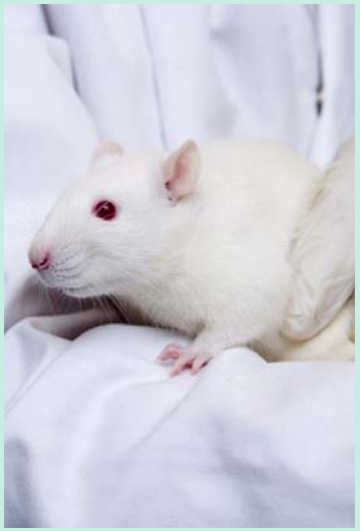
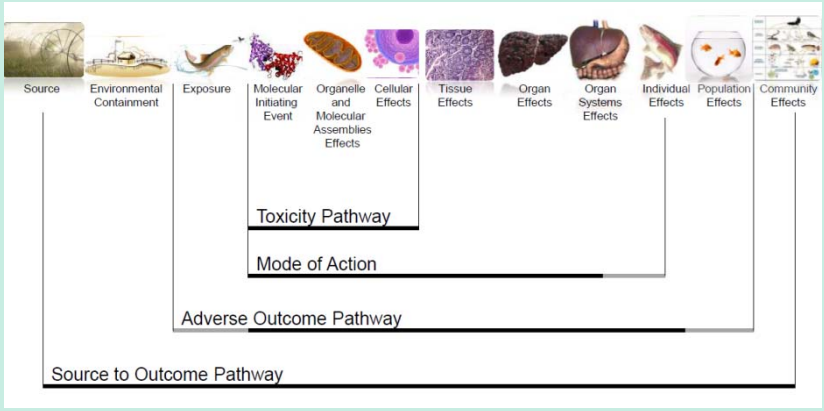
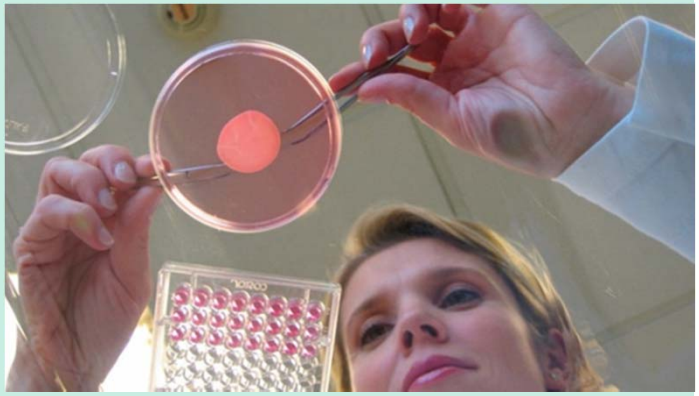






National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

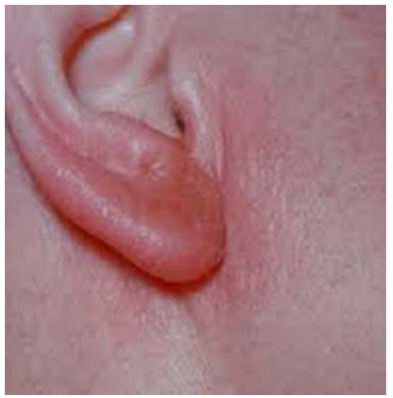
Non-animal testing in the assessment of Skin sensitization

A Sequential Testing Strategy

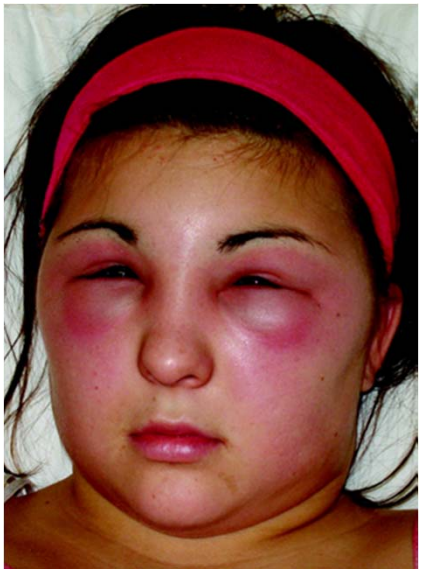
J.van der Veen, Emiel Rorije, R.Emter, A.Natsch, H.van Loveren, Janine Ezendam.

