# Mixtures prioritization based on exposure and hazard

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anses
alimentation, environnement, travail

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# Example : dietary exposure

Individuals are exposed to various substances which can interact

~ 200 Pesticides ~ 30 Inorganic contaminants and minerals
~ 10 Additives ~ 10 Phyto-oestrogens ~ 20 Mycotoxins
~ 50 Dioxins, PCB, furans, brominated flame retardants, Perfluoroalkyl acids

French Total Diet Study 2, Anses, 2011

Unrealistic to test all possible combinations:
Pesticides: $2^{200} = 1.6x10^{60}$ Combinaisons !!!

How to prioritize mixtures? → need methods to identify mixtures

# Current approaches for mixture selection

## Based on toxicological data (Efsa, US-EPA)

Targeted organ / specific effect / mode / mechanism of action

- ✓ Chemical families (Dioxins and PCB-DL, Triazoles, Organophosphates,…)
- ✓ Cumulative assessment group, CAG, EFSA
- ✓ Common mechanism group, CMG, US-EPA

## Based on exposure data (Périclès*, French ANR project)

- ✓ Directly from dietary co-exposure (1)
- ✓ Through cluster diet (2)

## Based on toxicological and exposure data (3)

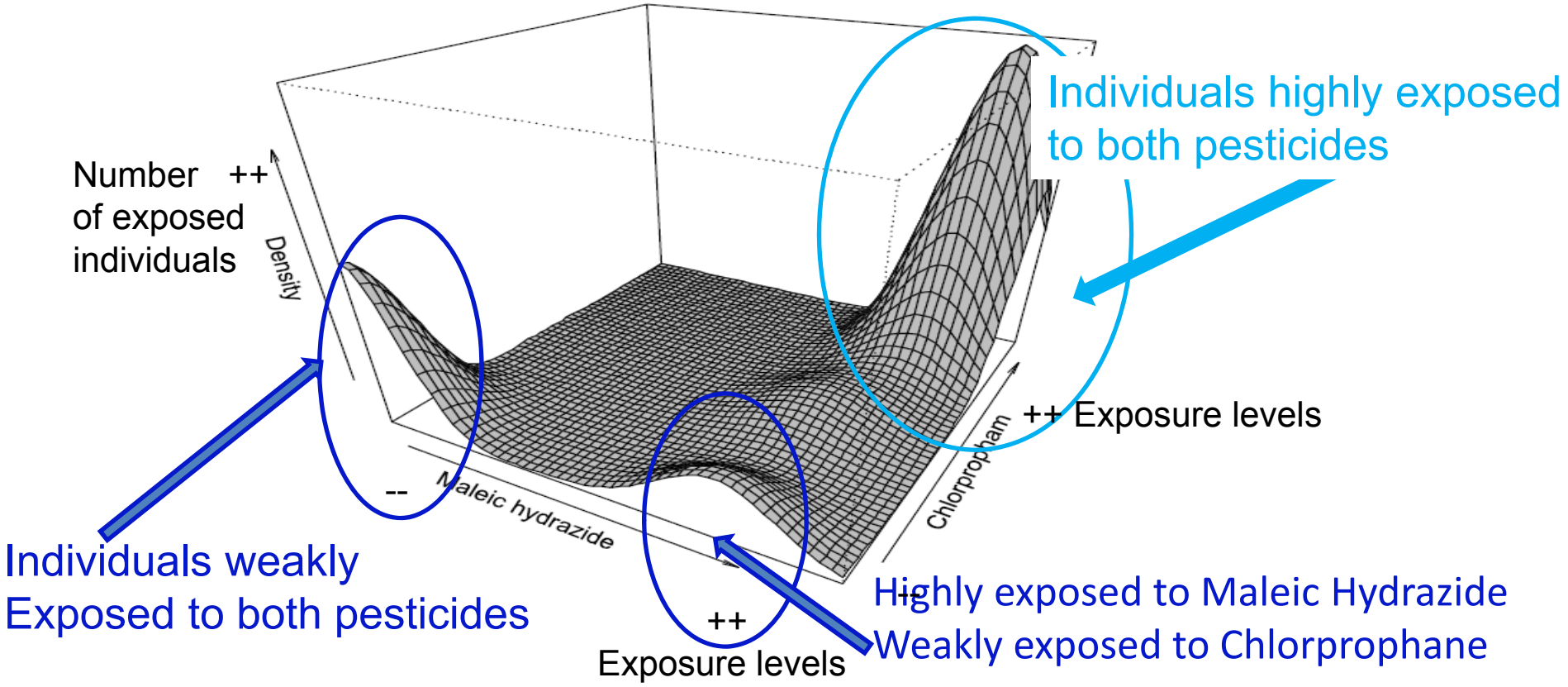
EuroMix

**Crépet A., et al. (2013) The PERICLES research program: an integrated approach to characterize the combined effects of mixtures of pesticides residues to which the French population is exposed. (Toxicology).*

