	Single model	Group contribution	Nearest neighbor
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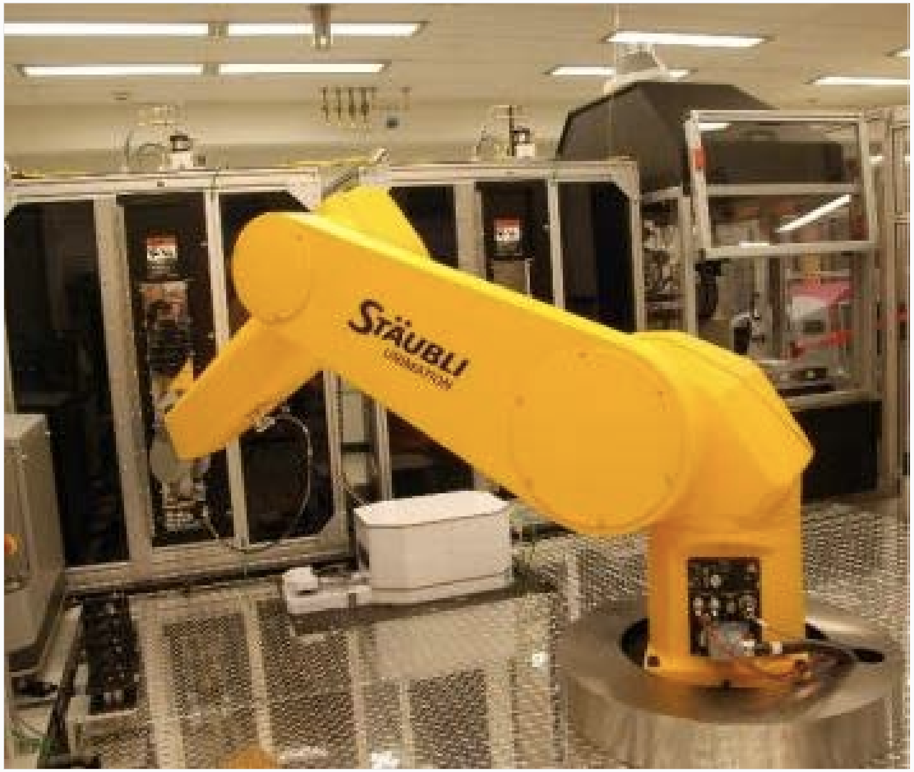
Normal boiling point		185.1 °C	232.6 °C		[illegible]	145.7 °C
Melting point		3.5 °C	-8.6 °C			-30.7 °C
Flash point						
Vapor pressure						-0.579 Log10(mmHg) 0.264 mmHg
Density		1.425 g/cm³	1.506 g/cm³			1.425 g/cm³

Type to enter a caption.

- ToxCast has data on over 1,800 chemicals- including industrial and consumer products, food additives, and potentially "green" chemicals that could be safer alternatives to existing chemicals.

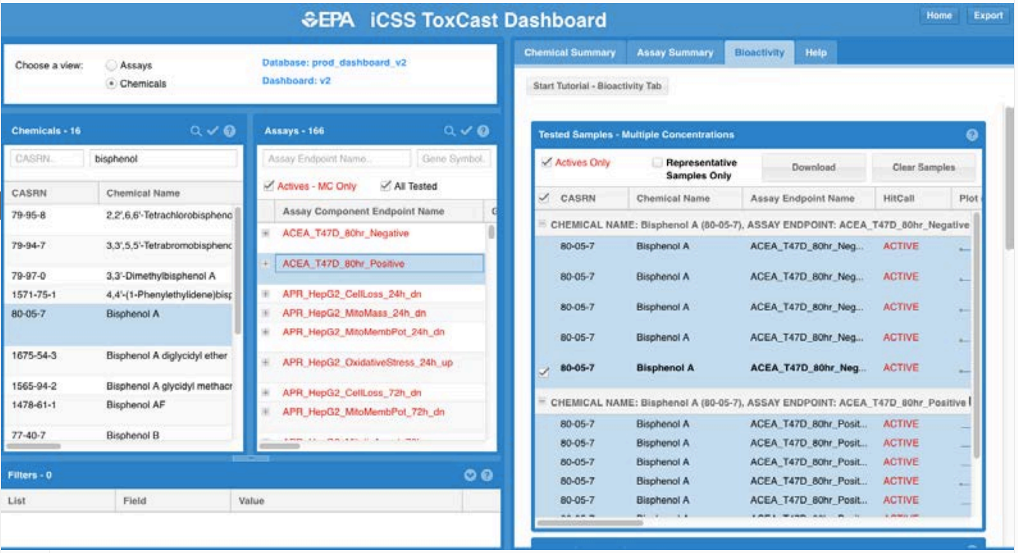
- ToxCast screens chemicals in over 700 high-throughput assays that cover a range of high-level cell responses and approximately 300 signaling pathways.