Dutch National Institute for Public Health and the Environment (RIVM)
Bilthoven, The Netherlands

A Sequential Testing Strategy

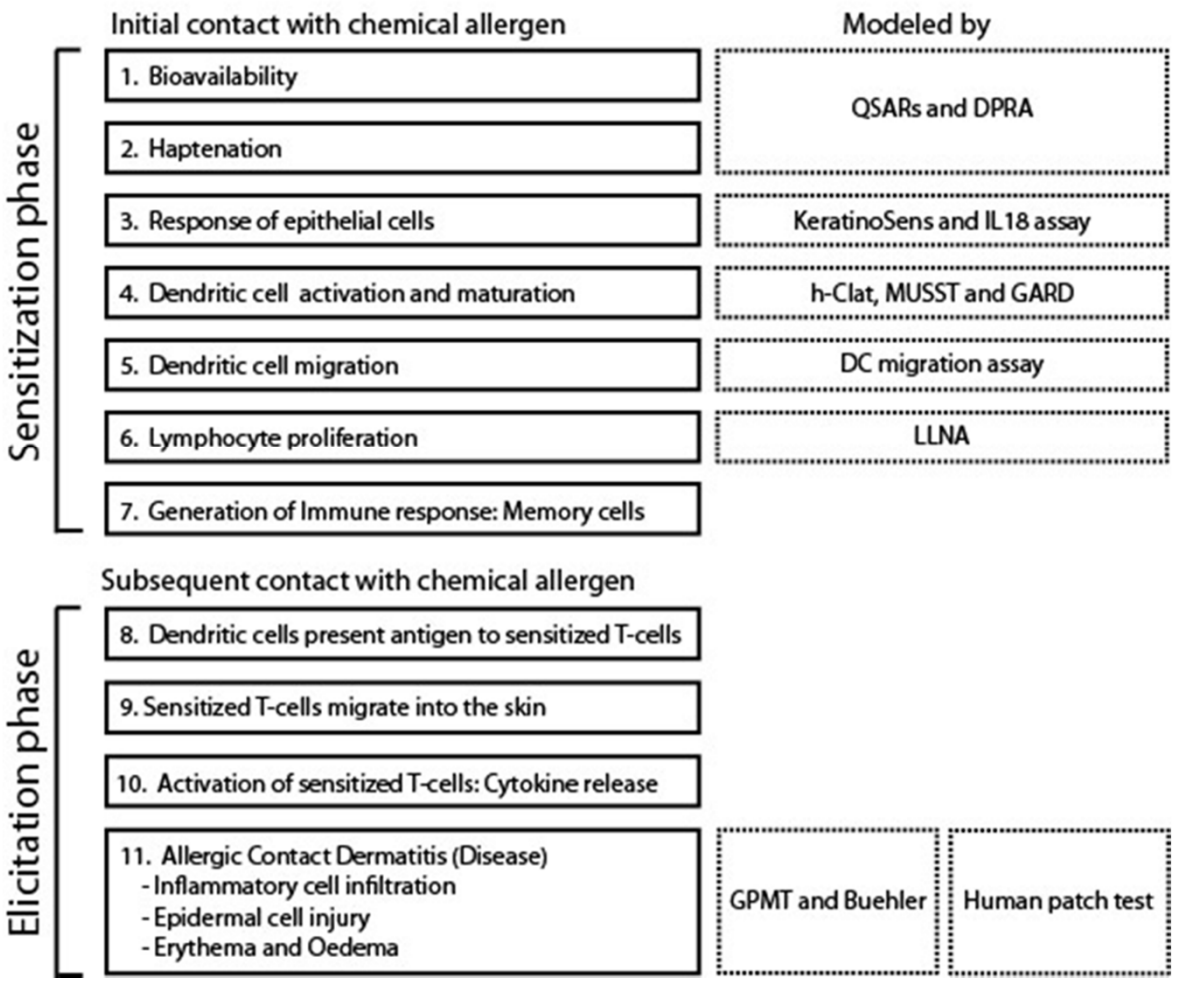


- Using three "tiers" or AOP key events, combining
  - *in silico ((Q)SAR)*
  - *in chemico (peptide reactivity)*
  - *in vitro (keratinocytes, dendritic cells)*
- Stepwise approach
- Decision making involves interim decision points
- Need for additional testing is determined after each step
- Outcome: Sensitizer / No Sensitizer



