In vitro effects of mixtures of potentially endocrine-disrupting food contaminants

Hélène MOCHE[1], Laurence DAHBI[2], Mathilde HAULIN[1], Julie SANTOS[1], Marie BAILLIEUX[1], Manon BOURDEAU[3], Gaud DERVILLY[3], Isabelle SÉVERIN[2], Bruno LE BIZEC[3], Marie-Christine CHAGNON[2], Fabrice NESSLANY[1], Anne PLATEL[1]

[1] Univ. Lille, CHU Lille, Institut Pasteur de Lille, ULR 4483 - IMPECS - IMPact de l'Environnement Chimique sur la Santé humaine, F-59000 Lille, France
[2] UMR INSERM 1231, équipe NUTOX, Université de Bourgogne Franche Comté, F-21000 Dijon, France
[3] Oniris, INRAE, LABERCA, Nantes, France

INTRODUCTION

Food is one of the main sources of general population exposure to chemical substances. Indeed, food results from a complex chain of actions, including vegetal and animal productions and transformation, preservation, packaging, distribution and preparation processes, each of these steps potentially leading to food contamination by various substances. Consumers are thus exposed to mixtures of food contaminants, likely to vary according to their diet. Among these substances, some are, or are suspected to be, endocrine disruptors (ED).

Determination of diet-associated mixtures of potentially ED food contaminants

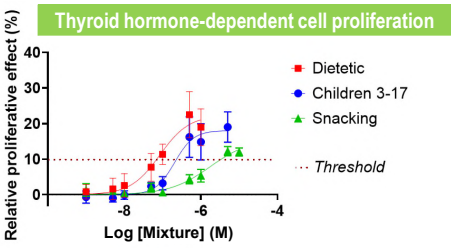
Based on food consumption data from the INCA2 survey (ANSES, 2009), we determined 7 main diets in the French adult population by Sparse and Unique Nonnegative Matrix Factorization and Hierarchical Clustering on Principal Components methods. Exposures to 78 substances of interest, selected using lists and databases of ED or suspected ED among contaminants measured in foods in the second French TDS (ANSES, 2011), were calculated for each of the 7 modelled adult diets as well as for a 3 to 17 years-old children diet. For these 8 diets, substances with highest exposures were selected to define the composition of diet-associated mixtures.
The endocrine activity of these substances, isolated and mixed according to proportions representative of dietary exposure for each diet, was then assessed using several bioassays.
For cell-based assays, only concentrations inducing less than 20% decrease in cell viability were analysed.

Molar proportions (%) of substances in diet-associated mixtures — Results of bioassays for substances / mixtures	Heavy metals		Pesticides					Phytoestrogens					ER transactivation	AR transactivation	TR T-Screen assay	Steroidogenesis		Iodide uptake (IC50)	TPO activity (IC50)
	Cadmium	Lead	Piperonyl butoxide	Chlorpropham	Imazalil	Iprodione	Propargite	Resveratrol	Daidzein	Genistein	Enterolactone	Equol				Estradiol	Testosterone		
Pleasant & convenient	26.3%	14.2%	10.9%	11.6%	5.6%	9.5%	8.5%	13.4%	-	-	-	-	Inactive	Inactive	Inactive	↘	↘	↘ (57 µM)	↘ (53 µM)
Basic	23.9%	11.5%	8.2%	15.4%	7.4%	8.4%	8.8%	16.4%	-	-	-	-	Inactive	Inactive	Inactive	↘	↘	↘ (62 µM)	↘ (52 µM)
Traditional	21.3%	17.1%	6.9%	8.9%	4.8%	11.5%	11.1%	18.3%	-	-	-	-	Inactive	Inactive	Inactive	↘	↘	↘ (65 µM)	↘ (60 µM)
Simplicity	24.4%	13.1%	8.9%	11.7%	6.1%	7.6%	10.1%	18.2%	-	-	-	-	Inactive	Inactive	Inactive	↘	↘	↘ (51 µM)	↘ (53 µM)
Mediterranean	22.1%	11.9%	7.6%	8.4%	6.2%	9.3%	12.8%	21.5%	-	-	-	-	Inactive	Inactive	Inactive	↘	↘	↘ (52 µM)	↘ (46 µM)
Dietetic	19.9%	11.1%	6.3%	-	-	11.8%	13.7%	12.9%	13.1%	11.2%	-	-	Agonist	Inactive	Partial agonist	↗	↘	↘ (126 µM)	↘ (70 µM)
Snacking	25.8%	13.1%	12.4%	16.1%	6.6%	8.0%	-	12.3%	5.7%	-	-	-	Agonist	Partial agonist	Partial agonist	↘	↘	↘ (32 µM)	↘ (79 µM)
Children 3-17 years-old	22.3%	11.0%	9.3%	14.1%	-	-	5.5%	21.0%	-	-	8.4%	8.5%	Agonist	Inactive	Partial agonist	↗	↘	↘ (26 µM)	↘ (51 µM)
ER transactivation	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Weak agonist	Agonist	Agonist	Partial agonist	Agonist							
AR transactivation	Inactive	Inactive	Inactive	Partial agonist	Inactive	Inactive	Inactive	Partial agonist	Partial agonist	Partial agonist	Inactive	Inactive							
T-Screen	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Partial agonist	Partial agonist	Inactive	Partial agonist							
Steroidogenesis: Estradiol	Inactive	↘	Inactive	↗	↘↘	Inactive	Inactive	↗	↗	↗	Inactive	↗							
Steroidogenesis: Testosterone	Inactive	Inactive	Inactive	Inactive	↘↘	↘	Inactive	↘	↘	↘	Inactive	↘							
Iodide uptake (IC50)	Inactive	Inactive	↘ (33 µM)	↘ (24 µM)	↘ (43 µM)	Inactive	↘ (partial)	Inactive	Inactive	Inactive	Inactive	Inactive							
TPO activity (IC50)	Inactive	Inactive	Inactive	Inactive	↘ (171 µM)	Inactive	Inactive	↘↘ (3 µM)	↘ (1947µM)	↘ (516 µM)	↘ (250 µM)	↘ (240 µM)							
Cytotoxicity	+++						+++												