- Data can be downloaded or accessed through the iCCS ToxCast Dashboard or Pubchem

- Can potentially evaluate mixtures (e.g. environmental contaminants of waste water)

- Used very cautiously in hazard assessments



iCSS ToxCast Dashboard

Choose a view: ○ Assays ● Chemicals

Database: prod_dashboard_v2
Dashboard: v2

Chemicals - 16

CASRN... | bisphenol

CASRN	Chemical Name
79-95-8	2,2',6,6'-Tetrachlorobisphenc
79-94-7	3,3',5,5'-Tetrabromobisphenc
79-97-0	3,3'-Dimethylbisphenol A
1571-75-1	4,4'-(1-Phenylethylidene)bisp
80-05-7	Bisphenol A
1675-54-3	Bisphenol A diglycidyl ether
1565-94-2	Bisphenol A glycidyl methacr
1478-61-1	Bisphenol AF
77-40-7	Bisphenol B

Assays - 166

Assay Endpoint Name... | Gene Symbol...

☑ Actives - MC Only ☑ All Tested

Assay Component Endpoint Name
ACEA_T47D_80hr_Negative
ACEA_T47D_80hr_Positive
APR_HepG2_CellLoss_24h_dn
APR_HepG2_MitoMass_24h_dn
APR_HepG2_MitoMembPot_24h_dn
APR_HepG2_OxidativeStress_24h_up
APR_HepG2_CellLoss_72h_dn
APR_HepG2_MitoMembPot_72h_dn

Filters - 0

List	Field	Value

Tested Samples - Multiple Concentrations

☑ Actives Only □ Representative Samples Only

✓	CASRN	Chemical Name	Assay Endpoint Name	HitCall	Plot
	CHEMICAL NAME: Bisphenol A (80-05-7), ASSAY ENDPOINT: ACEA_T47D_80hr_Negative				
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Neg...	ACTIVE	
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Neg...	ACTIVE	
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Neg...	ACTIVE	
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Neg...	ACTIVE	
✓	**80-05-7**	**Bisphenol A**	**ACEA_T47D_80hr_Neg...**	ACTIVE	
	CHEMICAL NAME: Bisphenol A (80-05-7), ASSAY ENDPOINT: ACEA_T47D_80hr_Positive				
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Posit...	ACTIVE	
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Posit...	ACTIVE	
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Posit...	ACTIVE	
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Posit...	ACTIVE	
	80-05-7	Bisphenol A	ACEA_T47D_80hr_Posit...	ACTIVE	

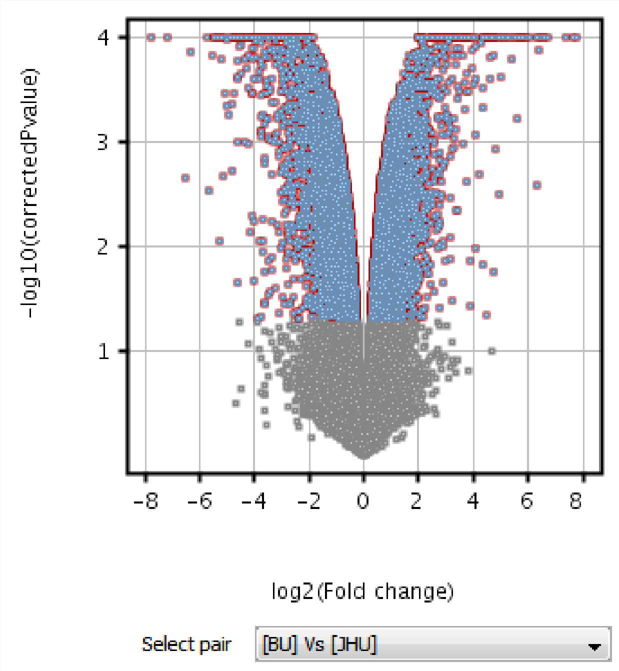
HTS Strengths

- Human Assays
  - Eliminates need for animal to human extrapolation
  - Eliminates need for animal testing
- Bulk chemical testing and analysis