# Mixture selection from exposure and cluster diet

1 2

# Combined exposures to two pesticides




In real life: more than 2 chemicals
Define combinations from a large number of chemicals

# Method

## Individuals are exposed to mixtures because

- ✓ They combine different food during a meal, a day, life…
- ✓ One food can contain several substances

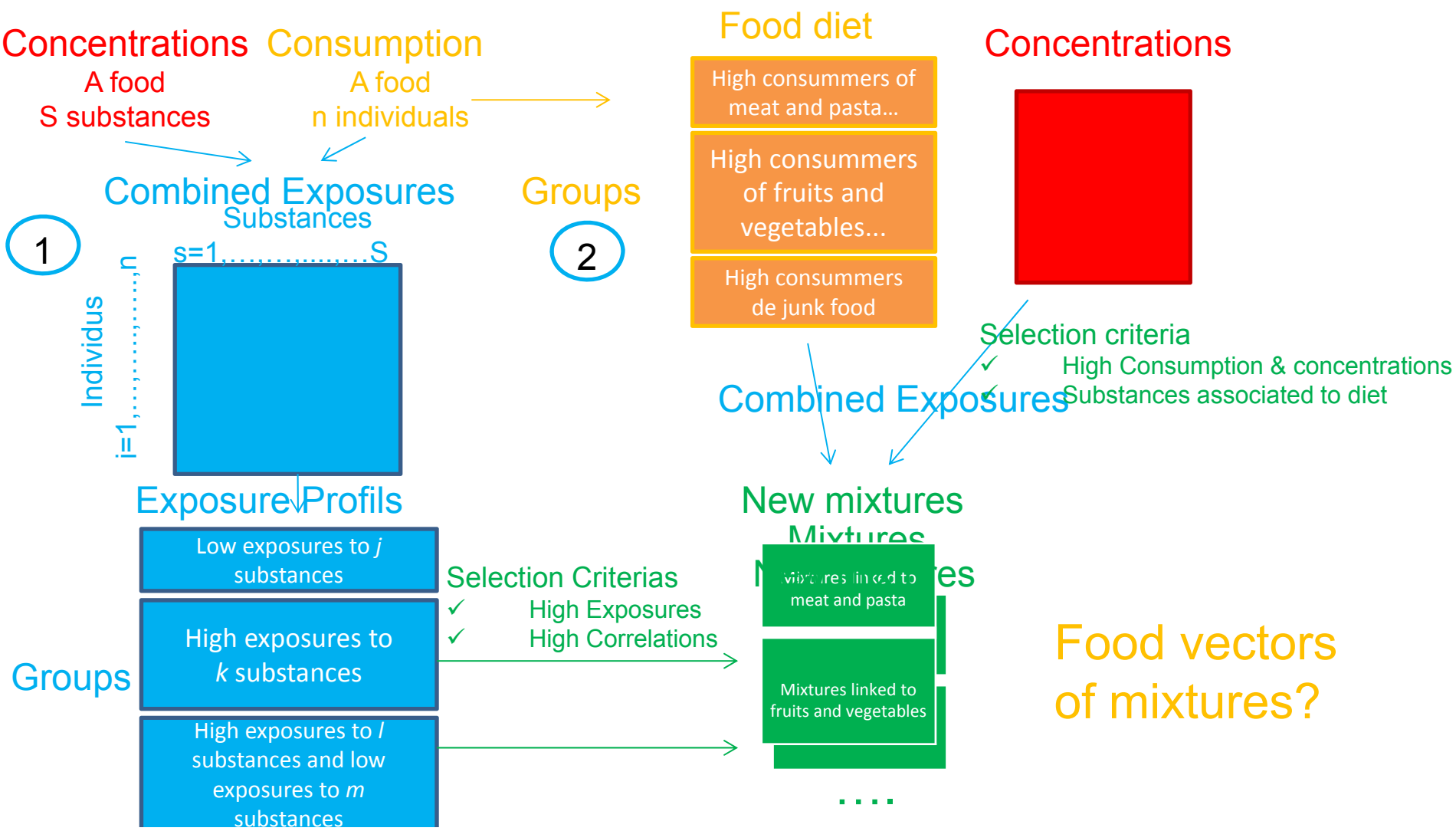
## The population can be divided in different subgroups