- Human Hazard identification

Adverse Outcome Pathway - Allergic Contact Dermatitis



RIVM HaCaT gene signature

STS – RIVM approach

- Dataset: 27 skin sensitizers; 14 non-sensitizers
  (incl. 2 LLNA false negatives, 4 LLNA false positives, 10 skin irritants)

- Bayesian QSAR battery (4 models)
- Direct Peptide Reactivity Assay (DPRA)

MIE: peptide binding

- Keratinosens assay
- RIVM keratinocyte gene signature

Key event Events in KC

- H-Clat (Dendritic cells) – published data only → **Key event Events in DC**

- **From simple to complex**

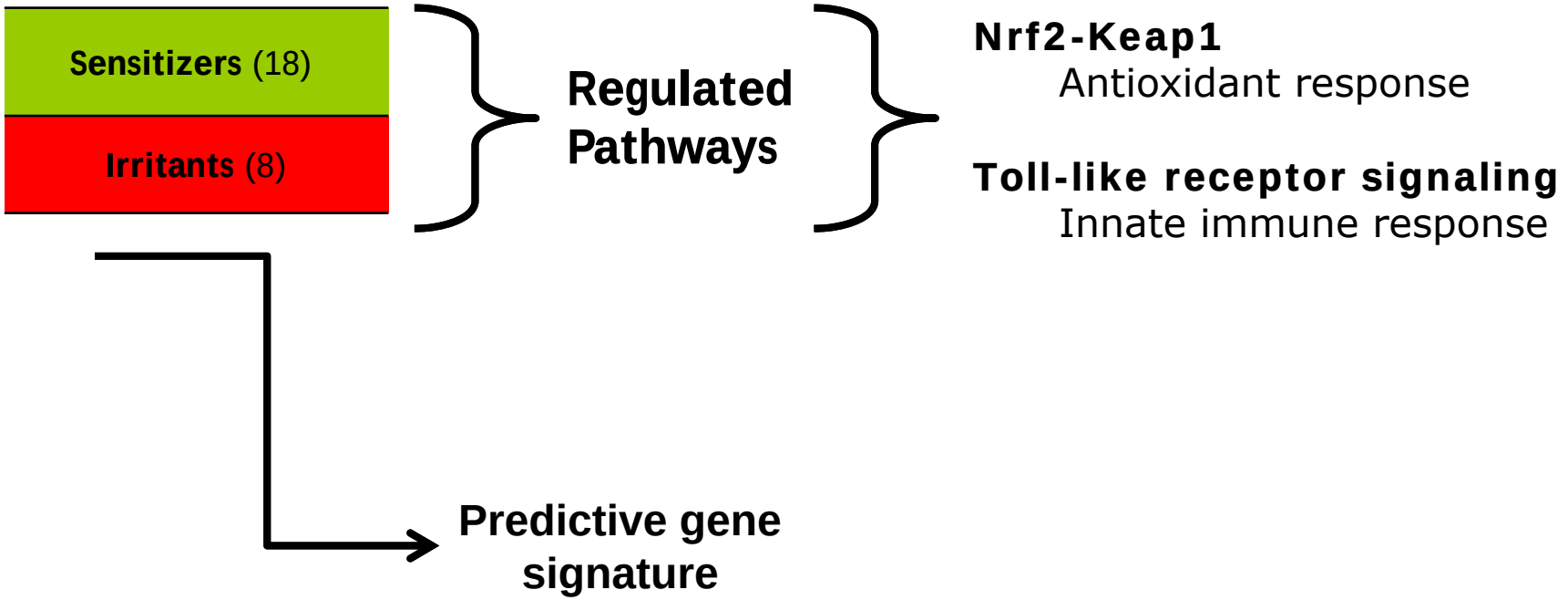
HaCaT gene signature

Metabolism of haptens
conversion to active form

