# The NociOcular Assay: A Novel Assay Looking at TRPV1 Channel Activity for Eye Sting Predictions Webinar

March 13, 2014 10am

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**Presenters:**

| | |
|---|---|
| Dr. Anna Forsby | University of Stockholm |
| Dr. Neena Tierney | Johnson & Johnson |
| Dr. Kimberly Norman | IIVS |

- Anna, Neena, and Kim will be available for questions at the conclusion of the webinar.
- Answers to questions and copies of the slides will be sent to participants within a week.

# Webinar Tool Bar

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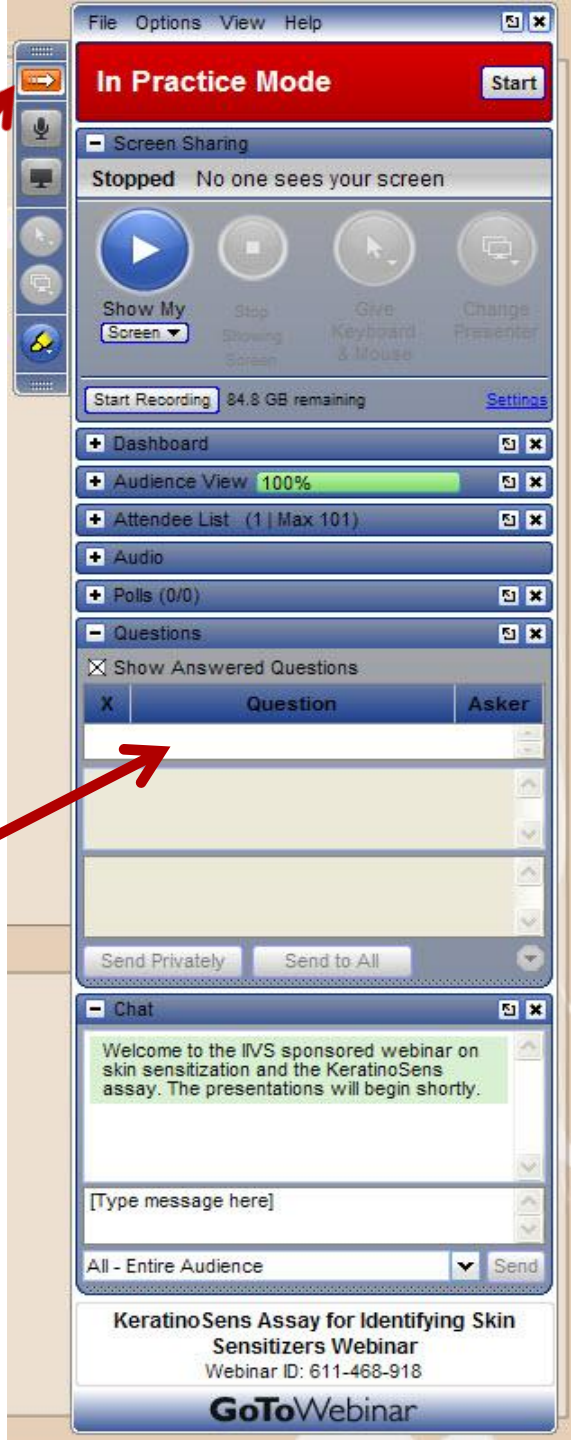
Type your questions here



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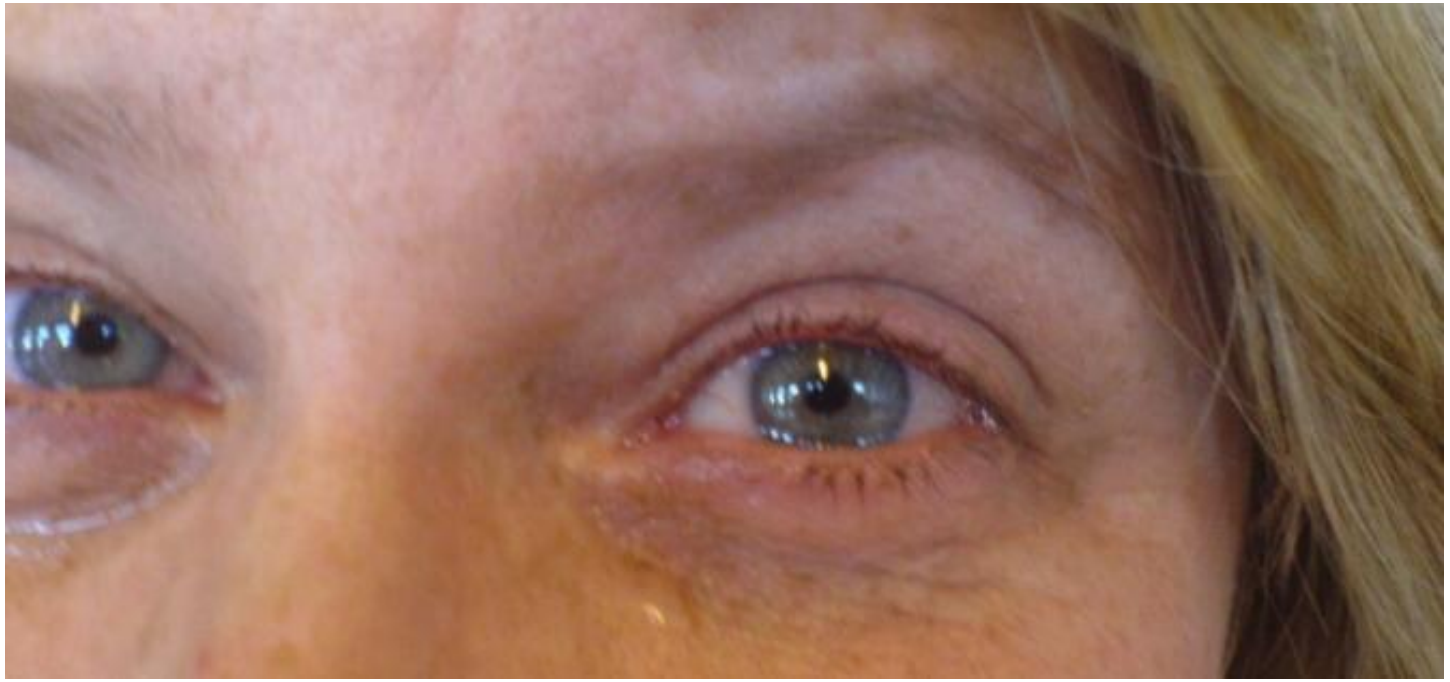
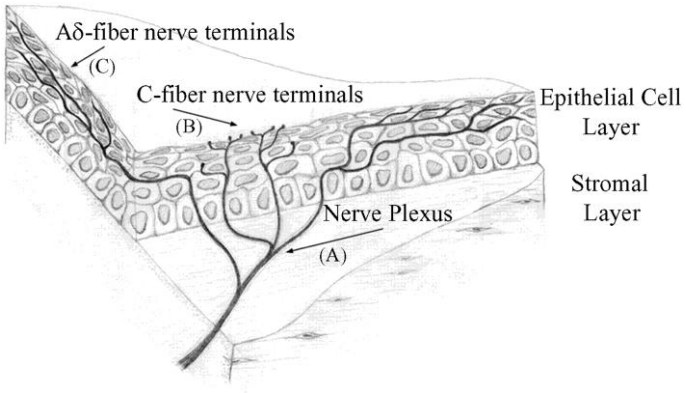
# Why does soap sting in the eyes?