- ✓ Due to particular combinations of consumed food : specific diet
- ✓ Due to particular contamination: regionality, high contaminated sites

Mathematical methods

- Cluster individuals with similar profiles in subgroups
- Extract mixtures by studying correlations/latent variables


- *Crépet A., Tressou J. (2011). Bayesian nonparametric model for clustering individual co-exposure to pesticides found in the French diet. Bayesian Analysis. 6(1), 127:144.*
- *Béchaux, C., Zetlaoui, M., J. Tressou, Leblanc, J.Ch., Héraud, F., Crépet, A. (2013) Identification of pesticide mixtures and connection between combined exposure and diet, Food Chemical and Toxicology, 59, 191–198.*


Concentrations
A food
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Food diet
High consummers of meat and pasta…
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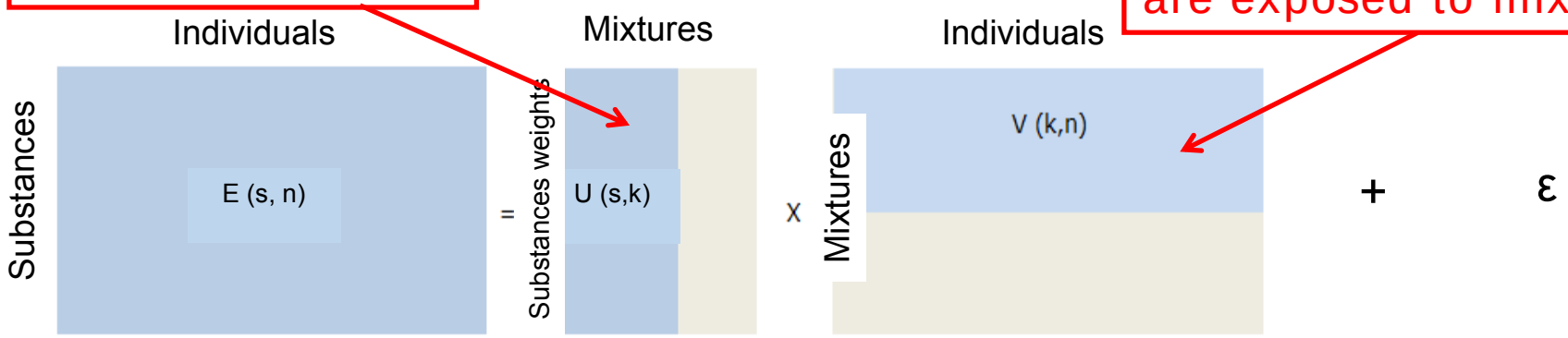
Selection Criterias
✓ High Exposures
✓ High Correlations
New mixtures
Mixtures linked to meat and pasta
Mixtures linked to fruits and vegetables
….
Food vectors of mixtures?

# Mixture selection method

A combination of Sparse Non-negative Matrix Underestimation (SNMU) and hierarchical classification

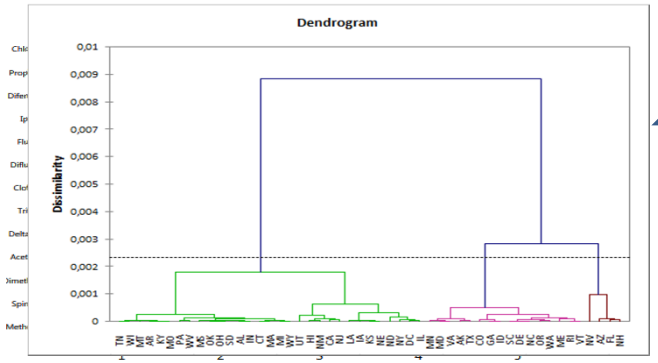
U shows mixtures

V shows how individuals are exposed to mixtures




Clustering

- Balance between
  - Exposure Correlations
  - Exposure levels
- Similar exposure profils
- Cluster diet
  - Relative potency factors