HTS Weaknesses

- Prominent amount of false positives
  - Statistical Validations
  - Certainty of assays
  - Physical Properties - this is extremely important for nanomaterials
- Lacks ADME (Absorption, Distribution, Metabolism, and Excretion)
  - Microsomal S9 Inserts
- Translating HTS to Adverse Outcome Pathway (AOP) problematic with regard to resources needed
- Cytotoxicity – false negatives/false positives

Comparison of MCF-7 in two laboratories



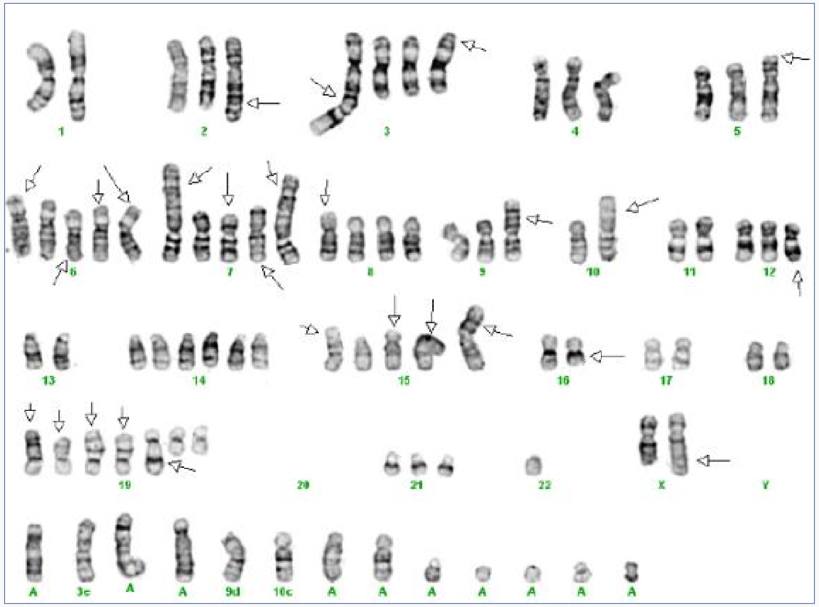
Select pair: [BU] Vs [JHU]

Same batch from ATCC

Method transfer

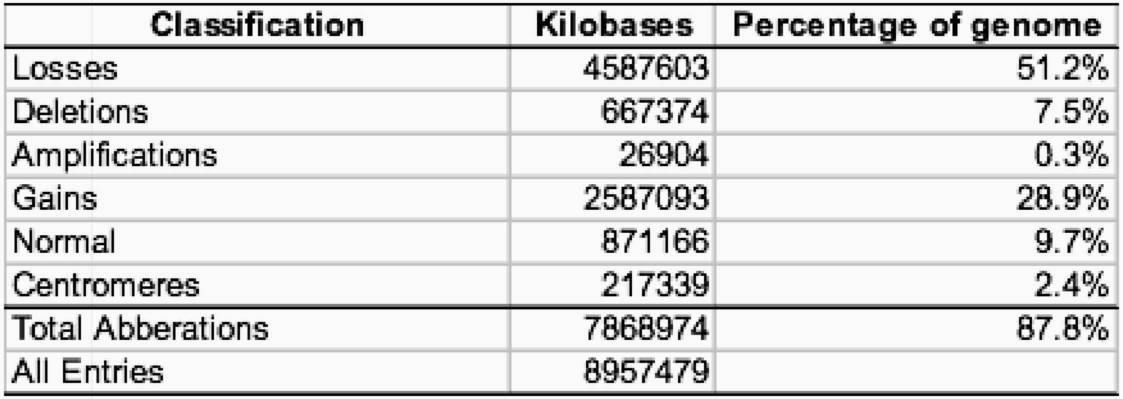
Transcriptomics

negative controls, 4h, gene level, *n* = 3 / group



Karyotyping

Extent of deviations from normal genome



Classification	Kilobases	Percentage of genome
Losses	4587603	51.2%
Deletions	667374	7.5%
Amplifications	26904	0.3%
Gains	2587093	28.9%
Normal	871166	9.7%
Centromeres	217339	2.4%
Total Abberations	7868974	87.8%
All Entries	8957479	

