Active Beauty

Neurophroline™ – Overall skin stress control

March 2016

Givaudan

engage your senses

Stress [noun]:
any environmental or physical pressure that elicits a response from an organism

Encyclopedia Britannica - 2016

A universal fact, well described in Chinese:

危 (DANGER)

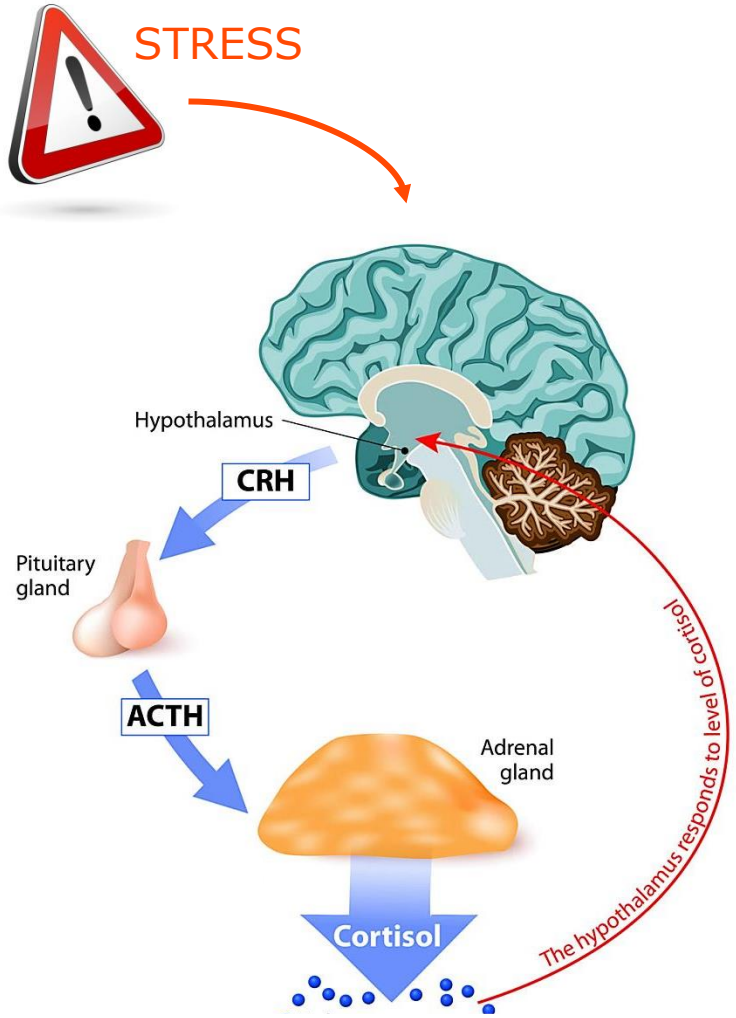
機 (OPPORTUNITY)

Stress & Evolution

The fight or flight response(1)

- Stress is a positive evolution factor, which forces our body to react and adapt to an identified threat
- The biological answer consists in the release of "stress hormones" by the HPA (Hypothalamic Pituitary Adrenal) axis, including adrenaline and cortisol
- Adrenaline increases heart rate, raises blood pressure and provides extra energy.
- Cortisol, known as the stress hormone, temporarily increases energy by triggering the release of glucose into the bloodstream, to help you fight or run away.

(1) Dr Walter Bradford Cannon



CRH: Corticotropin-Releasing Hormone

ACTH: AdenoCorticoTropic Hormone

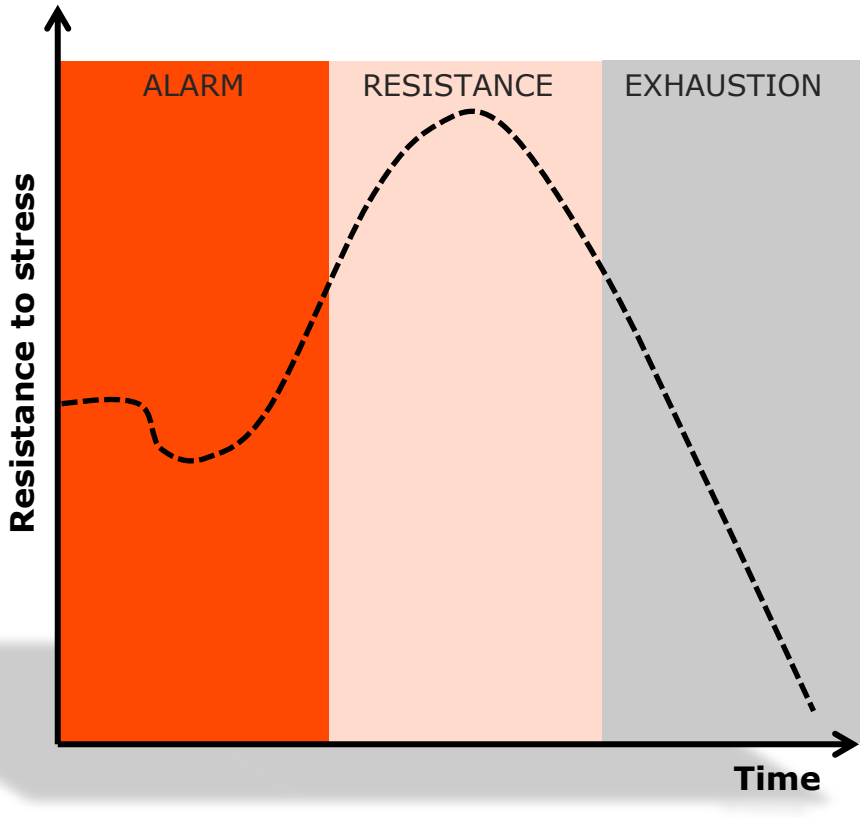
Chronic versus acute stress
General Adaptation Syndrome

A repeated accute stress modifies the biological answer in 3 steps (Dr Hans Selye 's model)

1. Alarm: after a short shock period, fast instant answer (release of hormones)

2. Resistance: high level of hormones in the body, adaptation of the body to the stressor

3. Exhaustion: depletion of ressources, feedback controls and failure to resist.



Stress causes
In our daily life

- Job pressure
- Money
- Health
- Relationships
- Bad nutrition
- Media overload
- Work conditions
- Pollution
- Sleep deprivation

Stress statistics
Social impact

77% of people regularly experience **physical** symptoms caused by stress

Source: Statistic Brain Research Institute, American Institute of Stress, NY

Stress statistics
Social evolution

48% of people

are **more stressed**

today than 5 years ago

Source: Statistic Brain Research Institute, American Institute of Stress, NY

Stress statistics

Economical cost

$300B/year

Worldwide annual cost to employers linked to stress

Source: Statistic Brain Research Institute, American Institute of Stress, NY

Stress statistics
Well being

33%

of people feel they are living with

extreme stress

Source: Statistic Brain Research Institute, American Institute of Stress, NY

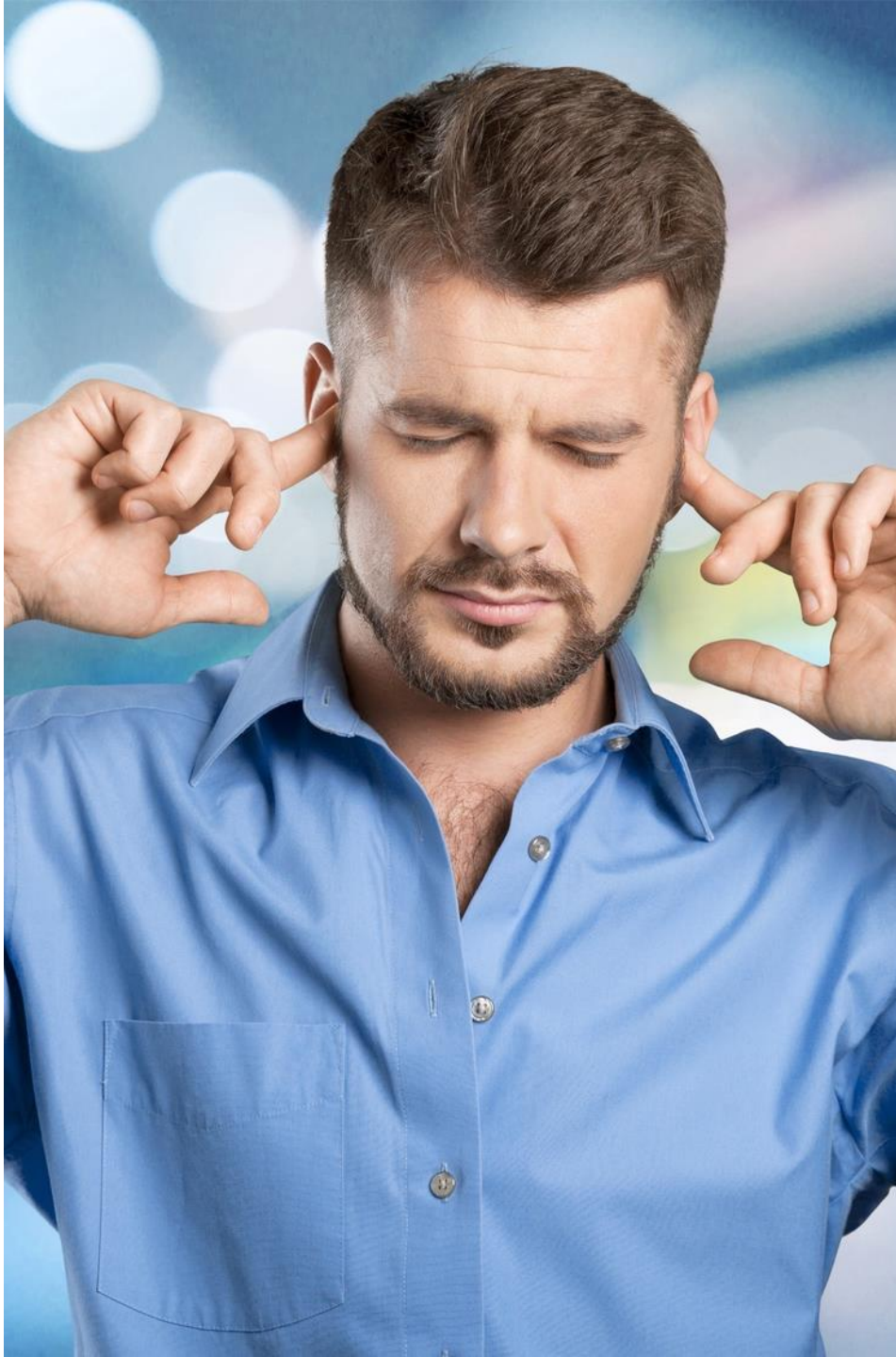
Stress
Health impact

73%

regularly experience

psychological

symptoms caused by stress



Source: Statistic Brain Research Institute, American Institute of Stress, NY

Skin stress causes

- UV radiations
- Polluants (PM10, ozone, aromatic compounds, formaldehyde, cigarette smoke)
- Chemicals
- Detergents
- Pathogens
- Oxidative agents
- Mechanical aggressions
- Hormones
- Central nervous system stress