Matrix V
3 groups

Matrix U
Weight in total exposure

# 2 Mixtures through cluster diet

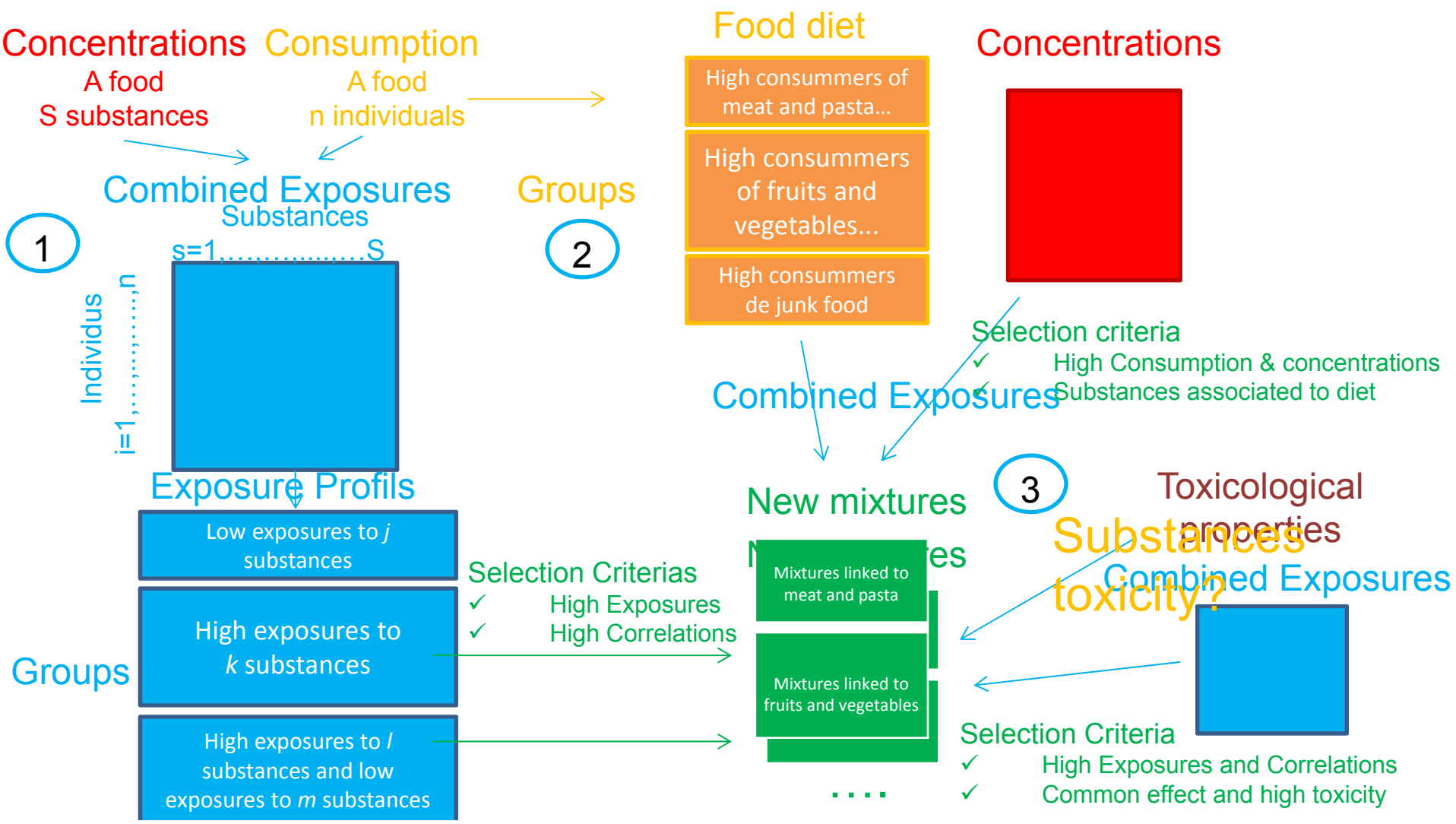
## Chronic exposures to 153 substances (TDS 2, Anses 2011)