SurePrint G3 ISCA CGH+SNP Microarray Kit, 4x180K

115234 CGH features.2440 CGH replicate probes, 59647 SNP features

reference mapping: caucasian female human reference DNA

Kleensang et al., Nature Sci Rep, 2016

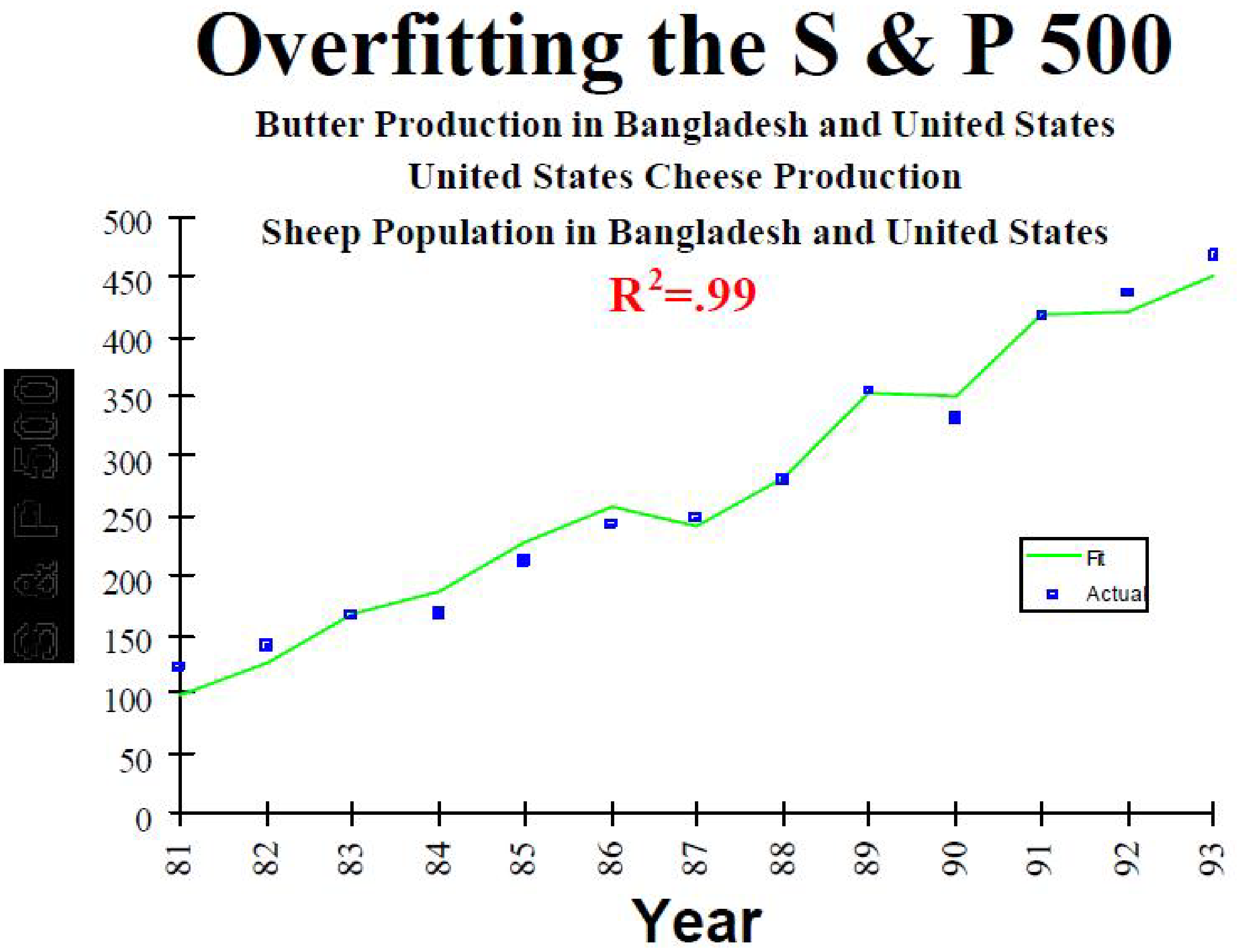
Future Directions

- Machine learning can already predict human skin sensitization with an accuracy similar to testing approaches just based on structural features; further improvement will require incorporating *in vitro* or much larger data sets
- It will be difficult to reproduce this success with other endpoints, although progress is being made
- The “Limiting Reagent” is human data - we can’t test new chemicals on humans for ethical reasons
    - . . . except we do

Future Directions

- We need better surveillance and reporting of human exposure before we can really model and predict human phenotypes
- Existing models may be poorly suited for newer chemicals that are likely to be economically important
  - Biobased chemicals
  - Nanomaterials
- “Big data” based on models are already work well for screening; they will soon be key component of any hazard assessment