Chronic stress consequences on skin[1,2]

Decreased immunity

- Acne
- Eczema
- Desquamation
- Bad wound healing

Triggered inflammation

- Redness
- Rosacea
- Psoriasis
- Dark circles
- Pigmentation disorders

Lower energy and detox

- Premature aging
- Low antioxidant response
- Low detox capabilities
- Lack of radiance
- Tired look

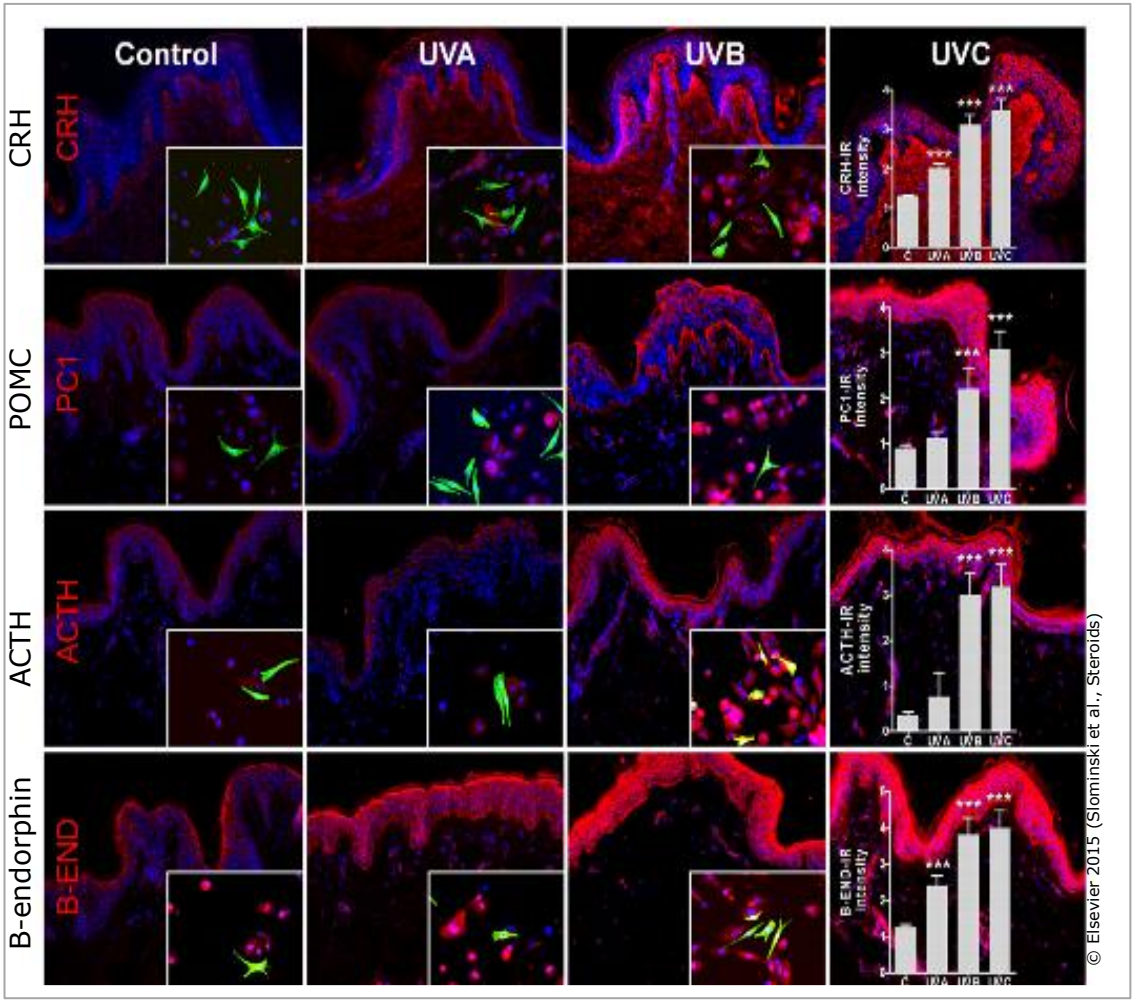
[1]Noise Health. 2003 Apr-Jun;5(19):41-50. Respiratory and dermatological diseases in children with long-term exposure to road traffic emissions.
[2]Psychosom Med. 2007 Nov;69(8):807-15. Epub 2007 Oct 17. Stress, social support, and delayed skin barrier recovery.

How does skin answer to stress ?
Skin as an "HPA like axis" pathway

Skin and brain share the same embyonic origin.

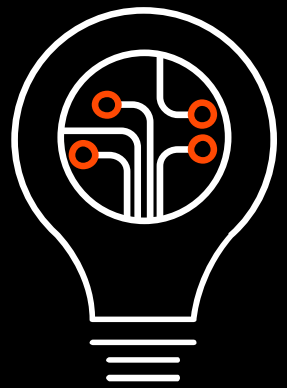
As neurons, skin cells under biological (emotional stress[1]), and physical[2,3] (UV…) conditions produce:

- CRH (corticotropin-releasing hormone)
- POMC (pro-opiomelanocortin)
- ACTH (adrenocorticotrophin hormone)
- Beta endorphin
- And Cortisol



1: Clin Exp Dermatol. 2015
2: J Invest Dermatol. 2015
3. Steroids.2015 .

"Can we inhibit skin cortisol production and its consequences?"



Pliha Satru

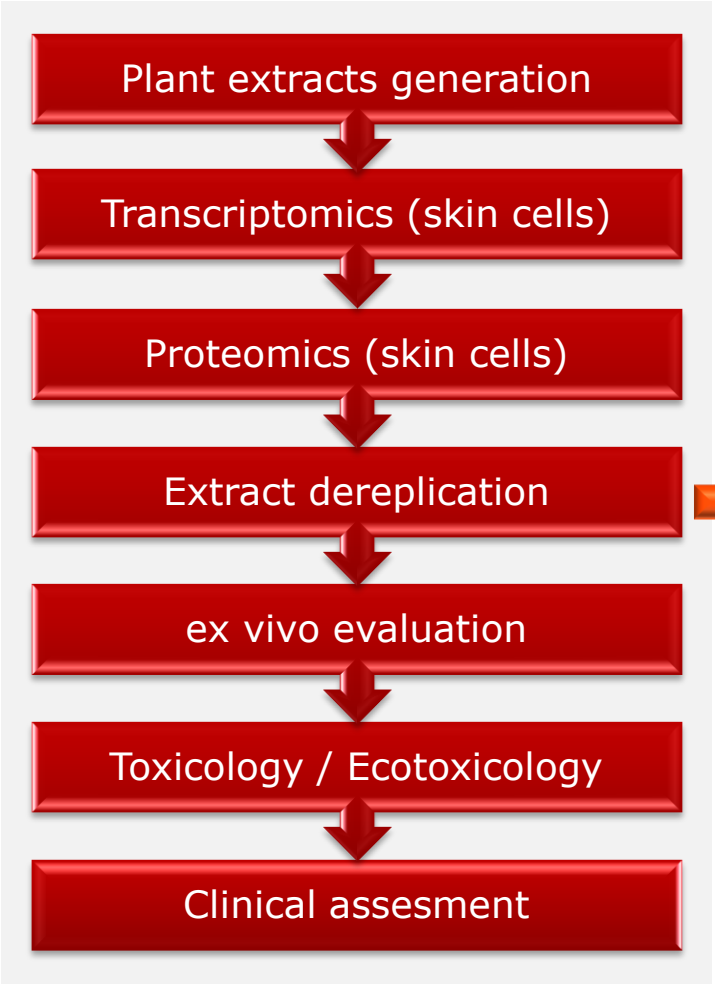
"*The enemy of splenic diseases*" (Sanskrit)

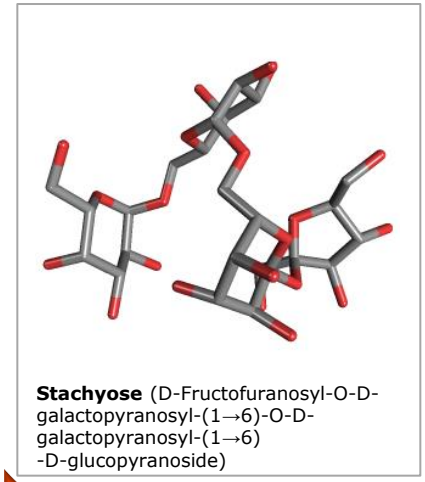
The healthy Indian wild indigo
Tephrosia purpurea

- *Tephrosia* is has been used for centuries in Ayurvedic tradition for its benefits in the treatment of body
- The plant is found throughout India, especially in Southern India and western Himalayas up to 6000 ft.
- All parts of the plant are used for health purposes.