Interference with thyroid hormones activity & synthesis

T-Screen assay

After a 5-day exposure period (-/+ T3), thyroid hormone-dependent GH3 cell proliferation was assessed.
Mixtures associated to **Dietetic, Children and Snacking** diets, containing daidzein, genistein and/or equol, **induced cell proliferation** in absence of T3 and increased T3-induced proliferation. Dietetic mixture decreased T3-induced cell proliferation at higher concentration.

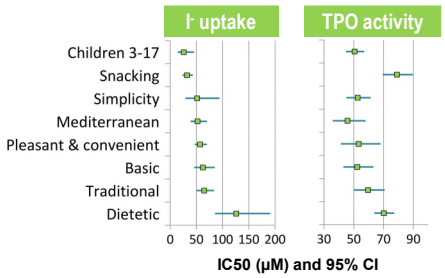


Iodide uptake assay based on Sandell-Kolthoff reaction

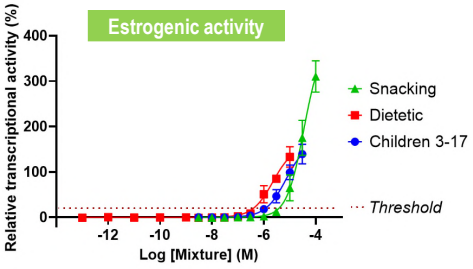
I^- uptake was measured in FRTL-5 cells exposed to mixtures or substances for 60 min in presence of NaI.
All mixtures decreased I^- uptake, with an higher IC50 for the chlorpropham-free Dietetic mixture.

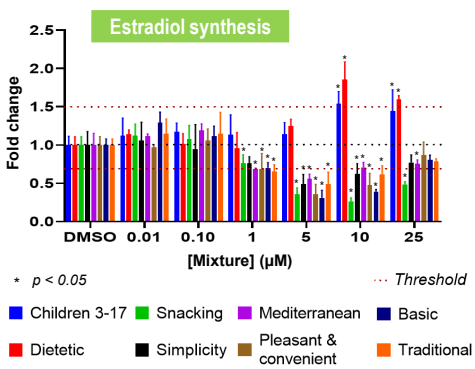
Thyroid peroxidase (TPO) inhibition assay

TPO activity was determined based on luminol oxidation method in Nthy-ori 3-1 cell lysates exposed for 30 min.
All mixtures inhibited TPO activity, with IC50 globally related to their proportion of resveratrol.



Interference with ER, AR & steroidogenesis (OECD TG Nos. 455, 458 & 456)





Estrogen receptor (ER) transactivation assay

Luciferase activity was measured in VM7Luc4E2 cells after a 24-h incubation (-/+ E2).
Mixtures associated to **Dietetic, Children and Snacking** diets, containing phytoestrogens other than resveratrol, were **ER agonists**.
None of the mixtures induced anti-estrogenic activity.

Androgen receptor (AR) transactivation assay

Luciferase activity was measured in AR-EcoScreen™ cells after a 24-h incubation (-/+ DHT).
Only the mixture associated to **Snacking** diet, with daidzein and without propargite, was **partial AR agonist**.
None of the mixtures induced anti-androgenic activity.

H295R steroidogenesis assay

H295R cells were exposed to mixtures or substances for 48h before estradiol and testosterone measurement.
Mixtures associated to **Dietetic and Children** diets **increased estradiol synthesis**. All other mixtures, containing imazalil, **decreased estradiol synthesis**.
All mixtures **decreased testosterone synthesis**.

CONCLUSION

Mixtures of potential ED associated to five adult diets were of close composition and induced similar effects. The presence of phytoestrogens other than resveratrol and/or the absence of imazalil, chlorpropham or propargite in mixtures associated to the other two adult diets and to the 3-17 years-old children diet could explain the different responses observed for these mixtures in some assays.

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