More Data Is Not Always Better



Overfitting the S & P 500

Butter Production in Bangladesh and United States

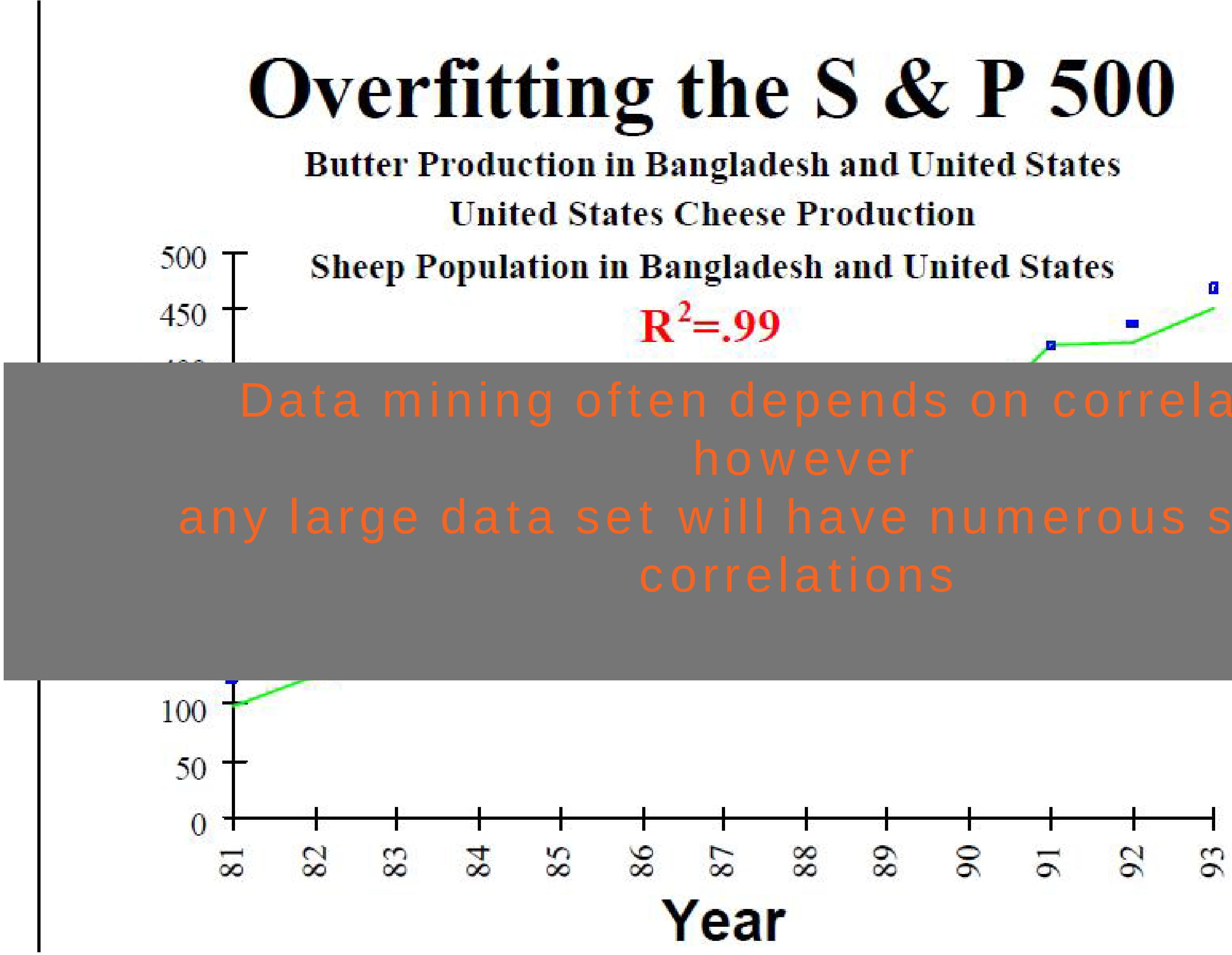
United States Cheese Production

Sheep Population in Bangladesh and United States

R^2=.99

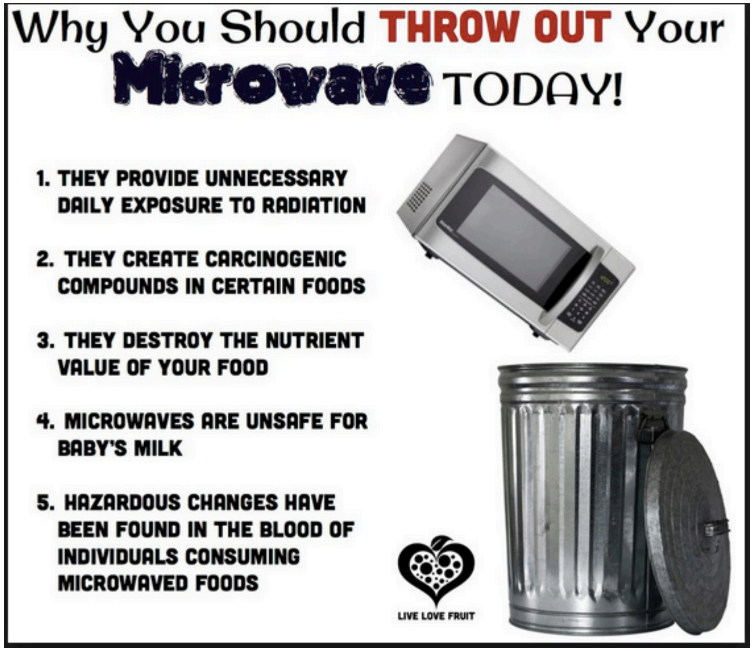
From: Leinweber, David J. Nerds on Wall Street: Math, machines and wired markets. John Wiley and Sons, 2009.

More Data Is Not Always Better



Data mining often depends on correlations however
any large data set will have numerous spurious correlations