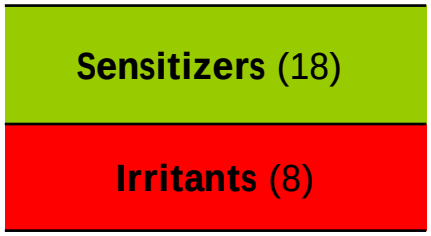
Sensitizers → **Keratinocytes** → **Haptenization**
binding to proteins

Initiation and regulation of immune response
IL-1, TNFα, IL-10, TGF-β, IL-18, IL-8

DNA Microarray in human keratinocytes (HaCaT)



DNA Microarray in human keratinocytes (HaCaT)

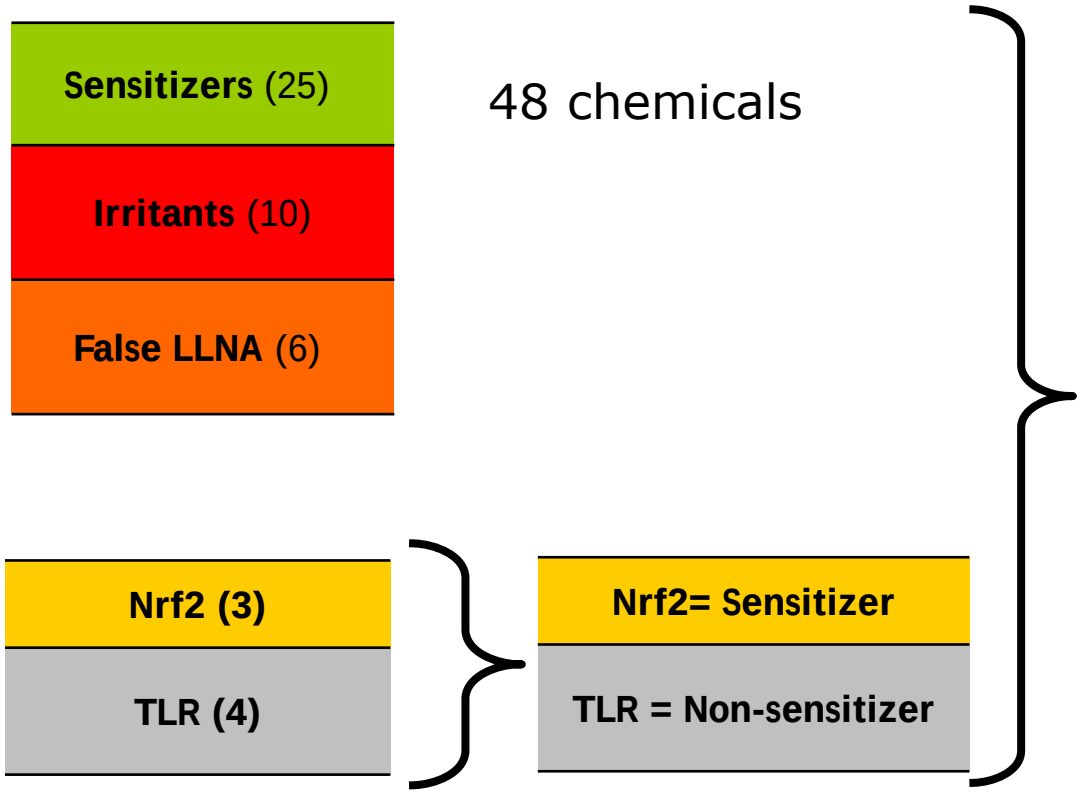


Predictive gene signature

Heme oxygenase 1	**Oxidative stress / Nrf2**
Sulfiredoxin 1	
Stanniocalcin 2	
Adrenomedullin	
FOS	**Stress response & TLR signaling**
FOSL1	
Ankyrin repeat domain 37	Unknown
DNA methyltransferase 3b	DNA folding
RNA binding motif protein 5	Alternative splicing
Cyclin-dependent kinase 12	