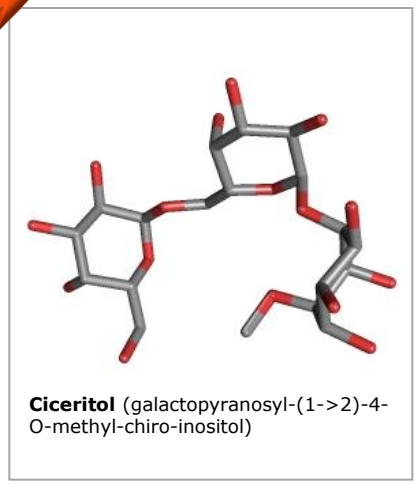
	Parts of the plant	Traditional usages
	Decoction of the leaves	Diuretic, coughs
	Decoction of the roots	Asthma, sore throat
	Topic application of oil	Itching, eczema

Green fractionation applied to phyto tradition
An ISO 9001 integrated process





Stachyose (D-Fructofuranosyl-O-D-galactopyranosyl-(1→6)-O-D-galactopyranosyl-(1→6) -D-glucopyranoside)



Ciceritol (galactopyranosyl-(1->2)-4-O-methyl-chiro-inositol)

Neurophroline™ is a sustainable vegetal extract obtained from the seeds of *Tephrosia purpurea* to protect the plant.

This new seed extract is enriched in two specific sugars:

- Stachyose and Ciceritol;

and three original polyphenol rutinosides:

- Kaempferol-3-O-rutinoside,
- Quercetin-3-O-rutinoside,
- Patuletin-3-O-rutinoside)[1]

(1) Hubert et al., J. Nat . Products, 2015

in vitro evaluations

Regulation of cortisol production (*in vitro* on human keratinocytes)

PROTOCOL

Cells: HNK (Human Normal Keratinocytes)

Treatment: Neurophroline™ at 0.5% and 1% and 1µM of cortisone (cortisol precursor)

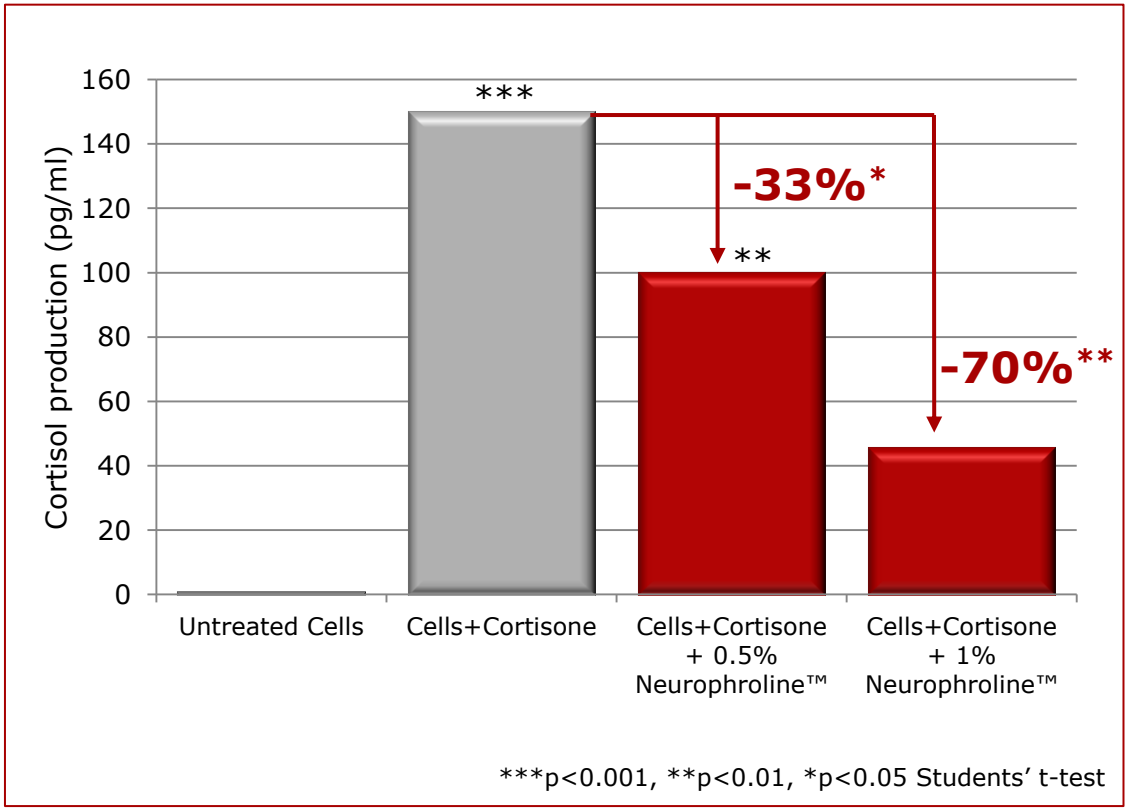
Time: 2 hours

Detection: quantification of cortisol release

→ Very fast action (2 hours)

→ Up to -70% cortisol production inhibition

Inhibitory effect on stress hormone release



Stimulation of β-endorphin (calming neuropeptide) (*in vitro* on human keratinocytes)

PROTOCOL

Cells: HNK (Human Normal Keratinocytes)

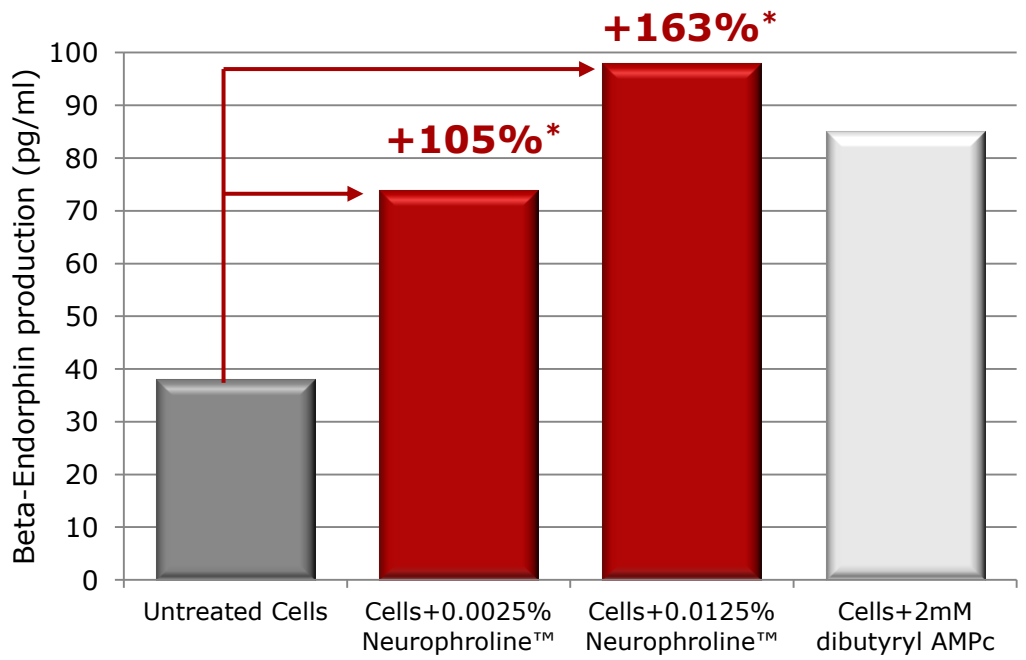
Treatment: Neurophroline™ at 0.0125% and 0.0025% Dibutyryl AMPc as positive control

Time: 24 hours

Detection: ELISA quantification of β-endorphin release

→ Up to +163% β-endorphin production

Induction of relaxing neuropeptide production



*p<0.05 Students' t-test

Activation of anti-stress genetic responses (*transcriptomic* on keratinocytes)

PROTOCOL

Cells: HNK (Human Normal Keratinocytes)