6 groups of individuals combining 1 or 2 of the 6 major consumption systems (CS) and therefore exposed to 1 or 2 mixtures among the 6 major mixtures

| Group | % of CS | Major Consumption Systems | Mixtures |
|---|---|---|---|
| Group 2<br><br>N=523<br>Age=32*<br>BMI=25.2*<br>%Men=55% | 36% | Soda, Pizza, Sandwich, Hamburger,<br>Chocolate spread, Sauteed potatoes or chips<br>Salted potato crisp, etc. | PHE, FA (PAHs),<br>Zer (Mycotoxins),<br>Pirimiphos-methyl, Piperonyl Butoxide<br>PBDEs<br>Co, Acrylamid, Ni, etc. |
| | 22% | Baguette, Beef steak, Pasta, Butter, Camembert, Sugar,<br>Eggs, etc. | HT2, Don, Niv, Zer (Mycotoxins), BPA,<br>PY, PHE (PAHs)<br>Cd, Co, Te, Pb , etc. |
| Group 3<br><br>N=164<br>Age=56*<br>BMI=22.9*<br>%Women=73* | 49% | Peach, Melon, Abricot, Tomato, Cucumber, Vinaigrette,<br>Bean, Radish, Strawberry, etc. | Λ-Cyhalothrin, Chlorpyrifos-ethyl, Acrinathrin,<br>Procymidone, Phosmet, Chlorothalonil,<br>Cyprodinil, etc. |

**significantly different from the whole population*

*T. Traoré, C. Béchaux, V. Sirot, A. Crépet. 2016 To which chemical mixtures is the French population exposed? Mixture identification from the second French Total Diet Study, Food and chemical toxicology*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Toxicological properties
Substances toxicity?
Combined Exposures
Selection Criteria
✓ High Exposures and Correlations
✓ Common effect and high toxicity

# Mixture selection from co-exposure and hazard

3

Euromix, projet européen, 2015-2019, https://www.euromixproject.eu/

# EFSA, Cumulative Assessment Groups (CAGs)

## For Pesticides

- Level 1: targeted organ
- Level 2: specific effect
- Level 3: mode of action
- Level 4: mechanism of action

Level 1 and 2 done
Done for 16 organs
EFSA reports 2012, 2015

NOAELs (no observed adverse effect)

**Relative potency factors (RPFs)**

Convert each susbtance s in toxicity of an index compound i

RPFs=NOAELi/NOAELs

# Mixture selection strategy

EFSA, Cumulative assessment groups (CAGs)

*RPFs=NOAELi/NOAELs*

Combined exposure

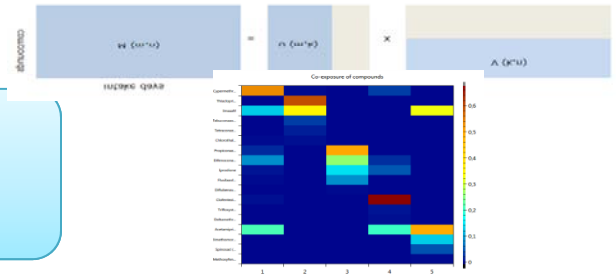
$E_{s,i}$

$E_{s,i} \times RPF_s$

Mixtures from co-exposures and hazard Risk based mixtures

Selection Criteria

- ✓ High Exposures and Correlations
- ✓ Common effect and high toxicity



EuroMix

# Applied to pesticides and liver CAG

EFSA, Cumulative assessment groups (CAGs)