From: Leinweber, David J. Nerds on Wall Street: Math, machines and wired markets. John Wiley and Sons, 2009.



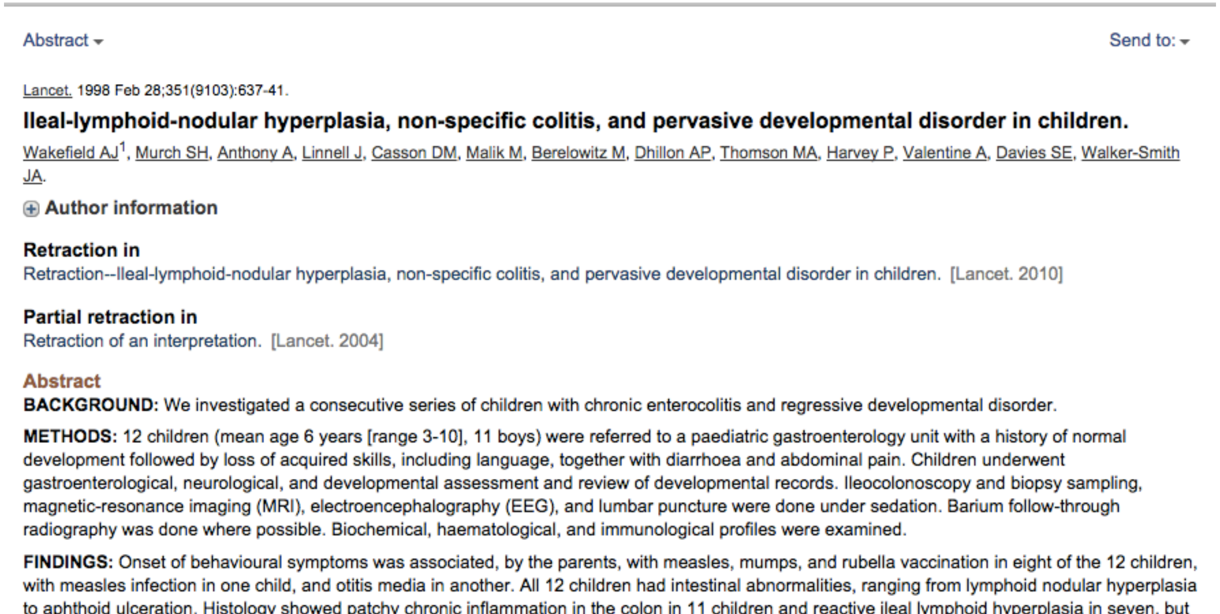
Why You Should THROW OUT Your Microwave TODAY!

1. THEY PROVIDE UNNECESSARY DAILY EXPOSURE TO RADIATION
2. THEY CREATE CARCINOGENIC COMPOUNDS IN CERTAIN FOODS
3. THEY DESTROY THE NUTRIENT VALUE OF YOUR FOOD
4. MICROWAVES ARE UNSAFE FOR BABY'S MILK
5. HAZARDOUS CHANGES HAVE BEEN FOUND IN THE BLOOD OF INDIVIDUALS CONSUMING MICROWAVED FOODS

There are lies. . .