Predictivity gene signature

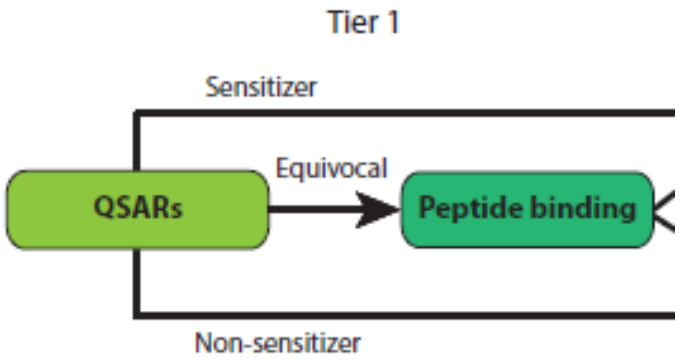
qPCR



	Signature	LLNA
Accuracy	85,4%	85,4%
Specificity	**100%**	88,2%
Sensitivity	71,4%	81,5%

TIER 1: protein binding

- Start with non-testing information
- Bayesian QSAR battery (DEREK, MultiCASE, CAESAR, OECD Toolbox)
- Equivocal results → perform DPRA

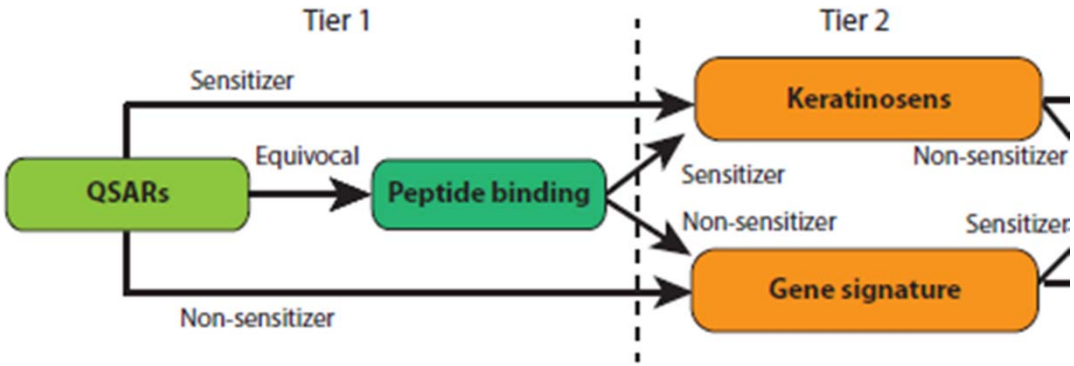


Tier 2: Keratinocyte assays

- KeratinoSens or HaCaT gene signature

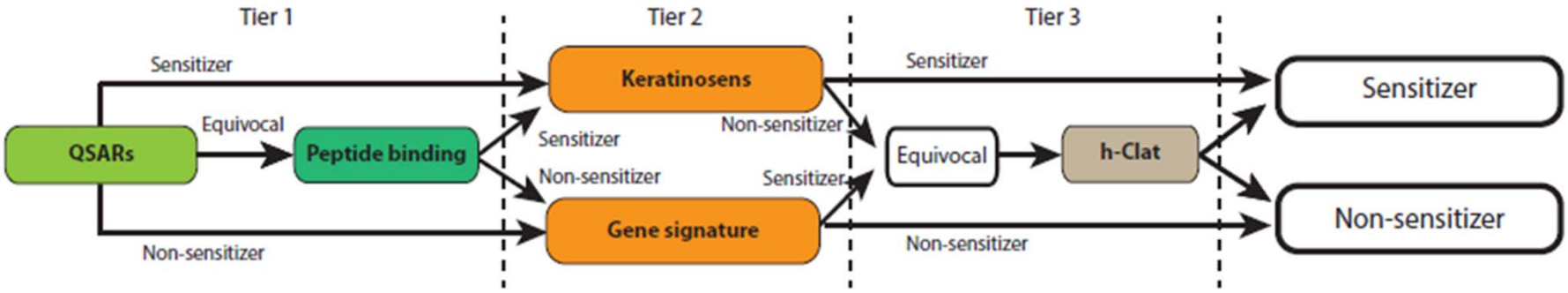
- Test **sensitizers** from tier 1 in assay with **highest PPV**
  - i.e. lowest number of false positive results