*RPFs=NOAELi/NOAELs*

➢Level 2: Steatosis, 144 pesticides

Combined exposure

France, 194 pesticides,

Chronic, adults, 2010-2014

$E_{s,i}$

120 pesticides

$E_{s,i} \times RPF_{s}$

**70 pesticides** $\geq LOD$

with censored value= 0

Mixtures from co-exposures and hazard Risk based mixtures

Selection Criteria

✓ High Exposures and Correlations

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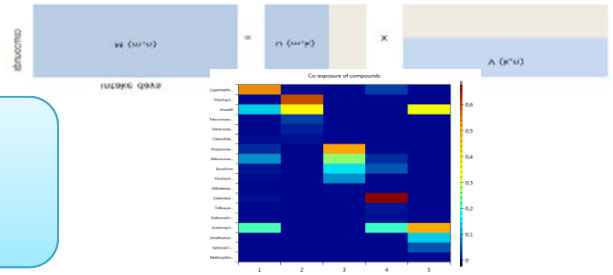
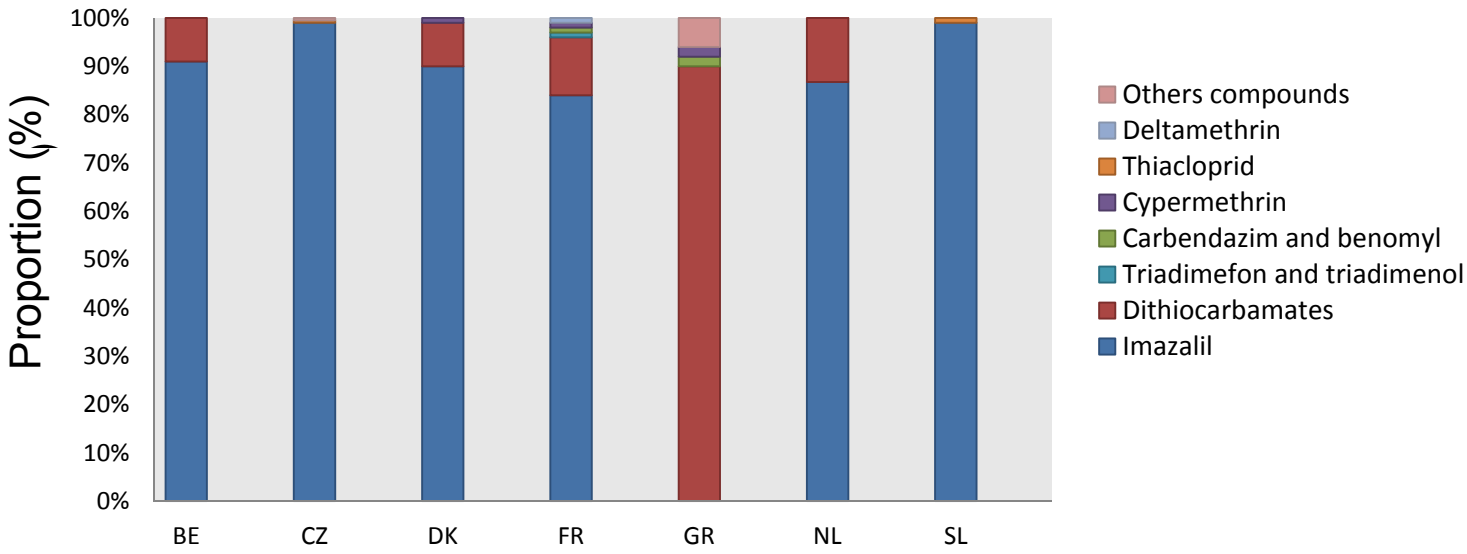


EuroMix

# Steatosis, Adults, Chronic exposure (France)

| Main mixture | | | | |
|---|---|---|---|---|
| Variance explained by the mixture : 77.2% | | | | |
| **Substances** | **SNMU weight** | **Exposure µg/kg bw.d Mean and P95** | | **RPF** |
| Imazalil | 84% | 0.75 | 2.53 | 0.13 |
| Dithiocarbamates | 12% | 0.14 | 0.35 | 0.53 |
| Carbendazim and benomyl | 1% | 0.02 | 0.05 | 0.2 |
| Cypermethrin | 1% | 0.03 | 0.06 | 0.28 |
| Delthamethrin | 1% | 0.02 | 0.05 | 0.53 |

# Compounds of the main mixture per country




## Food drivers

Imazalil: oranges, grapefruit, mandarins, bananas

Dithiocarbamates: fungi, wine grapes, lettuce, apple and concumbers for Greece

# Conclusions and limits

## The developed methods lead to identify mixtures

- ✓ Based on observed combined exposures
- ✓ Considering dietary habits
- ✓ Identifying populations exposed to specific mixtures
- ✓ Using toxicological information such as CAG and NOEAL

→ Could be applied to environmental/aggregated exposure, contamination and biomonitoring data

## Results depend of the data

- ✓ A snapshot at a given time → need actualized data at European level
- ✓ Censored data → need appropriate method for mixtures
- ✓ CAG → continue efforts, extend to other substances than pesticides

