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SCIENCE FOR A SUSTAINABLE DEVELOPMENT

FOOD INTERACTIONS : EFFECTS ON HEALTH, CONSUMER PERCEPTION AND IMPACT ON AGRO-FOOD INDUSTRIES

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M. MORMONT, M. MULLER, G. MAGHUIN-ROGISTER, M.-L. SCIPPO, E. VAN DER HEIDEN, L. RIBONNET, Y.LARONDELLE, Y.-J. SCHNEIDER, F. CALLEBAUT, L. PUSSEMIER, S. DE VOGHEL, A. COVACCI, I. NOBELS, J. ROBENS, S. DE SAEGER, J. DIANADIMAVUNGU

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FINAL REPORT PHASE 1
SUMMARY

FOOD INTERACTIONS : EFFECTS ON HEALTH, CONSUMER PERCEPTION AND IMPACT ON AGRO-FOOD INDUSTRIES

"FOODINTER"

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Marie-Louise Scippo, Guy Maghuin-Rogister, Marc Muller
Marc Mormont
Université de Liège (ULg)
Faculté de médecine vétérinaire
Département des sciences des denrées alimentaires
Bd de Colonster, 20, bat b43b, Sart Tilman, B-4000 Liège.
TEL : + 32 4 366 40 40
FAX : + 32 4 366 40 54
MLScippo@ulg.ac.be

Yves-Jacques Schneider, Yvan Larondelle
Université catholique de Louvain (UCL)

Luc Pussemier
Centre d'Etudes et de recherches vétérinaires
et agrochimiques (CERVA/CODA)

Ronny Blust, Wim De Coen, Johan Robbens
Universiteit Antwerpen (UA)

Sarah De Saeger, Carlos Van Peteghem
Universiteit Gent (UGent)

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BELGIAN SCIENCE POLICY

Rue de la Science 8
Wetenschapsstraat 8
B-1000 Brussels
Belgium
Tel: +32 (0)2 238 34 11 – Fax: +32 (0)2 230 59 12
http://www.belspo.be
project website : http://www.adaoa.ulg.ac.be/foodinter.htm

Contact person: Christine Mathieu
+32 (0)2 238 34 93

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Marc Mormont, Marc Muller, Guy Maghuin-Rogister, Marie-Louise Scippo, Edwige Van der Heiden, Laurence Ribonnet, Yvan Larondelle, Yves-Jacques Schneider, Fons Callebaut, Luc Pussemier, Simon de Voghel, Adrian Covacci, Ingrid Nobels, Johan Robens Sarah De Saeger, José Dianadimavungu. ***Food Interactions : Effects On Health, Consumer Perception And Impact On Agro-Food Industries "Foodinter"*** Summary Phase 1 Brussels : Belgian Science Policy 2009 – 8 p. (Research Programme Science for a Sustainable Development : Final Report Phase 1)

SUMMARY

Food supplements and functional food become of increasingly great interest, as they are now consumed by more and more people These food supplements (e.g. nutrients, vitamins, hormones, amino acids, anti-oxidants,), as well as functional food (e.g. phytosterols or omega-3 fatty acids enriched food) occupy a position between food and drugs. Botanical materials represent a large segment of this class of products (e.g. soy isoflavones, yam or hop extracts).

In many cases, there are still a number of unknowns such as the identification of specific active components and impurities, the effects of processing, the presence of toxic compounds, as well as their absorption and metabolism in the human body.

In the past, the attention of food toxicologists has been focused on the toxicity of single contaminating substances. The interactions between active and potentially toxic substances are poorly documented. Interactions can lead to additive or subtractive or even synergistic effects, which are being studied in FOODINTER.

Furthermore, this project aims to promote the communication between scientists and stakeholders (authorities, producers and consumers). In the field of food consumption, this objective is important because food safety depends not only on production and control, but also on consumption practices and good information must therefore be promoted. It is not only an education plan and the objective is also to promote a dialog between science and society in order to better identify the social preoccupations and need that research has to satisfy.

The objective of this project is thus to contribute to the risk assessment of chemicals, natural compounds and environmental contaminants, present in food supplements which could interact between them or with micro or macronutrients of normal human diet.

Interactions studies have been performed using existing *in vitro* models (based on culture of various cell types, prokaryotes and eukaryotes) with mixture of active substances at concentrations not yet studied until now and very close to the real situation in human nutrition. Extrapolation from the *in vitro* observations to the real risks for human will be attempted.

In phase I of the FOODINTER project, 3 steps were performed:

1. Preliminary information collection
2. Collection of samples, analysis of chemical contaminants and active ingredients, and construction of a data base
3. Biological *in vitro* assays

1. Preliminary information collection

Within this task, several sources of information were consulted in order to gather basic information on food supplements but also in order to prepare the collection of samples on which the experimental studies will be performed. Different approaches were followed such as overview of the Belgian and European legislations, list of products notified in Belgium, overview on the information and choice of products available on the internet and in commercials,

overview of the scientific literature dealing with health effects and interactions, contacts with producers and with consumers organized by means of surveys and focus group meetings, etc.

The European Directive 2002/46/EC stated food supplements (FS) as "*foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities*". Examples of FS are ampoules of omega-3, tablets of vitamin A, tablets of multi-vitamin and multi-mineral capsules or capsules of plant extracts such as valerian, garlic,…

The present project will focus more precisely on the botanical preparations marketed as FS.