**Anna Forsby, PhD**
*Associate professor, senior scientist*



2014-04-03

# Nociceptors…




Gover, et al. J.Neurosci, 23, 2003

**Are polymodal free ending afferent nerve fibres originating from dorsal root ganglia and trigeminal nerves**

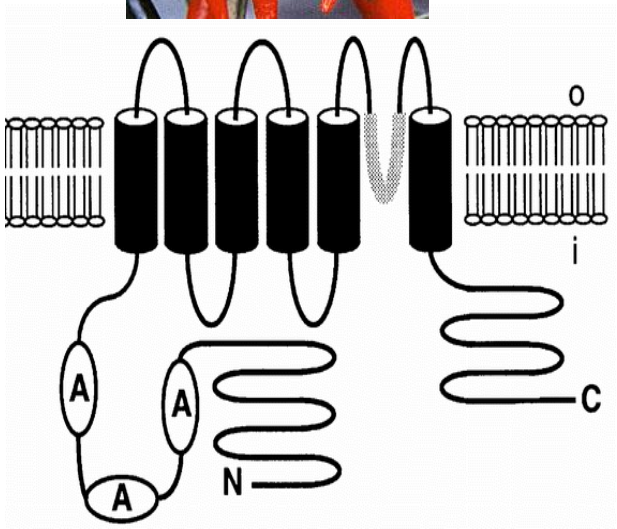
**Express receptors responding to potentially damaging stimuli by inducing pain in order to prevent tissue damage**

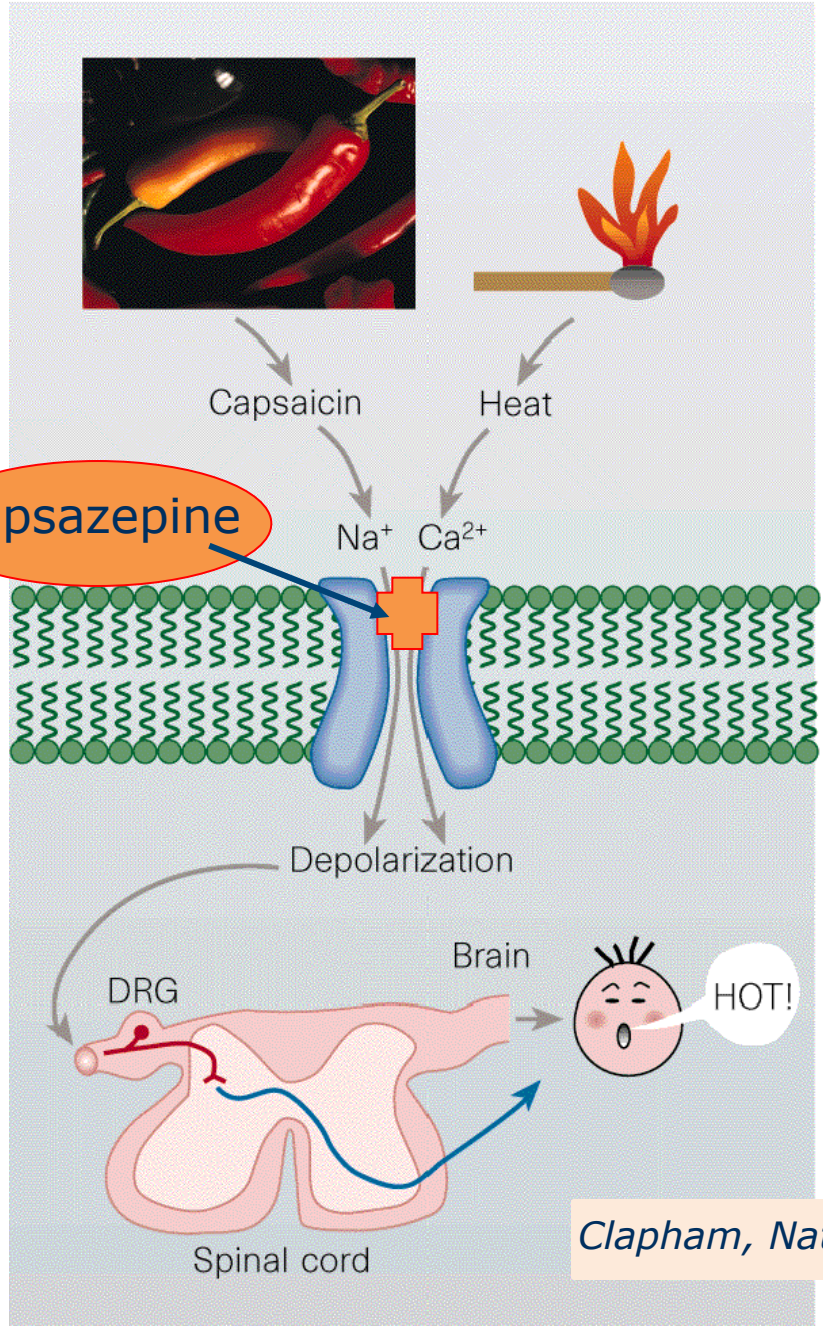
**Promote inflammatory states in the tissue such as pain, swelling and redness**

**Cornea and conjunctiva are innervated by afferent C-fibre neurons originating from the trigeminal nerve**

# Vanilloid Receptor, Transient Receptor Potential (TRPV1)

- The "capsaicin receptor"
- Expressed in polymodal nociceptors; afferent sensory C-fibres originating in dorsal root ganglia and the trigeminal nerve.
- Innervating conjunctiva and cornea.
- Releases $Ca^{2+}$ (and $Na^{+}$) into the cytoplasm when gated by:
  - Capsaicin (and other chemicals)
  - Inflammatory mediators
  - Acids
  - Heat (>43°C)
- Promotes neurogenic inflammation by local release of Substance P and Calcitonin Gene Releasing Peptide.





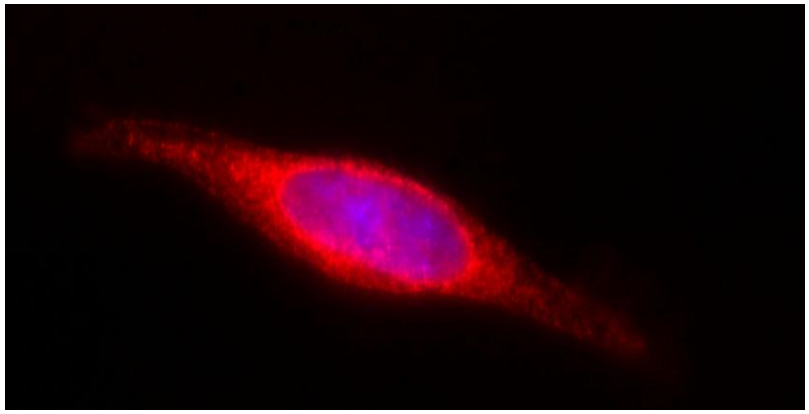

*Clapham, Nature, 389, 1997*

Is activation of the **TRPV1 ion channel** a common mode of action for chemically induced eye nociception?