There are damn lies. . .

And then there are. . .



Lancet. 1998 Feb 28;351(9103):637-41.

Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children.

Wakefield AJ[1], Murch SH, Anthony A, Linnell J, Casson DM, Malik M, Berelowitz M, Dhillon AP, Thomson MA, Harvey P, Valentine A, Davies SE, Walker-Smith JA.

⊕ Author information

Retraction in
Retraction--Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. [Lancet. 2010]

Partial retraction in
Retraction of an interpretation. [Lancet. 2004]

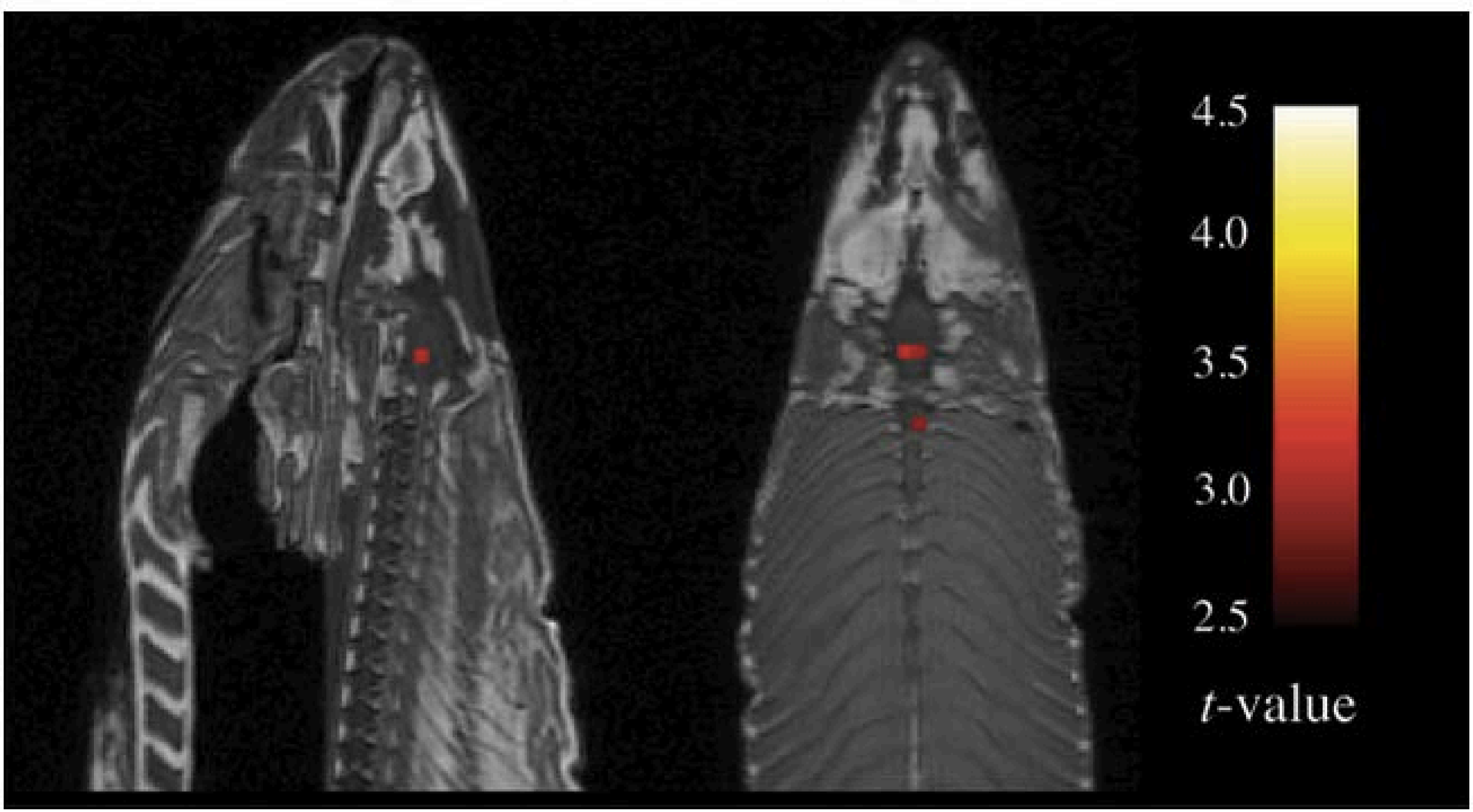
Abstract

BACKGROUND: We investigated a consecutive series of children with chronic enterocolitis and regressive developmental disorder.