Treatment: Neurophroline™ at 1%

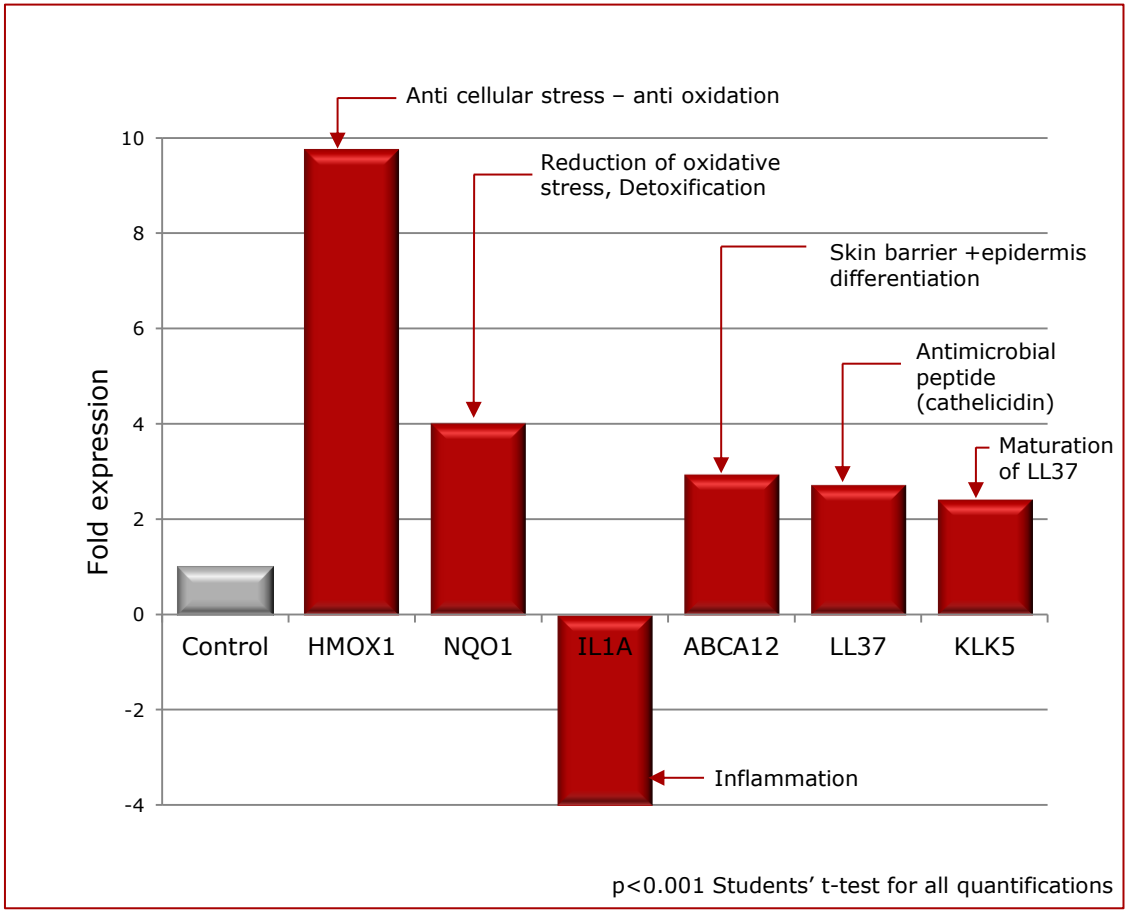
Time: 24 hours

Detection: qRT-PCR analysis of gene expression

→Activation of stress regulating genes

- **Detoxification**
- **Antioxidant response**
- **Antimicrobial peptide**
- **Skin barrier function**
- **Anti-IL1a**

Genes expression activation by Neurophroline™



Activation of anti-stress genetic responses (*transcriptomic* on fibroblasts)

PROTOCOL

Cells: HFN (Human Normal Fibroblasts)

Treatment: Neurophroline™ at 1%

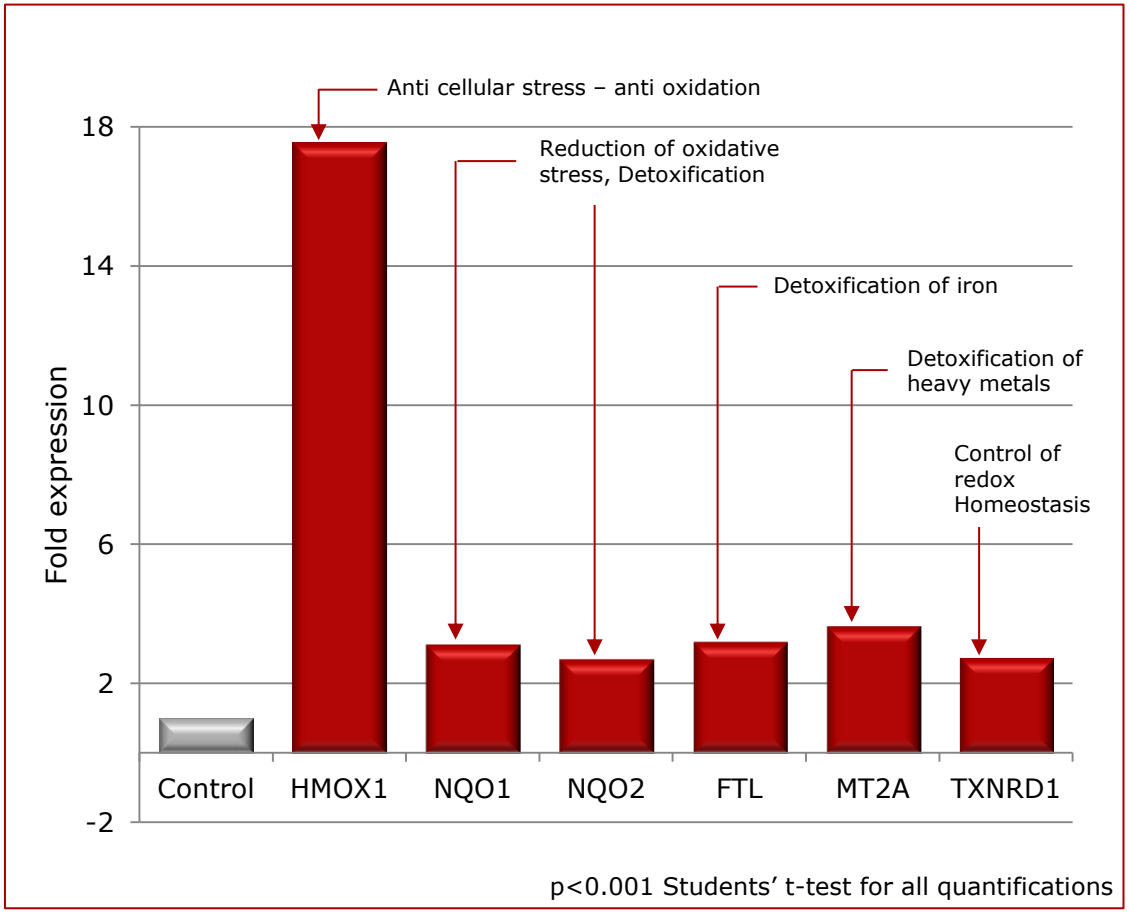
Time: 24 hours

Detection: qRT-PCR analysis of gene expression

→Activation of stress regulating genes

- **Global detoxification**
- **Antioxidant response**
- **Metals detoxification**
- **Redox control**

Genes expression activation by Neurophroline™



Activation of anti-stress proteins (*proteomic* on keratinocytes)

PROTOCOL

Cells: HNK (Human Normal Keratinocytes)

Treatment: Neurophroline™ at 1% - Celastrol or sulforaphane as controls

Time: 24 hours or 48 hours

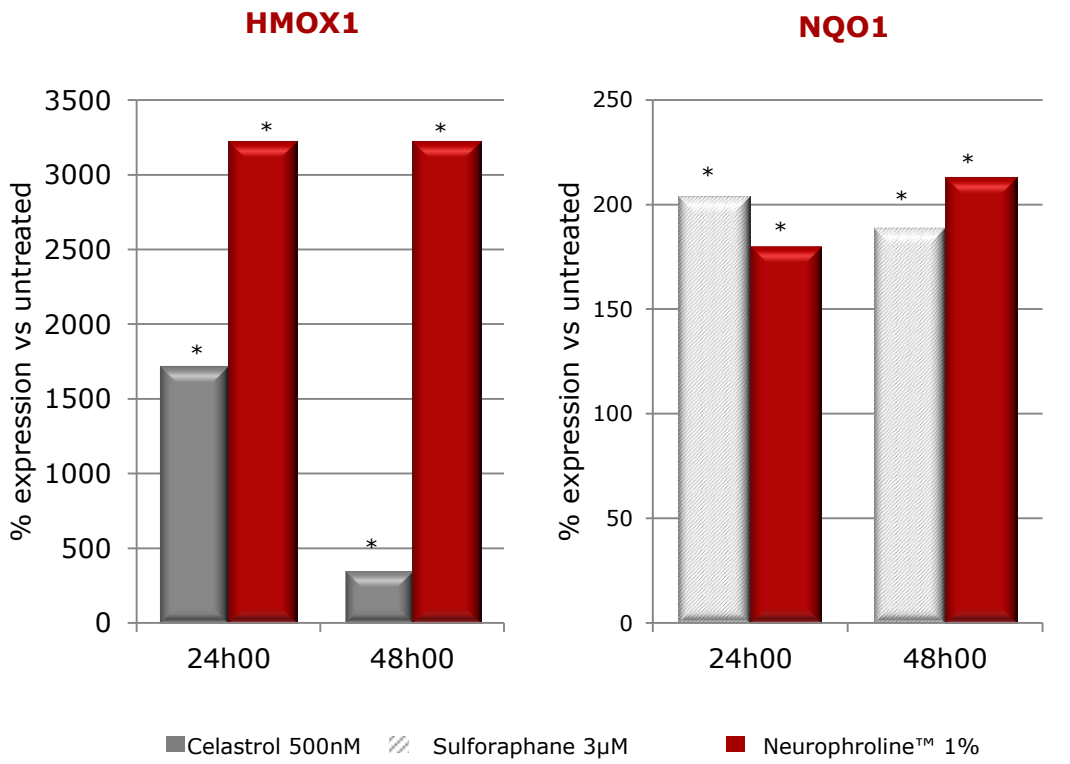
Detection: ELISA quantification of HMOX1 and NQO1

→ High expression of major anti-stress proteins:

- **Up to +3000% HMOX1**
- **Up to +200% NQO1**

→ Constant efficacy over 48 hours

Activation of HMOX1 and NQO1



*p<0.001 ANOVA & Dunnett comparison

Activation of anti-stress proteins (*proteomic* on fibroblasts)

PROTOCOL

Cells: HNF (Human Normal Fibroblasts)