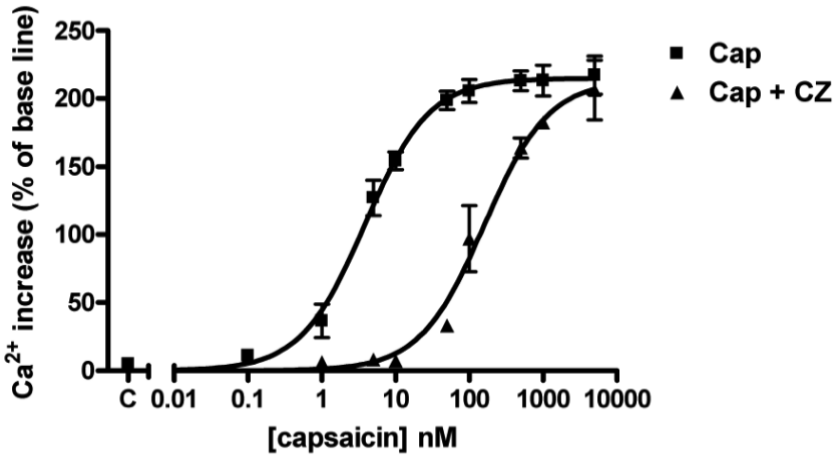
# SH-SY5Y
# Human neuroblastoma cells

# SH-SY5Y/TRPV1 sensory neuron

Stockholm University



Human neuroblastoma cells with stable expression of TRPV1. Anti-rat TRPV1 visualised by goat-anti-rat IgG-conjugated by Alexa fluo red 568. Nucleus stained with Hoechst.




Capsaicin-induced concentration-effect curve of $Ca^{2+}$-transients in TRPV1-SH-SY5Y cells as measured with the $Ca^{2+}$-binding and fluorescent probe Fura-2/AM. (■) Capsaicin-induced $Ca^{2+}$ increase from basal level (♦) 10 µM capsazepine was added to the wells 15 min before capsaicin addition and measurements. Plots represents 3-5 individual experiments and SEM.

# Semi-HTS of intracellular $Ca^{2+}$ measurements

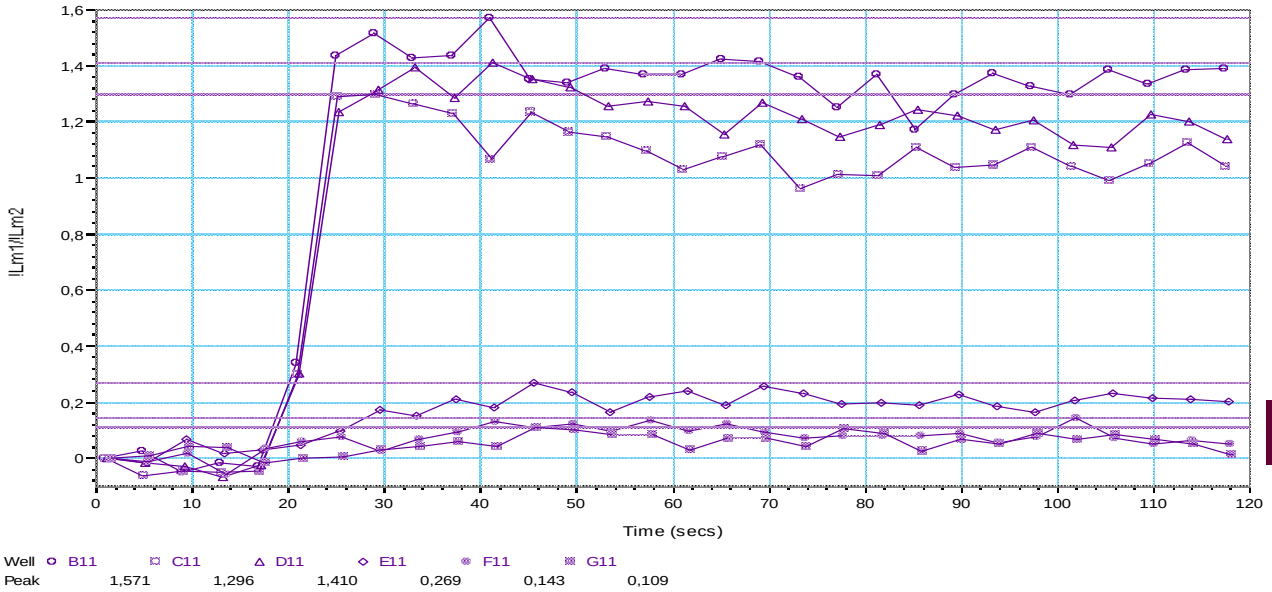
FlexStation II, Molecular Devices

Intracellular free $Ca^{2+}$ concentration by Fura-2 fluorescence

Ratio: Ex 340 nm ($Ca^{2+}$-Fura-2)/ 380 nm (free Fura-2)