- Test **non-sensitizers** from tier 1 in assay with **highest NPV**
  - i.e. lowest number of false negative results

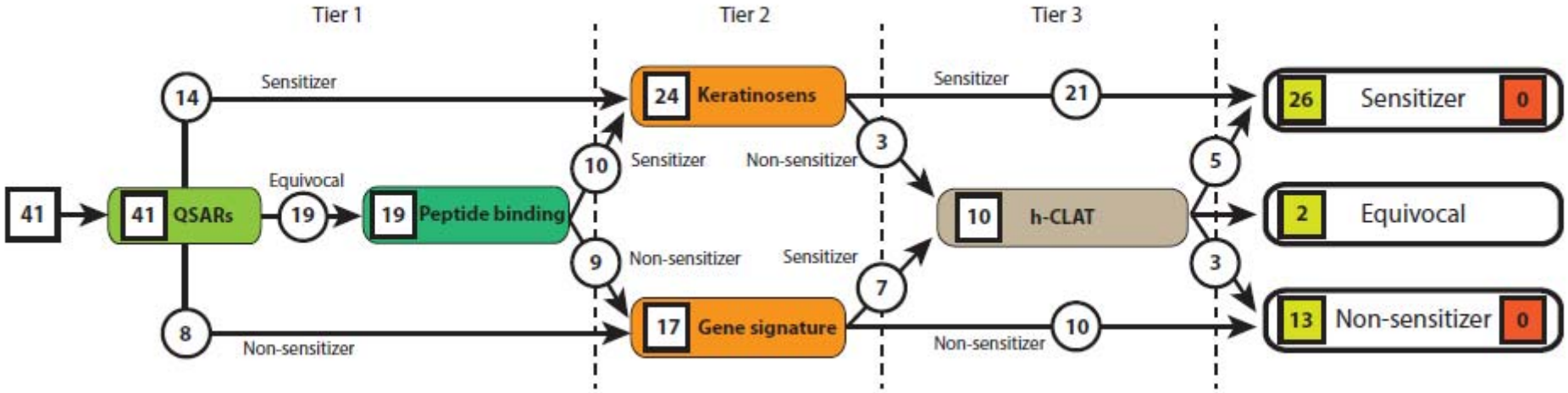


Tier 3: Dendritic cell assay

- Consistent call in tier 2 → decision: sensitizer / non-sensitizer
- Equivocal call → additional information from h-CLAT required
- Decision then based on the h-CLAT outcome (majority voting)



Results


Tier 1
Tier 2
Tier 3
41
41 QSARs
14
Sensitizer
8
Non-sensitizer
Equivocal
19
19 Peptide binding
10
Sensitizer
9
Non-sensitizer
24 Keratinosens
17 Gene signature
Sensitizer
Non-sensitizer
3
7
10 h-CLAT
Sensitizer
21
Non-sensitizer
10
5
3
26 Sensitizer 0
2 Equivocal
13 Non-sensitizer 0

Predictivity

N=41	LLNA	Sequential Test Strategy
Accuracy	82.9%	100% (95.8%)
Sensitivity	92,6%	100% (96.4%)
Specificity	64,3%	100% (95.0%)
No prediction	0	2*

* h-CLAT data was not available for all chemicals

Conclusions and limitations

- RIVM STS provides an accurate prediction model by using the strengths of the individual tests:
  - Only using reliable QSARs data (battery) in tier 1
  - Using the most relevant in vitro assay in tier 2
  - Simple approach, limits (*in vitro*) testing for "known" substances

- Predicitivity based on a limited number of chemicals
  - Including 4 LLNA false positives and 2 LLNA false negatives
  - Will probably decrease for a larger dataset?

- Does not provide potency information!
- Limited metabolic coverage

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Evaluating the performance of integrated approaches for hazard identification of skin sensitizing chemicals

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