Treatment: Neurophroline™ at 1% - Celastrol as controls

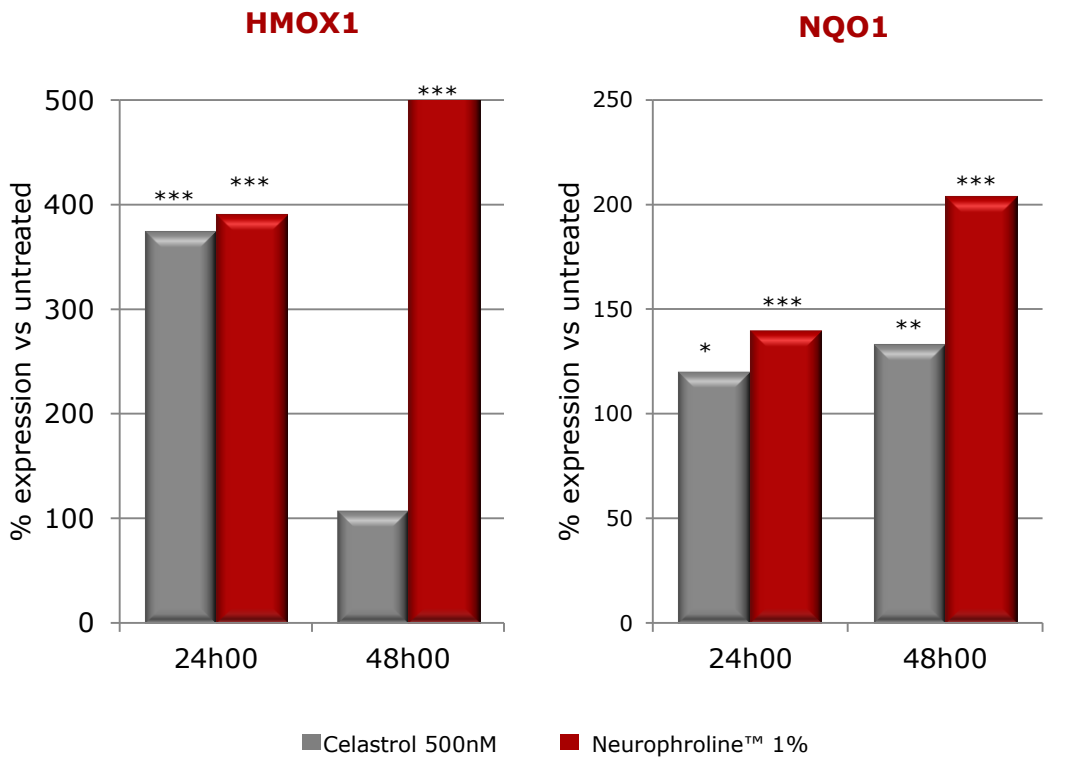
Time: 24 hours or 48 hours

Detection: ELISA quantification of HMOX1 and NQO1

→ **High expression of the major anti-stress proteins:**

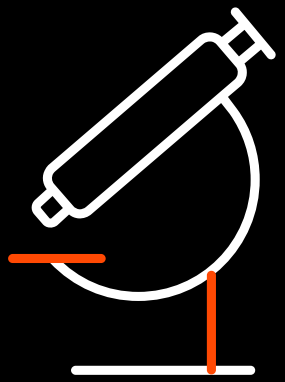
- **Up to +500% HMOX1**
- **Up to +200% NQO1**
- **Increased efficacy between 24 and 48 hours**

Activation of HMOX1 and NQO1



***p<0.001, **p<0.01, *p<0.05 ANOVA & Dunnett comparison

ex vivo evaluations



Visible anti-stress effect in the epidermis (*Immunolabeling on RHE*)

PROTOCOL

Topical application on RHE (reconstructed human epidermis) of formula containing either

- Nothing (placebo), or
- 1% Neurophroline™, or
- 750 mM Celastrol or 3µM sulforaphane as controls

Time: 48 hours

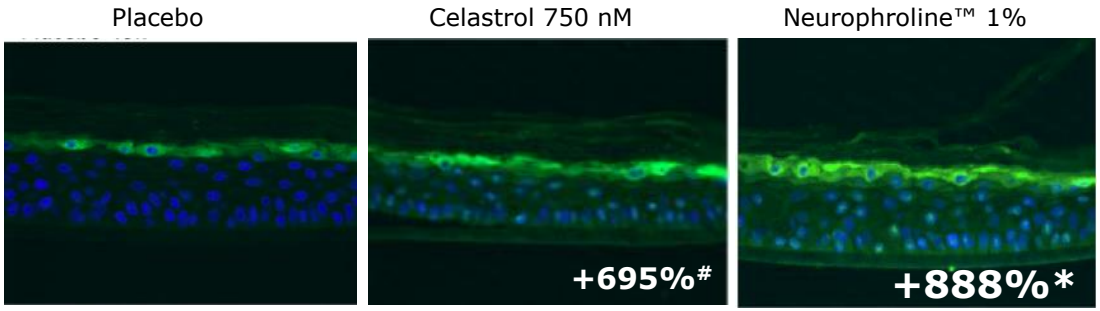
Detection: Immunolabeling and quantification of HMOX1 and NQO1 expression

→ Visible epidermis activation

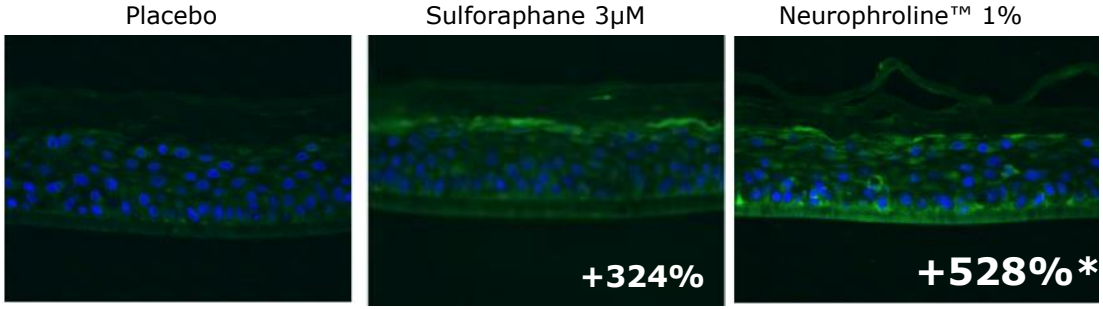
- **Up to +888% HMOX1**
- **Up to +528% NQO1**

Visualization of anti-stress markers HMOX1 and NQO1

HMOX1



NQO1



*p<0.05 compared to placebo; #p<0.05 compared to control - Student's t-test

Clinical assessment

Clinical test conditions
Protocol

PROTOCOL

Double blind versus placebo

24 women, 40 to 67 years old (53±2 years)

Test period: summer time

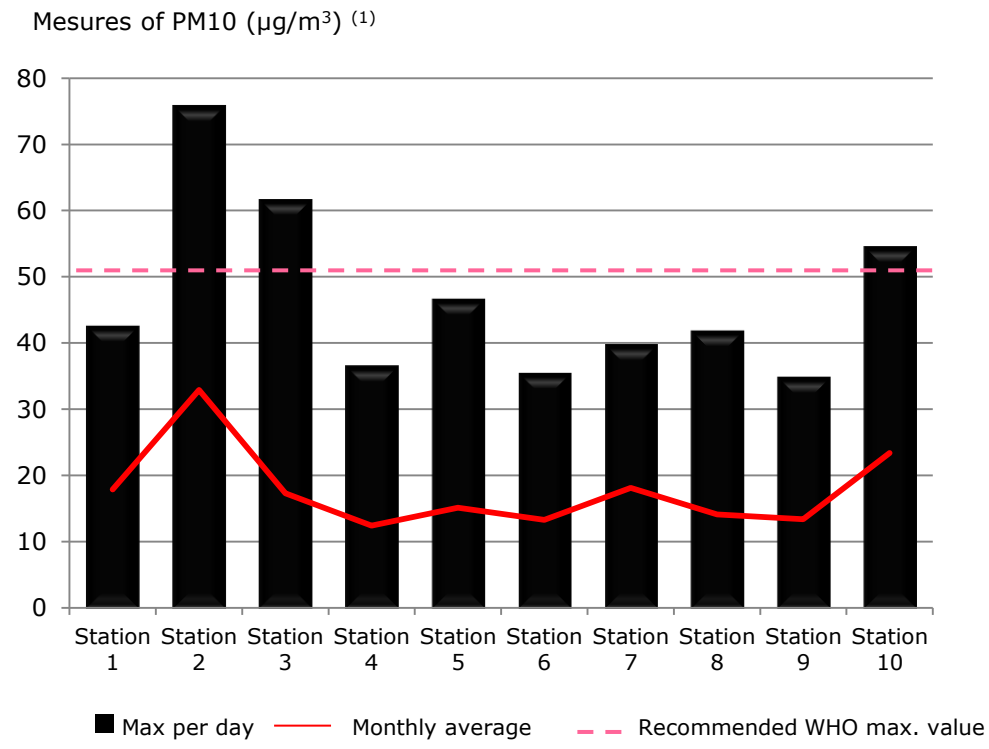
Length: one month

Test conditions: skin stressing conditions (peaks of pollution)

Usage: application twice a day on the most sensitive part of the face (under the eyes) of the placebo on one side, or placebo + 2% Neurophroline™ on the other side.