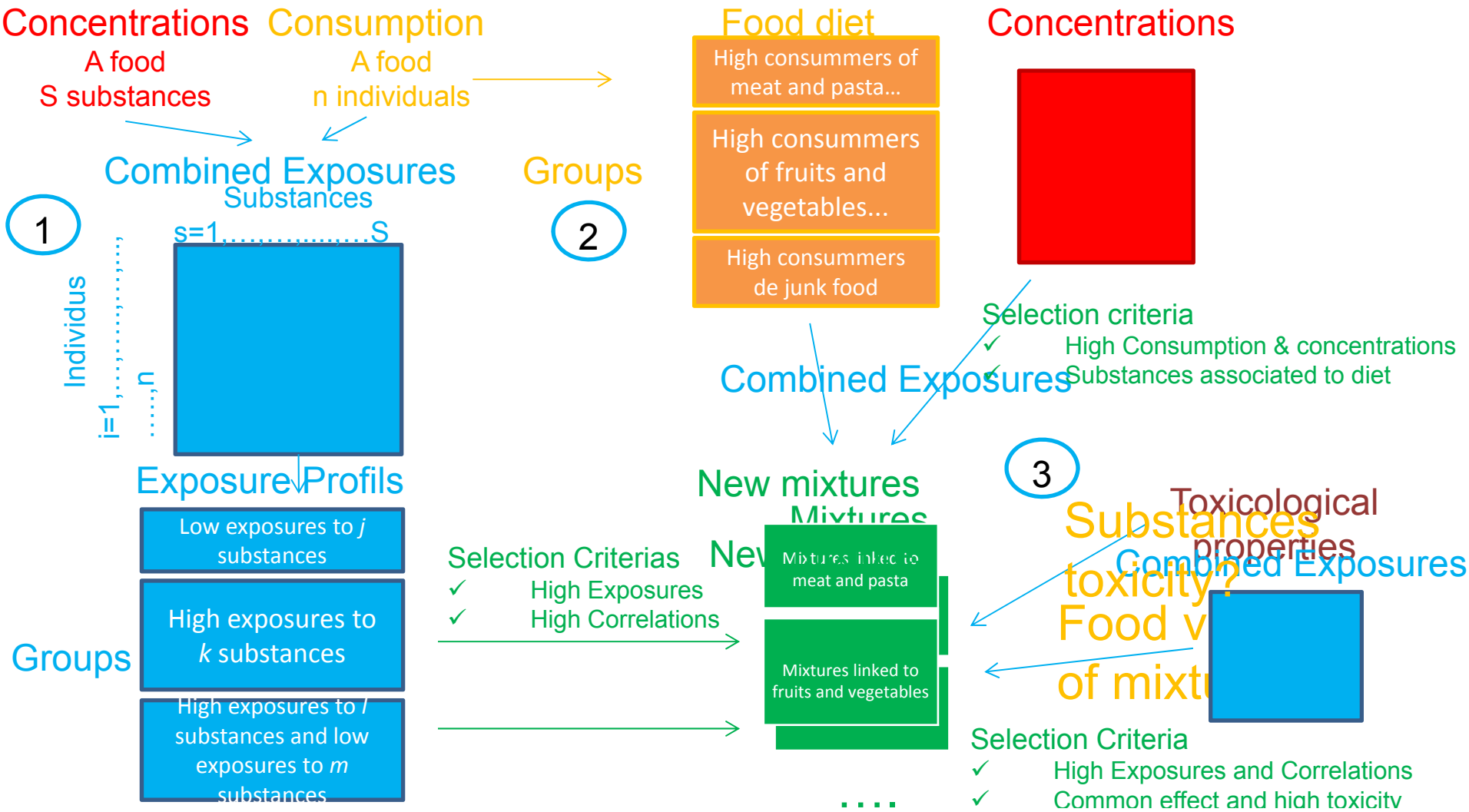
# Thanks to my coworkers

Anses-DER : Thiéma Traore, Marie Vanacker, Louis Trocellier, Véronique Sirot

PERICLES participants: Fanny Héraud, Jean-Charles Leblanc, Jessica Tressou, Vanessa Graillot, etc.

Euromix partners: in particular Jacob van Klaveren, Corinne Sprong, Hilko van der Voet, Waldo de Boer, Ad Peijninburg, Emiel Rorije, Angelo Moretto etc.

Thank you for your attention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