Em: 510 nm

# Intracellular free $Ca^{2+}$ concentration by Fura-2 fluorescense

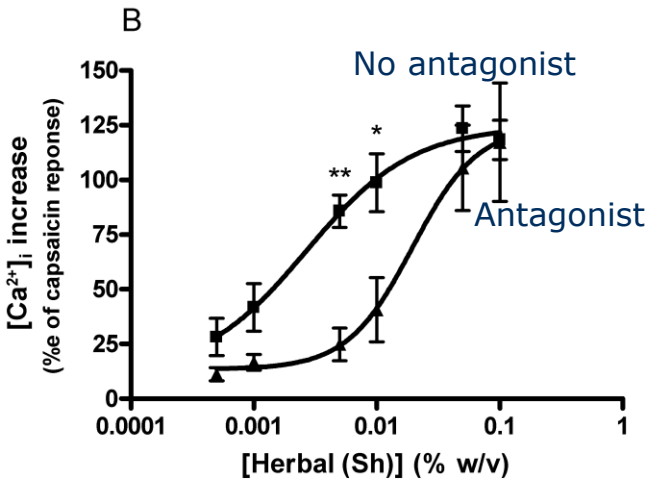


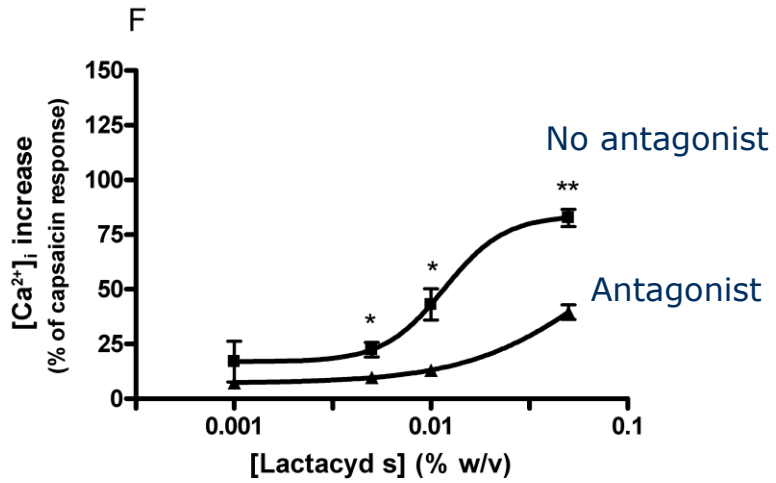

**Agonist**

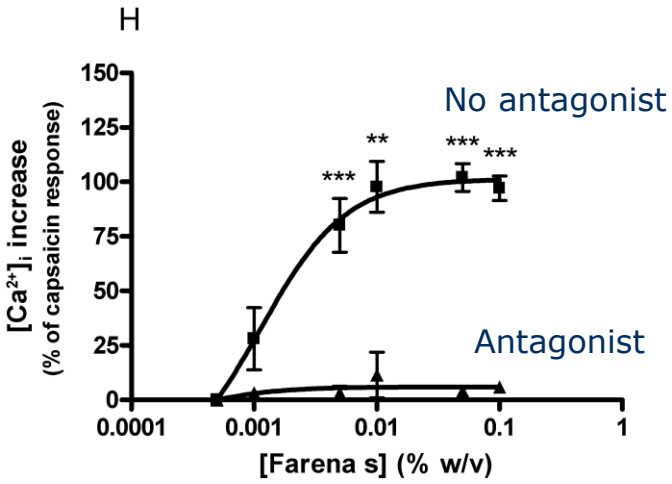
**Agonist + Antagonist**

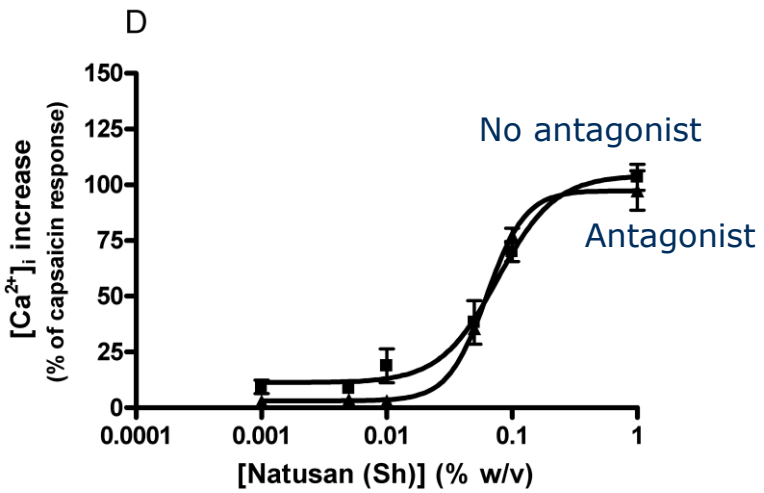
Ratio: Ex 340 nm ($^{Ca^{2+}\text{-bound}}$Fura-2)/ 380 nm ($^{free}$Fura-2), Em 510