INCI: AQUA/WATER, CAPRIC/CAPRYLIC TRIGLYCERIDE, CETEARYL WHEAT STRAW GLYCOSIDES, CETEARYL ALCOHOL, PHENOXYETHANOL, METHYL PARABEN, PROPYL PARABEN, ETHYL PARABEN, DIMETHICONE, FRAGRANCE, HEXYL CINNAMAL, BUTYLPHENYL, ETHYLPROPIONAL, CITRONELLOL, ALPHA ISOMETHYL IONONE, HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE, SODIUM HYDROXIDE **+/-2% Neurophroline™**

Air pollution by PM10 during the clinical test



(1) Source Airpomenia – Tricity location

Clinical test conditions
Protocol

PROTOCOL

Double blind versus placebo

24 women, 40 to 67 years old (53±2 years)

Test period: summer time

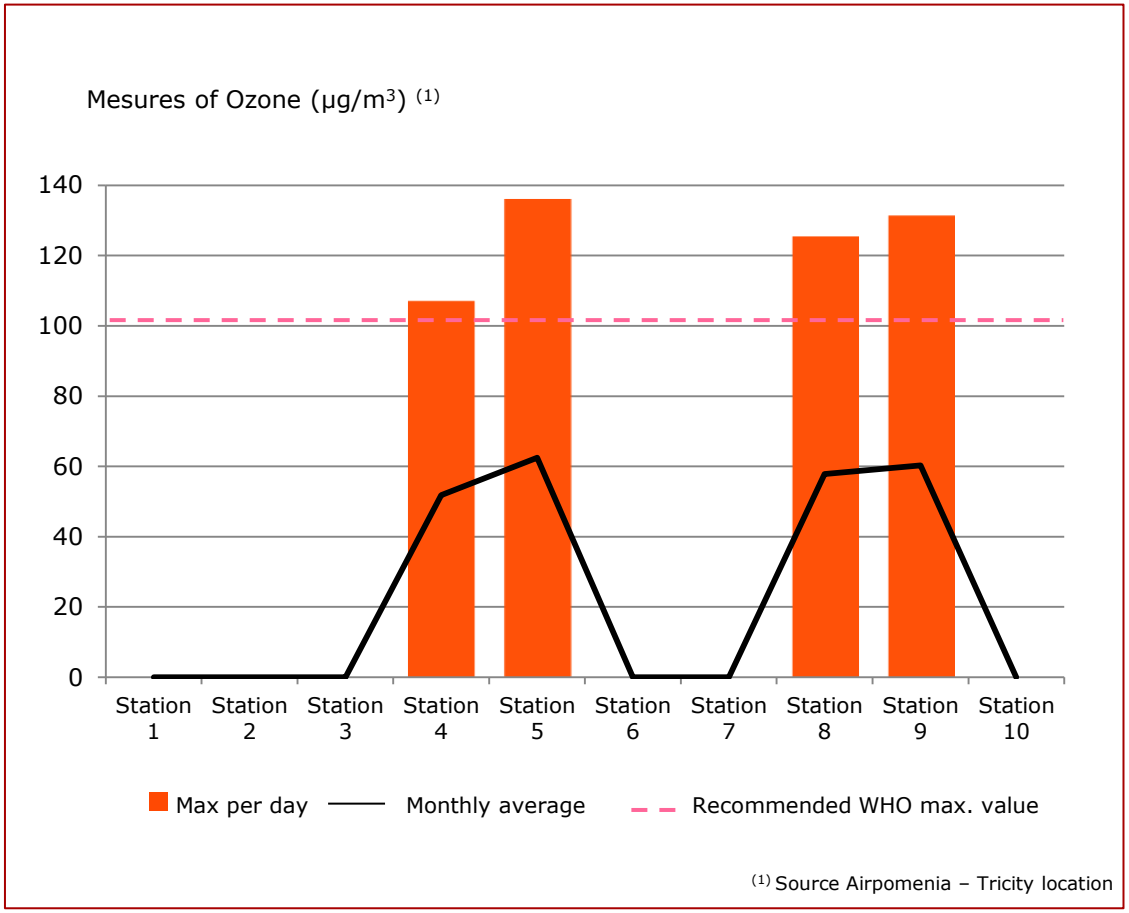
Length: one month

Test conditions: skin stressing conditions (peaks of pollution)

Usage: application twice a day on the most sensitive part of the face (under the eyes) of the placebo on one side, or placebo + 2% Neurophroline™ on the other side.

INCI: AQUA/WATER, CAPRIC/CAPRYLIC TRIGLYCERIDE, CETEARYL WHEAT STRAW GLYCOSIDES, CETEARYL ALCOHOL, PHENOXYETHANOL, METHYL PARABEN, PROPYL PARABEN, ETHYL PARABEN, DIMETHICONE, FRAGRANCE, HEXYL CINNAMAL, BUTYLPHENYL, ETHYLPROPIONAL, CITRONELLOL, ALPHA ISOMETHYL IONONE, HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE, SODIUM HYDROXIDE **+/-2% Neurophroline™**

Air pollution by Ozone during the clinical test



Fast recovery of skin luminosity (*spectrocolorimeter*)

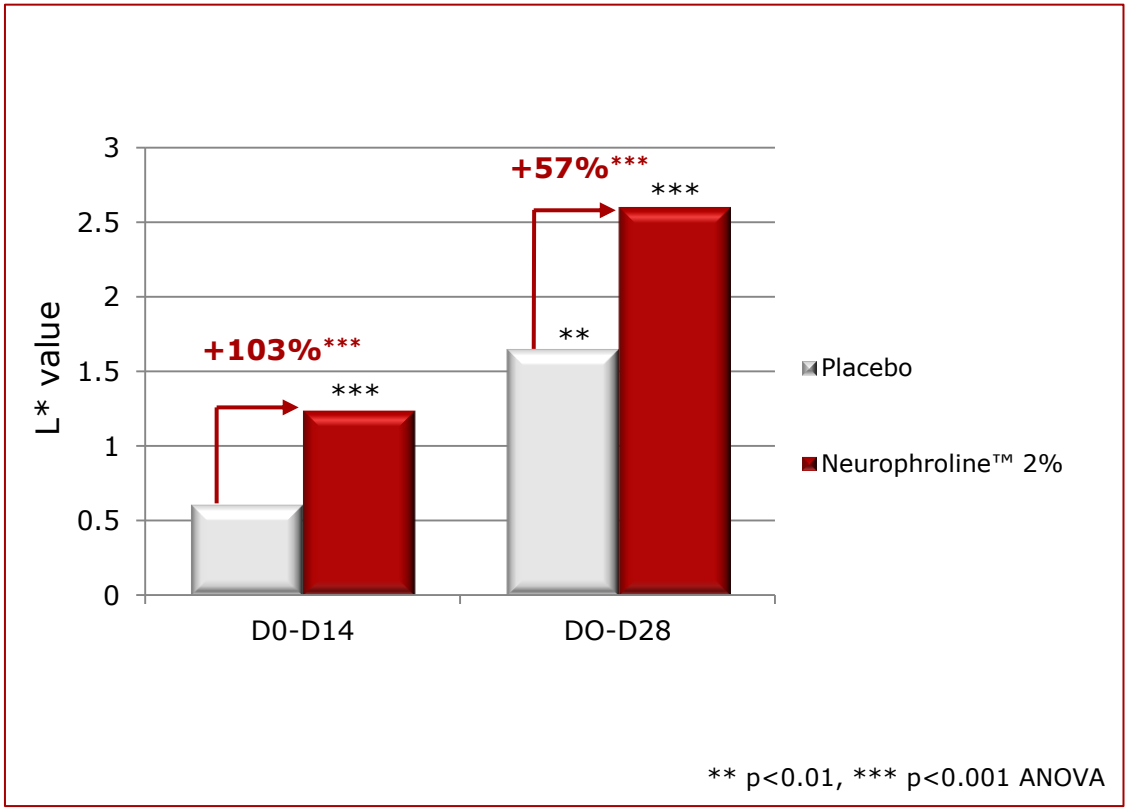
PROTOCOL

Measure of skin luminosity with a spectrocolorimeter at D14 and D28.

→Fast results in 2 weeks

→Up to 57% luminosity of the skin

Skin luminosity evolution (L* value)



Fast improvement of skin redness (*spectrocolorimeter*)

PROTOCOL

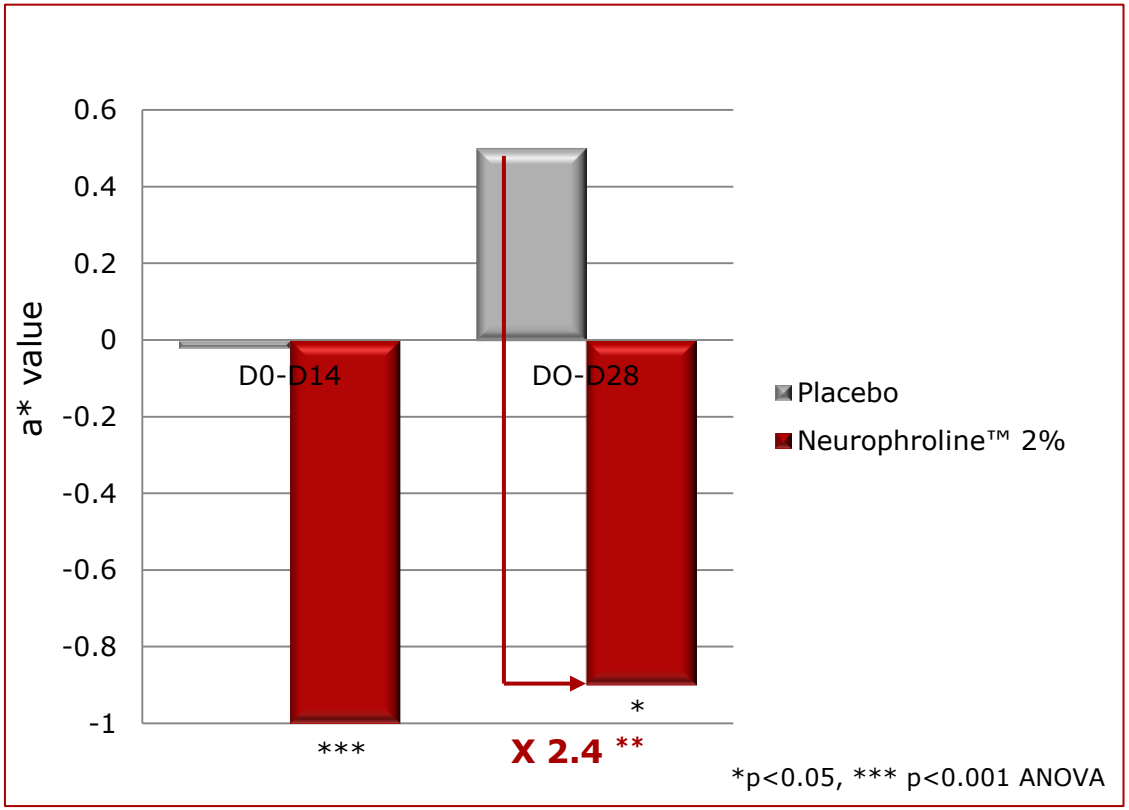
Measure of skin redness with a spectrocolorimeter at D14 and D28.