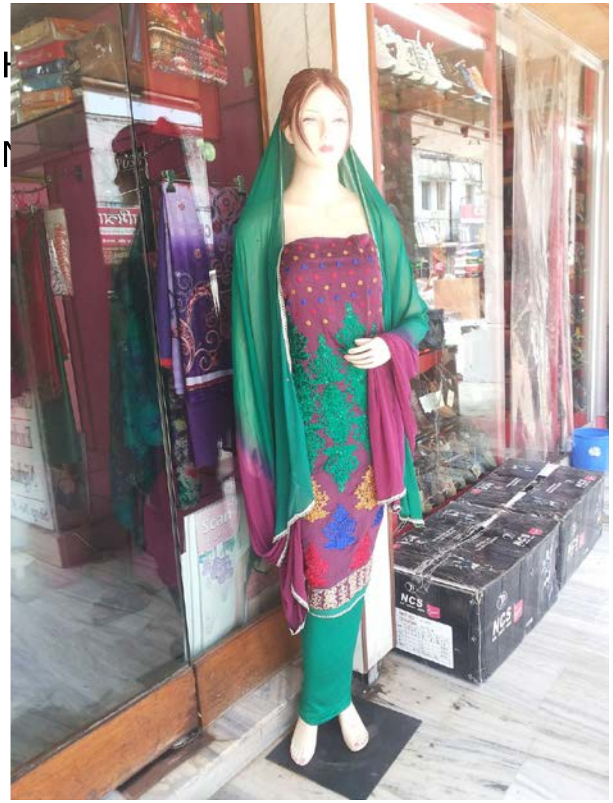
METHODS: 12 children (mean age 6 years [range 3-10], 11 boys) were referred to a paediatric gastroenterology unit with a history of normal development followed by loss of acquired skills, including language, together with diarrhoea and abdominal pain. Children underwent gastroenterological, neurological, and developmental assessment and review of developmental records. Ileocolonoscopy and biopsy sampling, magnetic-resonance imaging (MRI), electroencephalography (EEG), and lumbar puncture were done under sedation. Barium follow-through radiography was done where possible. Biochemical, haematological, and immunological profiles were examined.

FINDINGS: Onset of behavioural symptoms was associated, by the parents, with measles, mumps, and rubella vaccination in eight of the 12 children, with measles infection in one child, and otitis media in another. All 12 children had intestinal abnormalities, ranging from lymphoid nodular hyperplasia to aphthoid ulceration. Histology showed patchy chronic inflammation in the colon in 11 children and reactive ileal lymphoid hyperplasia in seven, but

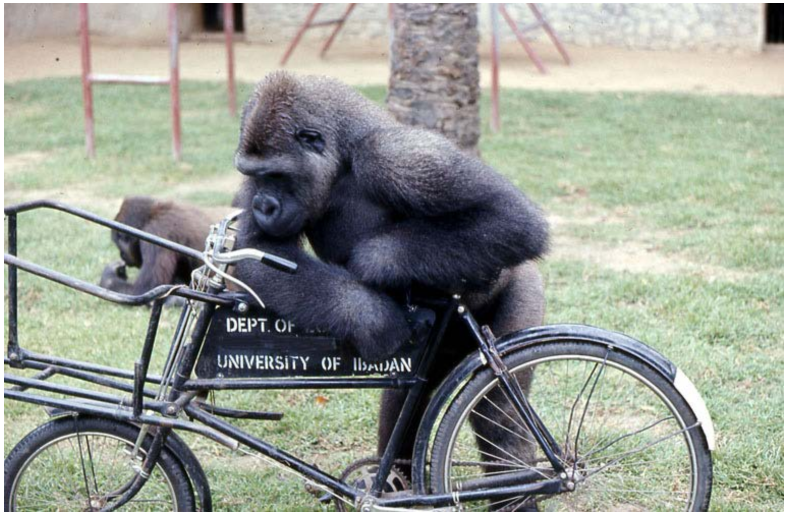
High dimensional data - lies with statistical significance and convinc



Computers perform better than humans, but make *different* mistakes



[x] Human
[ ] Not a human



[x] Human
[ ] Not a human

Thanks! Questions?

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Green Toxicology @ CAAT