# Selected hygiene detergents

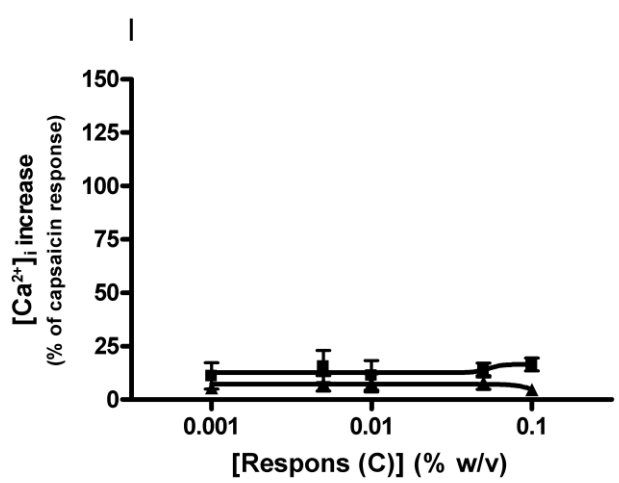


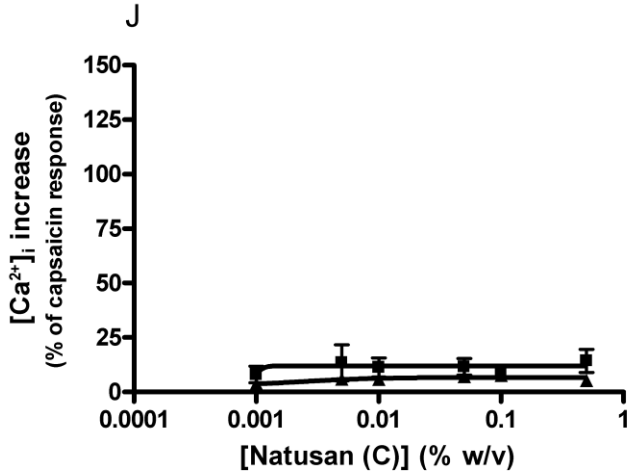



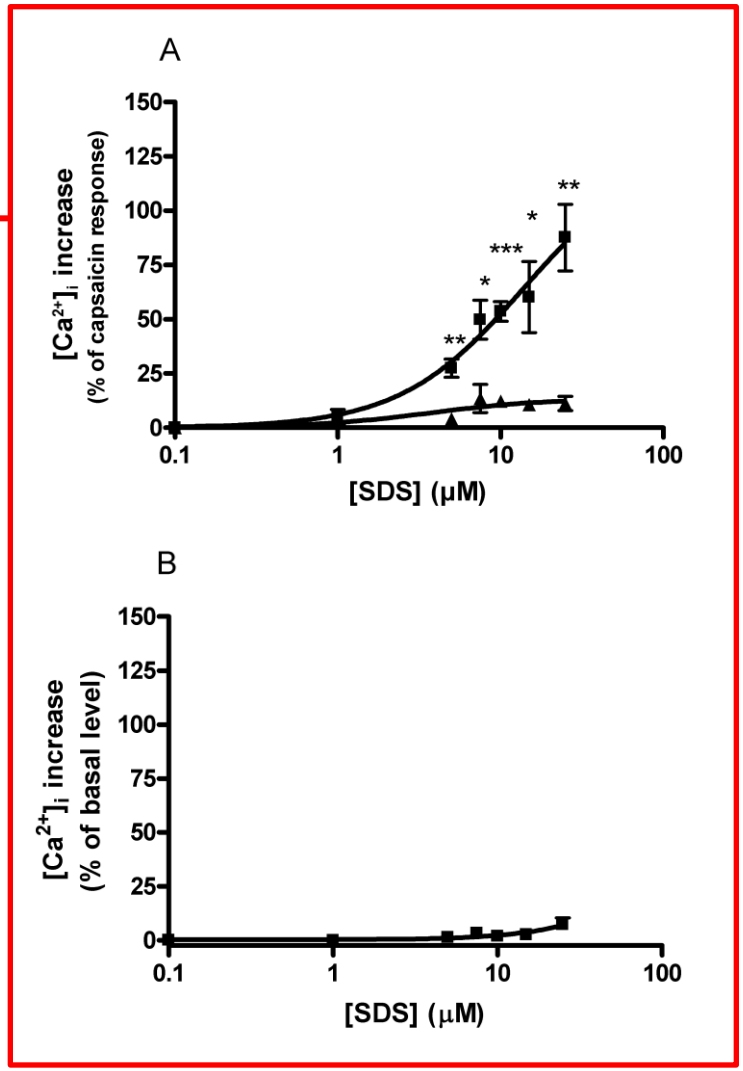
# Hair conditioner



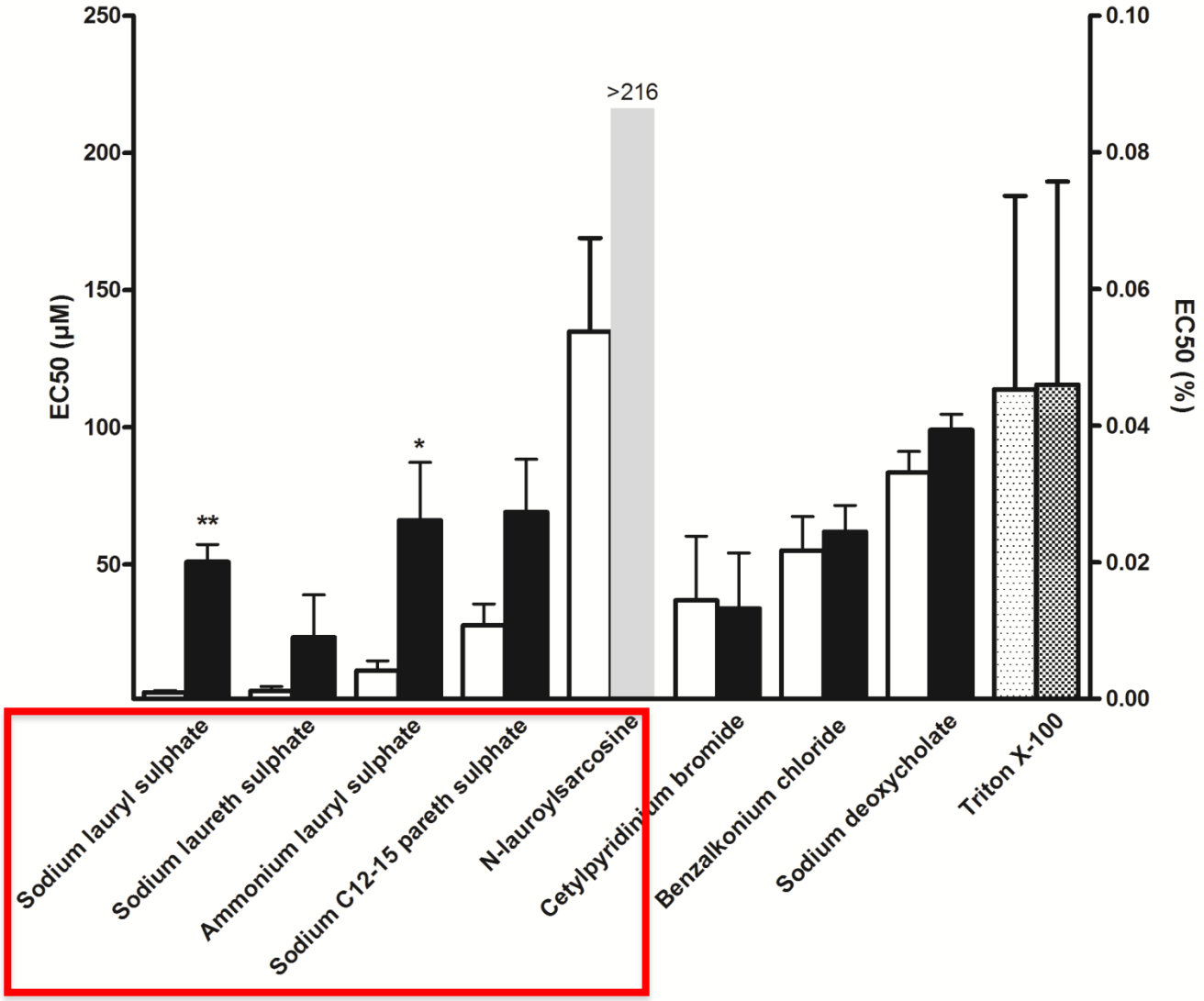
# What compound in shampoo activates TRPV1?

- Water
- Sodium lauryl sulfate (SLS)
- Sodium laureth sulfate (SLES)
- Ammonium lauryl sulfate
- Cocamidopropyl betaine
- Citric acid
- Other…



# $EC_{50}$-values of compounds

Stockholm University



| Compound | Ionic properties | TRPV1 agonist |
|---|---|---|
| Sodium lauryl sulphate | Anionic, linear aliphatic sulphate | Yes |
| Sodium laureth sulphate | Anionic, linear aliphatic sulphate | Yes |
| Ammonium lauryl sulphate | Anionic, linear aliphatic sulphate | Yes |
| Sodium C12-15 pareth sulphate | Anionic, linear aliphatic sulphate | Yes |
| N-lauroylsarcosine (sodium salt) | Anionic, linear aliphatic | Yes |
| Sodium deoxycholate | Anionic | No |
| Sodium taurocholate | Anionic | No |
| Cocamidoproyl betaine | Zwitterionic | No |
| Benzalkonium chloride | Cationic | No |
| Cetylpyridinium bromide | Cationic | No |
| Triton X-100 | Non-ionic | No |
| Tween 20 | Non-ionic | No |

# NociOcular Study Background And Test Products

**Neena K Tierney, PhD**

**Associate Director, Fellow**
**Medical & Clinical Affairs**

Johnson & Johnson
CONSUMER & PERSONAL PRODUCTS WORLDWIDE
Division of Johnson & Johnson Consumer Companies, Inc.

# Safety Assessment of Personal Care Products

- As part of the safety assessment of products that are designed to be used in or around the eyes, such as cosmetics, facial moisturizers, and sunscreens, the evaluation of ocular irritation potential is of primary importance.

- For baby personal care products such as shampoo or bath products, where the product can inadvertently come into contact with the baby's eyes, testing for ocular irritation, including erythema, lacrimation, and stinging, is conducted to ensure the absence of irritation and pain associated with their use.

# Background/Rationale

❖ The TRPV1 channel is a well characterized pain–inducing receptor that is expressed in sensory nociceptors which can be activated by chemical stimuli. Corneal and mucosal tissue in conjunctiva are rich in innervations which express TRPV1 channels

❖ Our hypothesis is that TRPV1 may be a general mediator of chemically induced pain on the surface of the eye

❖ No study had demonstrated the ability of *in vitro* assay to predict the human sting potential of personal care products which may come in contact with the eyes

❖ An *in vitro* assay capable of identifying the eye sting potential of personal care products would be very beneficial as a pre-clinical screening tool. Also, since *in vitro testing can be* more readily conducted, it can be used as a tool to advance the understanding of the relative contributions to ocular sting of various ingredients within personal care products.

# Test Products

❖ We sought to test our hypothesis by evaluating the eye sting (pain) potential of 19 baby shampoos which had been previously evaluated in human clinical eye sting tests

❖ Baby shampoo and bath test products were formulated with standard surfactants, conditioning agents, thickening agents including polymers, preservatives, fragrances, pH adjusters, and in some cases other skin benefit agents.