→Fast results in 2 weeks

→Over 2 times lower skin redness

→Untreated zone shows an increase skin redness

Skin redness evolution (a* value)



Global of skin color correction (ITA) (*spectrocolorimeter*)

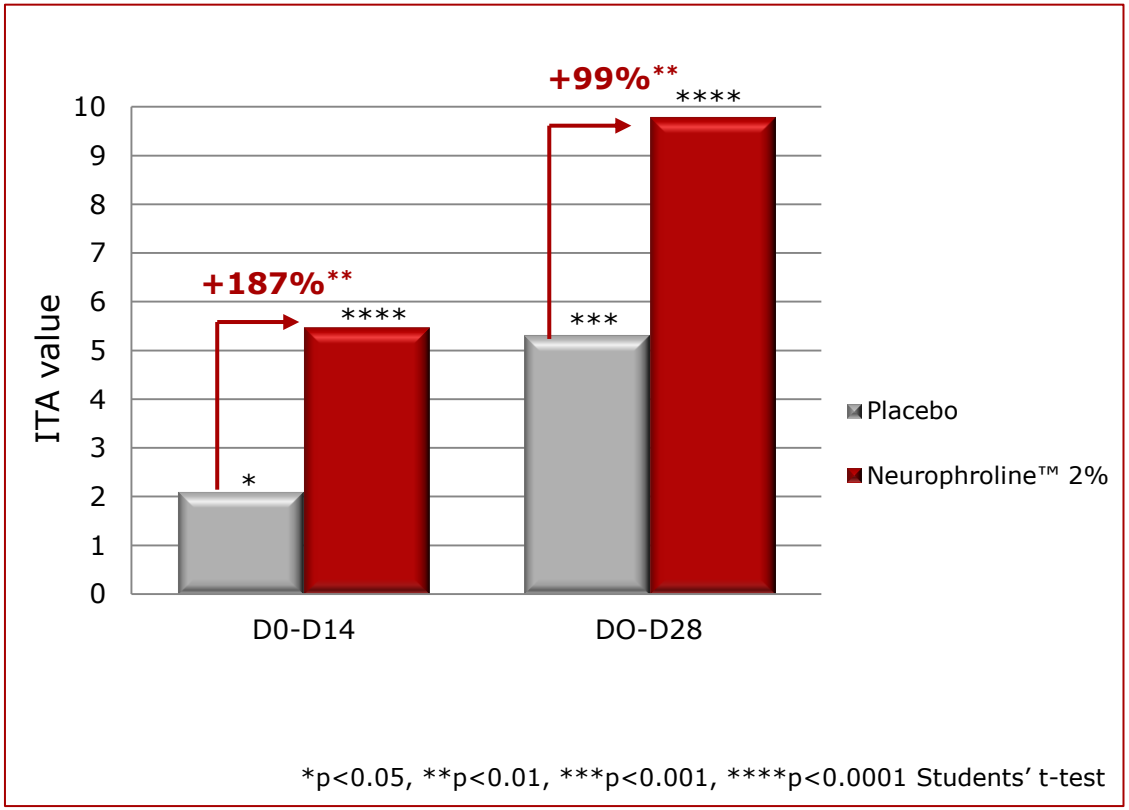
PROTOCOL

Measure of skin b* value with a spectrocolorimeter at D14 and D28, in order to calculate the ITA angle.

→Fast results in 2 weeks

→Up to 99% color improvement

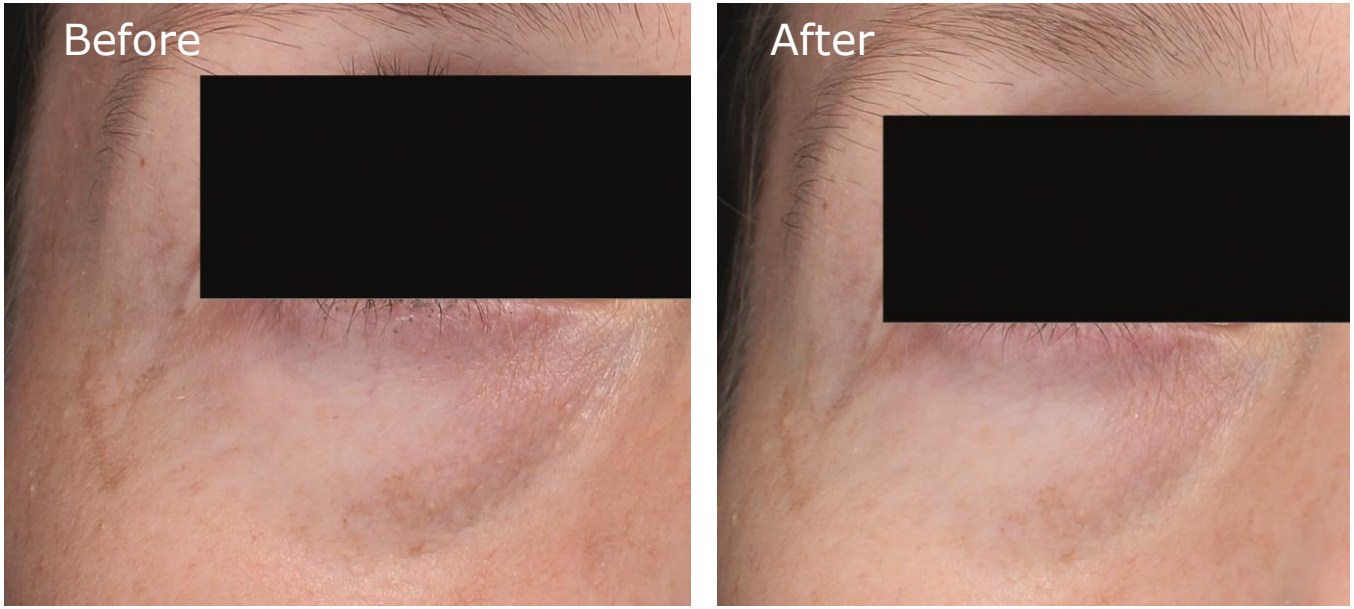
Skin color evolution (ITA angle)



*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 Students' t-test

Visible benefits
(*macro pictures after one month*)

Volunteer #6

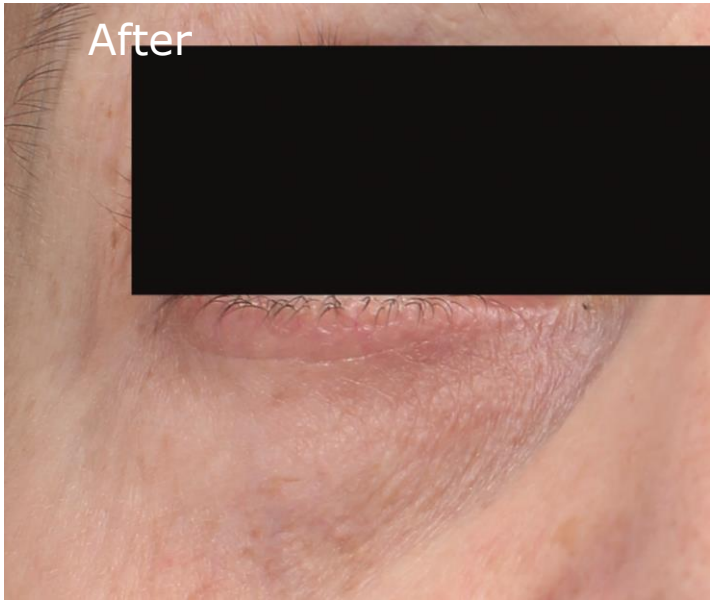


Visible benefits
(*macro pictures after one month*)

Volunteer #7

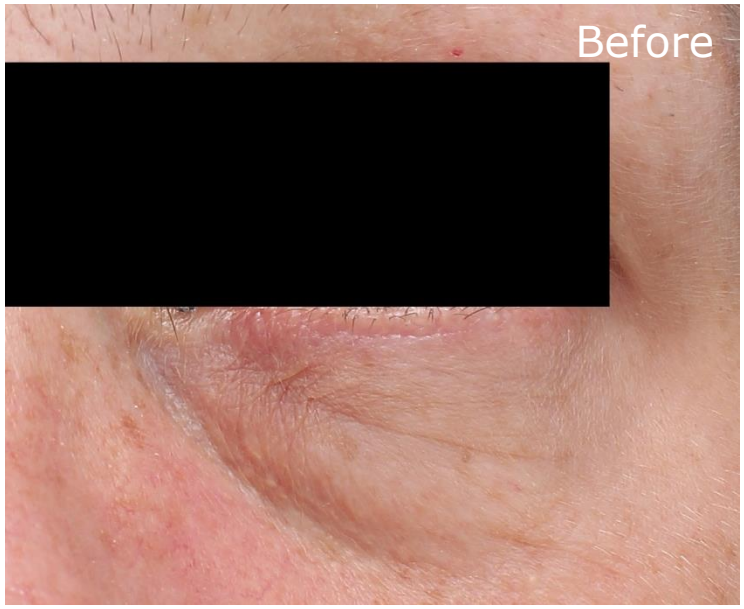
Visible benefits
(*macro pictures after one month*)