❖ It is well understood that for certain combinations and at high levels, some surfactants, conditioning agents, preservatives, and fragrances can result in ocular sting or pain. Example ingredients within these test products included sodium laureth sulfate, trideceth sulfate, cocamidopropyl betaine, sodium lauroamphoacetate, cocoglucoside, polyquaternium-10, PEG 80 sorbitan laurate, sodium benzoate, quaternium-15, and phenoxyethanol.

# NociOcular Study Results and Next Steps

*Kimberly Norman, Ph.D., DABT*

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Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

# Eye Irritation Assays

- Mild eye irritation may be assessed by sensitive *in vitro* assays including:
  - *Neutral red uptake assay (NRU)*
  - *Cytosensor microphysiometer assay*
  - *Transepithelial permeability assay (TEP)*
  - *EpiOcular ™ assay*
- None of these assays have been shown effective as sensitive biomarkers for stinging sensation
- 12 baby formulations evaluated in these assays and human clinical sting test


# Eye Irritation Assays

| Product | Human Ocular Instillation Test for Sting (Yes/No) | EpiOcular $ET_{50}$ (hours) | Cytosensor $MRD_{50}$ (mg/mL) | NRU $NRU_{50}$ (µg/mL) | TEP $EC_{50}$ (%) |
|---|---|---|---|---|---|
| **A1** | **Yes** | 7.8 | 3.99 | 103 | 2.29 ± 1.35 |
| A2 | No | 11.6 | 2.36 | 29.8 | 3.96 ± 0.053 |
| A3 | No | 22.1 | 3.72 | 106 | NA |
| **A4*(+)** | **Yes** | <1 | 0.519 | 16.2 | NA |
| A5 | No | 11.4 | 2.05 | 41.6 | 4.31 ± 0.59 |
| A6 | No | 3.0 | 1.31 | 53.0 | NA |
| A8 | No | 9.6 | 2.17 | 45.2 | NA |
| A12 | No | 9.0 | 2.88 | 44.3 | NA |
| **A13** | **Yes** | 18.6 | 5.19 | 195 | 6.17 ± 0.59 |
| A18 | No | 12.0 | 2.84 | 132 | 3.47 ± 0.81 |
| **A19** | **Yes** | 3.3 | 1.62 | 55.1 | 3.11 ± 0.38 |
| A20[1](-) | No | 8.3 | 2.87 | 80.3 | 4.19 ± 1.25 |

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# Eye Irritation Assays

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# Study Design

- 20 coded samples (19 baby bath products and one adult shampoo) were supplied to Stockholm University for NociOcular testing
- All (except adult shampoo) had been clinically tested for sting
- Coded results sent to IIVS for decoding and comparison to clinical results

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# Assay Set-up

- The TRPV1 SH-SY5Y cells were seeded in 96-well plates and cultured to confluency.
- Acute increases in the intracellular free $Ca^{2+}$ level was measured in a fluorescence reader before and after addition of sample .
- The TRPV1 antagonist capsazepine was added to each concentration of the sample to confirm TRPV1-mediated $Ca^{2+}$ influx.

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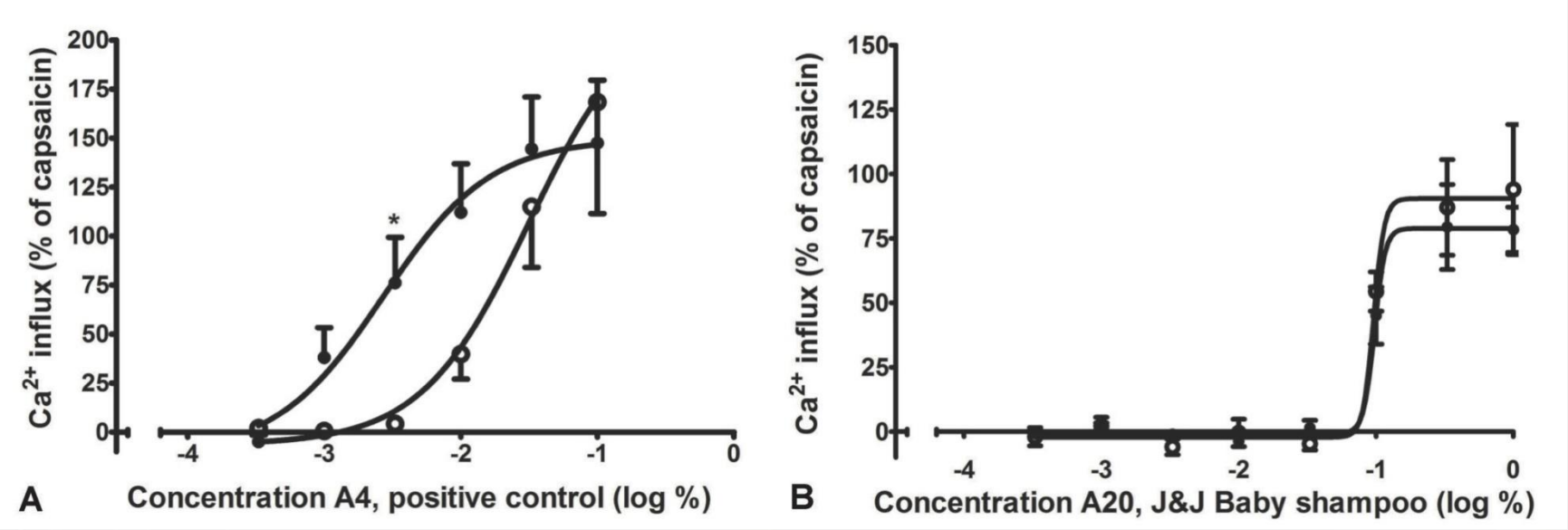
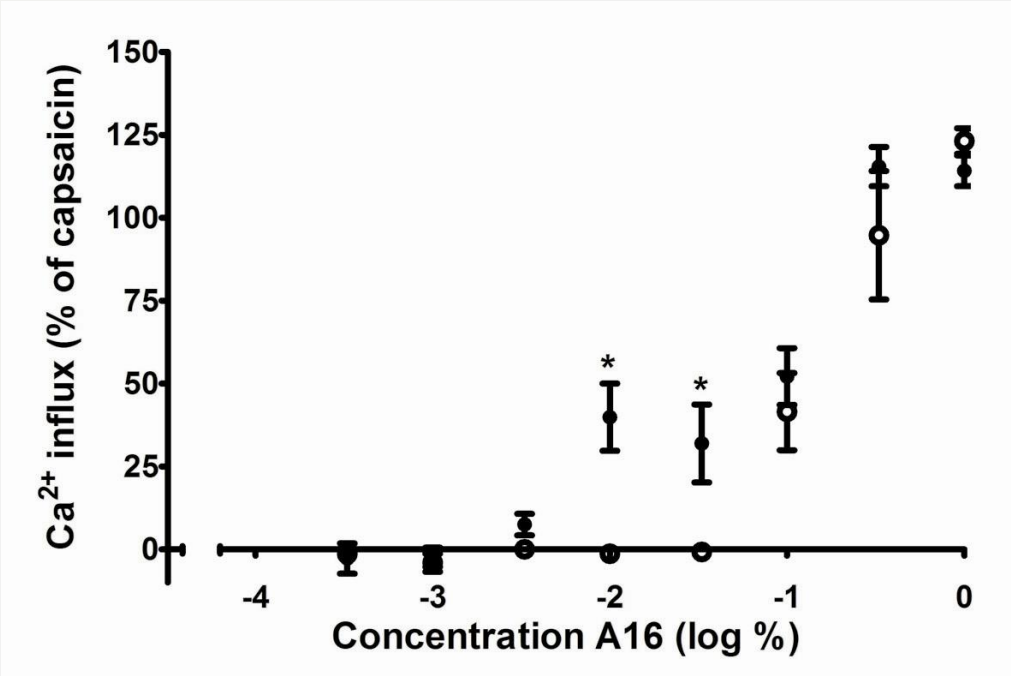
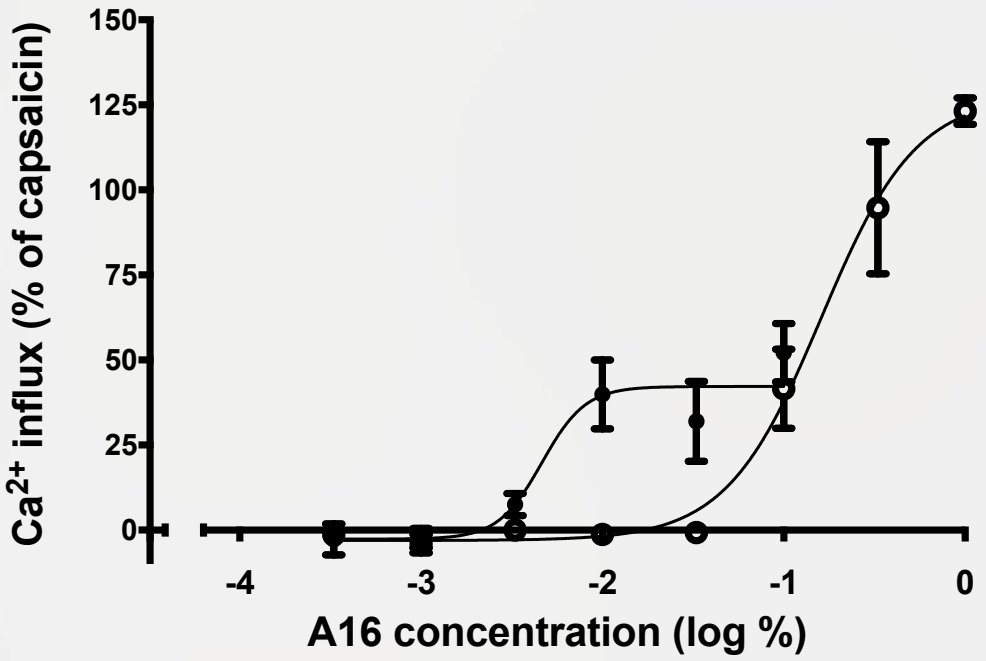
# Positive and negative controls



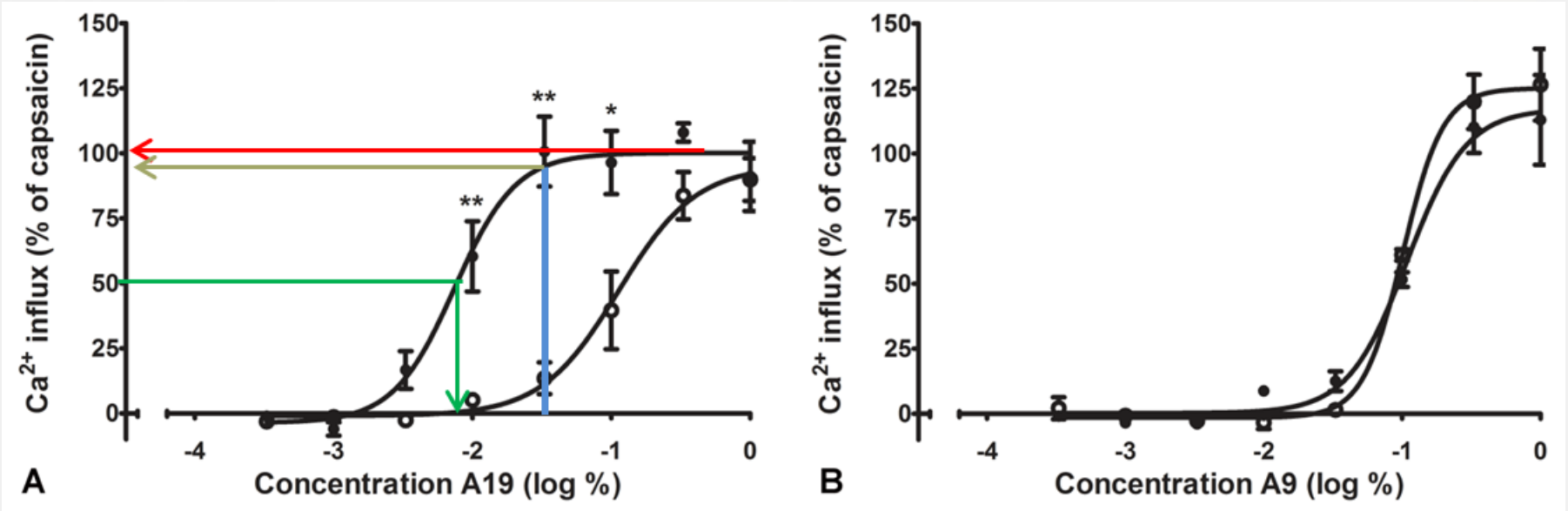
# Bi-phasic response; TRPV1-specific and unspecific $Ca^{2+}$ influx



# Use TRPV1-specific response for calculations



# Data and Prediction Model




| Test parameter | Cut off level |
|---|---|
| Emax (% of capsaicin response) | ≥ 24 |
| EC50 (concentration inducing 50% effect of Emax) | ≤ 0.03 |
| Effect at the concentration 0.032% | ≥ 22 |

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# Study Results

| Test Sample | EC50±S.E.M. (%) | Emax±S.E.M. (% of capsaicin effect) | Effect at 0.032% ±S.E.M. (% of capsaicin effect) | Stinger according to NociOcular | Human Ocular Instillation Test for Sting (Yes/No) |
|---|---|---|---|---|---|
| **A1** | **0.0077±0.0017** | **60±23** | **54±24** | **Yes** | **Yes** |
| A2 | N.C. | <20 | <10 | No | No |
| A3 | N.C. | <20 | <10 | No | No |
| **A4* (+ control)** | **0.0029±0.0015** | **159±41** | **144±38** | **Yes** | **Yes*** |
| A5 | 0.29±0.027 | 1227±16 | 95±19 | No | No |
| A6 | N.C. | <20 | 21±15 | No | No |
| A7 | N.C. | <20 | <10 | No | No |
| A8 | N.C. | <20 | <10 | No | No |
| A9 | N.C. | <20 | <10 | No | No |
| **A10** | **0.0091±0.0009** | **30±7** | **23±8** | **Yes** | **Yes** |
| A11 | 0.020±0.011 | 105±29 | 75±7 | Yes | No |
| A12 | 0.043±0.0016 | 51±15 | 3±3 | No | No |
| **A13** | **0.011±0.0011** | **54±21** | **52±24** | **Yes** | **Yes** |
| **A14** | **N.C.** | **<20** | **<10** | **No** | **Yes** |
| A15 | 0.013±0.0035 | 87±11 | 79±6 | Yes | No |
| **A16** | **0.0056±0.003** | **44±17** | **32±17** | **Yes** | **Yes** |
| **A17** | **0.025±0.015** | **42±1** | **22±8** | **Yes** | **Yes** |
| A18 | 0.014±0.0032 | 23±10 | 21±6 | No | No |
| **A19** | **0.0084±0.0032** | **101±23** | **101±23** | **Yes** | **Yes** |
| A20[1] (- control) | N.C. | <20 | <10 | No | No |

orange- false positive; blue- false negative

# Study Results

- There was no correlation between the clinical sting results and data generated from the four sensitive *in vitro* eye irritation assays.
- 6/7 formulations that induced stinging in the human test were positive in the NociOcular assay (sensitivity= 85.7%), as was the positive control.
- 10/12 that did not induce sting in the human test were negative in the NociOcular assay (specificity = 83.3%).
- Overall accuracy ~85%