Volunteer #13

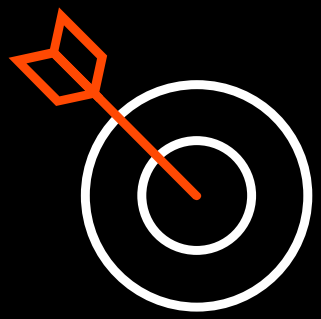


Visible benefits
(*macro pictures after one month*)

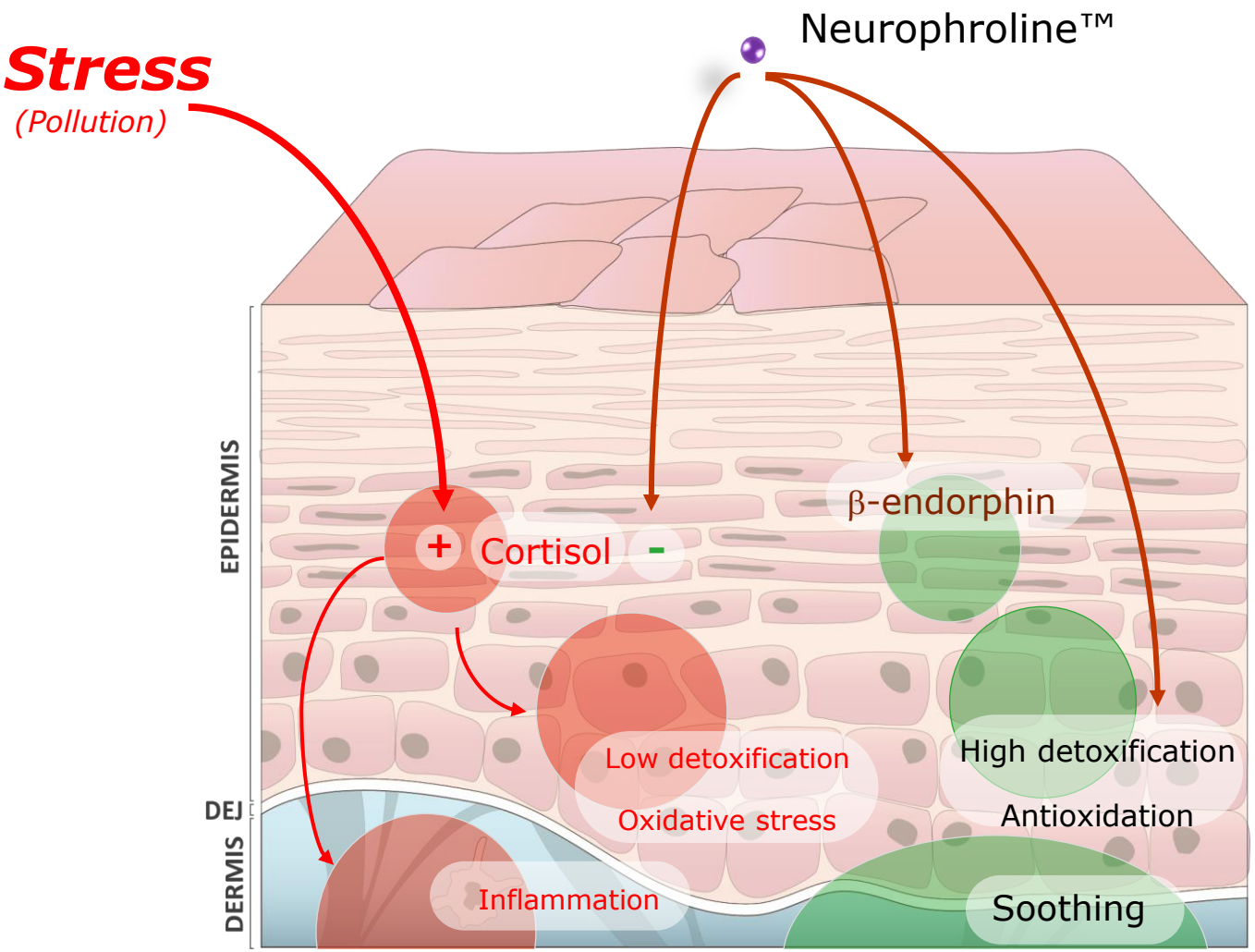
Volunteer #10



Summary



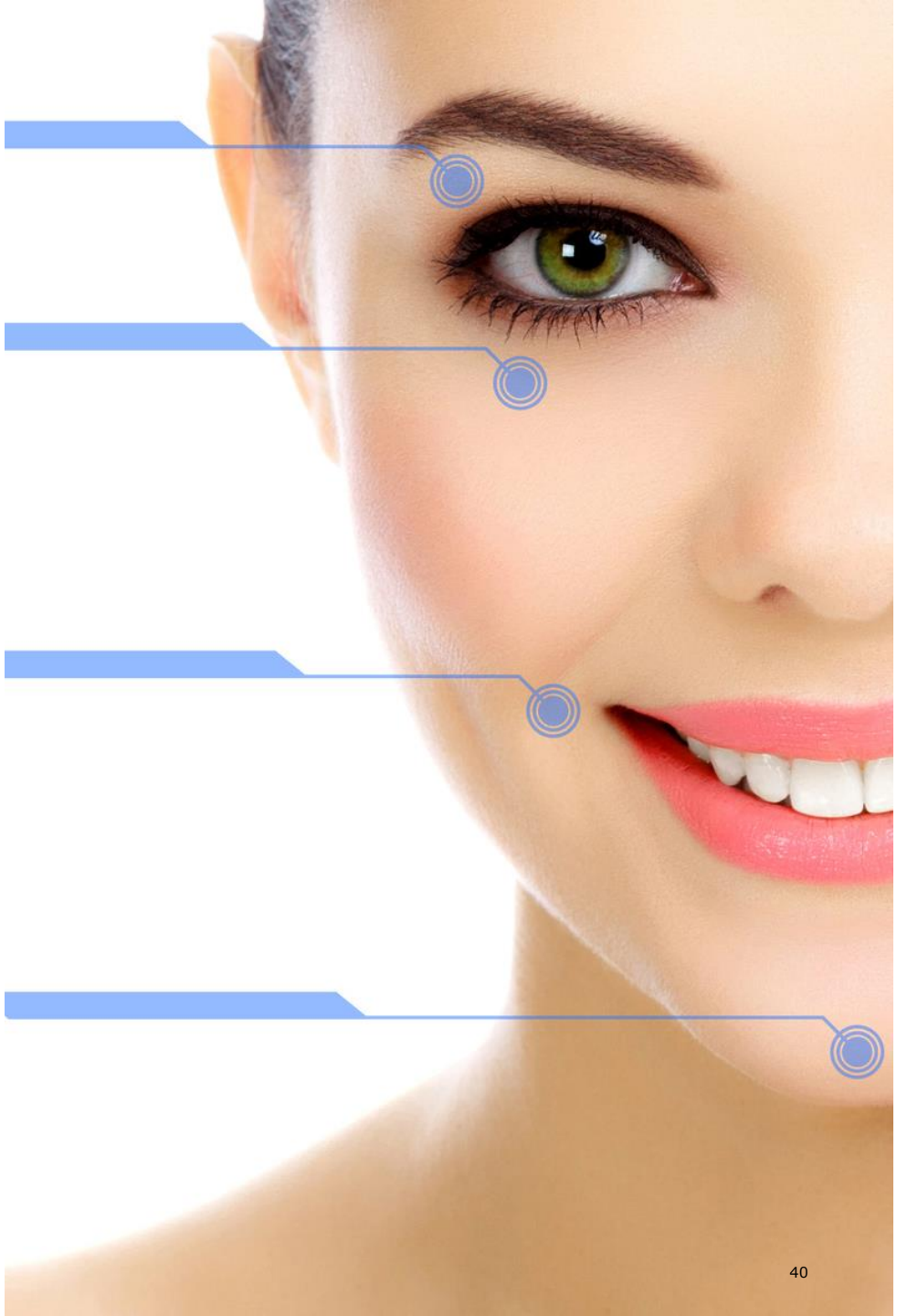
Neurophroline™ mode of action
A direct action on stress to bring back homeostasis



Main claims

Creating new opportunities

- Overall skin stress control
- Anti-tiredness
- Anti-hangover
- Anti-pollution
- Anti PM10
- Anti Ozone aggressions
- Anti-stress
- Anti-aging for sensitive skins
- Anti-redness
- Anti-dark circles



Applications

Make consumers look and feel good

Face care & Make-up

- Anti stress serum
- Full recovery (night) cream
- Anti pollution hydrating spray
- Total serenity foundation
- Happy primer
- Skin therapist cream
- Active detox essence

Body care

- Absolute homeostasis body lotion
- Calming body milk

Neurocosmetic product line

(in connection with Mariliance™ , Rubixyl™)

Technical information

INCI: WATER, PROPANEDIOL, TEPHROSIA PURPUREA SEED EXTRACT

Origin: Vegetal extraction

Preservation: Preservative-free

Appearance: Clear, light yellow liquid

Solubility: Water soluble

Dosage: 0.1-2%

Ecocert compliant (on going registration)

Cosmos compliant

Processing:

Can be added at the end of the formulation process under stirring or homogenizing. Can be heated for a short time with the water phase of formulation. Formulate at temperature below 50°C, and final pH below 6.0.

Neurophroline™

by Soliance technology

"Happiness in a cream"

Thank you.

Givaudan Active Beauty

Global.cosmetic@givaudan.com