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# Study Conclusion

Conclusion: TRPV1 channel activation may be a principle mechanism for eye-stinging sensation induced by soaps and the NociOcular assay may serve as a simple bioassay to ascertain this sensory response in the eye

**Editor's Choice:** Using Novel In Vitro NociOcular Assay Based on TRPV1 Channel Activation for Prediction of Eye Sting Potential of Baby Shampoos

*Anna Forsby, Kimberly G. Norman, Johanna EL Andaloussi-Lilja, Jessica Lundqvist, Vincent Walczak, Rodger Curren, Katharine Martin, and Neena K. Tierney*

Toxicol. Sci. (2012) 129 (2):325-331

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# Technology Transfer

- Johnson & Johnson and Anna Forsby have supported transfer of assay to IIVS
  - IIVS staff training at Stockholm University
  - donation of FlexStation by J&J
- IIVS has performed the assay in-house and data evaluated by Anna Forsby
- Following successful assay transfer, IIVS aims to offer this efficacy assay commercially

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# Future Directions

- Perform additional comparative studies
- Evaluate the ability of the assay to predict the stinging potential of other product types
- Determine if the assay may be predictive of skin and mucosal tissue stinging

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# Acknowledgements


## Stockholm University

*Anna Forsby, Johanna EL Andaloussi-Lilja, Jessica Lundqvist, Tom Gatsinzi, Helene Lindegren, Hanna Mogren, Sofia Holback, Helena Gustafsson*

## Johnson & Johnson

*Neena Tierney, Vincent Walczak, Katharine Martin, Michael Southall*

## IIVS

*Rodger Curren, Lindsay Krawiec, Elizabeth Sly*

# Questions

# Thank you for your participation!

- Answers to all questions will be provided to the participants within a few business days.
- Announcements of future webinars will be sent via email and will be advertised on our website
- Presenters:


Anna Forsby – annaf@neurochem.su.se

Kim Norman – knorman@iivs.org

Neena Tierney – ntierne@cpcus.jnj.com


**www.iivs.org**

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# Chemicals that were tested in the NociOcular assay
# No irritation, irritation

| Compound | EC50 of Capsaicin (mM) | Emax (% of capsaicin) | In vitro category | Risk class (eye) | GHS category(1) | Draize's MMAS |
|---|---|---|---|---|---|---|
| Carbamide/Urea | >700 | 36*** | N | NC | | |
| PEG 200 | 237 | 71,54 | N | NC | | 0 |
| Glycerol/glycerine, 86-89% purity | 1008 | 138 | N | NC | No category | 1,7 |
| DMSO | 340 | 126 | N | NC | | |
| Ethyl 2-methylacetoacetate | 1,82 | 71,5 | M | NC | 2B | 18 |
| Sodium deoxycholate | 0,114 | 124 | M | R36 | | |
| Ammonium nitrate | 7,24 | 101 | M | R36 | 2A | 18,3 |
| 2, 6-Dichlorobenzyl chloride | 2,91 | 83 | M | R36 | 2A | 23,8 |
| Citric acid | 0,208 | 91,3 | M | R36 | 2A | |
| Dibenzyl phosphate | 0,166 | 121 | M | R36 | 2A | 30 |
| Methyl acetate | 26,6 | 118 | M | R36 | 2A | 39,5 |
| Pidolic acid (pyrrolidone carboxylic acid) | 0,663 | 79,4 | M | R36 | 2 | |
| Ammonium lauryl sulphate | 0,0218 | 122 | I | R36 | 2 | 30 |
| N-lauroylsarcosine (sodium salt) | 0,0572 | 52 | M-I | R36 (R41) | 1 | |
| Cocamidopropyl betaine | 0,0375** | 35*** | M-I | R36, R41 | | |
| Sodium lauryl sulphate (15%) | 0,0186 | 118 | I | R41 | 1 | 59,2 |
| Sodium C12-15 pareth sulphate | 0,0776 | 146 | I | R41 | | |
| Benzalkonium chloride (1%) | 0,0507 | 104 | I | R41 | 1 (2A) | 45,3 |
| Cetylpyridinium bromide (6%) | 0,0275 | 107 | I | R41 | 1 | 85,8 |
| Benzoic acid | 0,704 | 104 | M | R41 | 1 | |
| Lactic acid | 0,368 | 84,6 | M | R41 | 1 | |
| Sodium laureth sulphate | 0,0031 | 107 | I | Irritaiting | | 30 |

# Chemicals that were tested in the NociOcular assay
# No irritation, mild irritation, severe irritation

| Compound | EC50 of Capsaicin (mM) | Emax (% of capsaicin) | In vitro category | Risk class (eye) | GHS category(1) | Draize's MMAS |
|---|---|---|---|---|---|---|
| Carbamide/Urea | >700 | 36*** | N | NC | | |
| PEG 200 | 237 | 71,54 | N | NC | | 0 |
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| Citric acid | 0,208 | 91,3 | M | R36 | 2A | |
| Dibenzyl phosphate | 0,166 | 121 | M | R36 | 2A | 30 |
| Methyl acetate | 26,6 | 118 | M | R36 | 2A | 39,5 |
| Pidolic acid (pyrrolidone carboxylic acid) | 0,663 | 79,4 | M | R36 | 2 | |
| Ammonium lauryl sulphate | 0,0218 | 122 | I | R36 | 2 | 30 |
| N-lauroylsarcosine (sodium salt) | 0,0572 | 52 | M-I | R36 (R41) | 1 | |
| Cocamidopropyl betaine | 0,0375** | 35*** | M-I | R36, R41 | | |
| Sodium lauryl sulphate (15%) | 0,0186 | 118 | I | R41 | 1 | 59,2 |
| Sodium C12-15 pareth sulphate | 0,0776 | 146 | I | R41 | | |
| Benzalkonium chloride (1%) | 0,0507 | 104 | I | R41 | 1 (2A) | 45,3 |
| Cetylpyridinium bromide (6%) | 0,0275 | 107 | I | R41 | 1 | 85,8 |
| Benzoic acid | 0,704 | 104 | M | R41 | 1 | |
| Lactic acid | 0,368 | 84,6 | M | R41 | 1 | |
| Sodium laureth sulphate | 0,0031 | 107 | I | Irritaiting | | 30 |