In Belgium, regulation of FS is grounded on two main Royal Decrees. The first one tackles the issue of Nutrients and their use into food supplements (AR 3/03/92). Among definitions stated in the documents, FS are defined as "*pre-dosed foodstuffs containing one or several nutrients, plants or plant preparations, or any other substance having a physiological or nutritive effect and which goal is to supplement normal diet.*" whereas nutrients are "*nutritive substances needed by the human organism*".

This Royal Decree mentions the notifying process through which a FS has to go in order to be marketed in Belgium. Maximal and minimal limits in terms of % of the Dietary Reference Intake (DRI) are fixed for different nutrients used in FS. Guidelines are detailing the labelling and the advertising of these FS.

The second Royal Decree concerns plants and plant preparations (AR 29/08/1997). It contains a list of dangerous plants whose use for direct consumption or as ingredient of preparation is strictly prohibited. Besides, another list gathers all plants allowed for direct consumption or as ingredient of preparation as long as a notification file has been accepted by federal authorities.

The Food Consumption Study performed by the Scientific Institute of Public Health in 2004 provides us data on FS consumption within the Belgian population (De Vriese et al., 2006). The study showed that 12 % of the population has included FS in their diet.

Consumer opinion was collected via surveys and focus group. From a first analysis of the results, it appeared that : 1) people do not exactly know what kind of preparations can be categorized as food supplements (a lot of hesitation for vitamins and plant extracts), 2) women seem to consume food supplements more often than men, 3) 37% of questioned people do consume food supplements from their own initiative (without medical advice) , 4) the main purpose of consuming food supplements is, according to consumers, to reinforce the immune system of the organism and to fight against tiredness (obviously for vitamins and mineral), 5) a lot of consumers are regular customers but mostly the money spend for buying food supplements is less than 50 Euros, 6) most of the consumers do read the label and are convinced of the beneficial effects as they are described in the label, 7) the majority of the questioned people do believe that food supplements are "natural" but seem to be aware that simultaneous intake of drugs can pose a health risk.

2. Collection of samples, analysis of chemical contaminants and active ingredients and construction of a database

The final selection was made of six different FS all made from one specific plant material:

- **Garlic (G):** Decreases arterial tension; very common botanical product, interactions with drugs

- **Ginkgo biloba (B)**: Improves blood circulation and cerebral oxygenation; very common botanical product, interactions with drugs

- **Sint-John's Wort (W):** Against mild depressions; very common botanical product, interactions with drugs

- **Soy isoflavones (I):** Reduces menopause effects; frequently used; hormonal activity

- **Maca (M):** Increases libido and limit sexual disorders; plant toxins (alkaloids); less studied

- **Black radish (R):** Stimulation of bile secretion and of intestine activity; plant toxins (glucosinolates); less studied

In total, 61 samples were thus collected. They were purchased from 37 companies via internet (36 samples), drugstores (18 samples) and specialized shops (7 samples). 25 are notified in Belgium whilst 36 are not notified (and generally available via the internet). This material was used to perform the analyses of chemical contaminants, of active ingredients as well as for the *in vitro* studies.

A data base has been compiled using all the relevant information on the uses of FS, the nature of their active ingredients, the **contents of active ingredient** s in the FS, the intake of active ingredient according to the recommended doses, the methods of analysis, the **biological studies performed on the active ingredients**, the **chemical contamination** of FS, etc

Analysis of chemical contaminants

Analysis of mineral elements

Seventeen trace elements (As, Ba, Bi, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Tl, Zn) were quantified by inductively coupled plasma with mass spectrometer (ICP-MS). Mercury (Hg) was quantified by Advanced Mercury Analyzer (AMA).

There were 10 non compliant (NC) samples with respect to the Belgian legislation for toxic element in FS (7 NC for Pb and 4 NC for Cd; one sample exceeded the norm for both elements).

Analysis of mycotoxins

The target mycotoxins included nivalenol (NIV), deoxynivalenol (DON), neosolaniol (NEO), fusarenon-X (F-X), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), diacetoxyscirpenol (DAS), HT-2 toxin (HT-2), T-2 toxin (T-2), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A

(OTA), altenuen (ALT), alternariol (AOH), alternariol methylether (AME), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), zearalenon (ZEA), beauvericin (BEAU), sterigmatocystin (STERIG). They were analyzed using gradient reversed-phase liquid chromatography (RP-LC) with electrospray ionization tandem mass spectrometry (ESI-MS/MS).

The toxins FB1, FB2, FB3 and OTA were detected in some samples. In 2 samples (one of Gingko Biloba and one of Maca), OTA was found at a level above 2 µg/kg (EC norm for wine and grape juice, Regulation 1881/2006/EC). The levels of FB1, FB2 and FB3 were largely below 800 µg/kg (EC norm for the sum of FB1 and FB2 in breakfast cereals, Regulation 1881/2006/EC) in all samples.

Analysis of PAHs

High performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector (HPLC/UV-FLD) has been used to detect the 15(+1) EU priority PAHs in the sixty food supplements selected in this project.

The results have shown that S^{t}-John's wort and ginkgo biloba extracts presented the most frequent contaminations and the highest average values for PAHs concentrations.. The most contaminated samples with the sum of the 16 PAHs were generally detected in St-John's wort and ginkgo products, except one sample of soy isoflavones .

From a preliminary risk assessment, we found five that food supplements were health concern (the daily intake from FS could be higher that the tolerable daily intake), one Black radish FS, one Ginkgo biloba FS, two St John's Wort FS and one Gralic FS. All are not notified.

Analysis of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and of polybrominated diphenyl ethers (PBDEs), and dioxins in oily FS

Low amounts of p,p'-DDE or p,p'-DDD (< 10 µg/Kg) were sdetected in 3 garlic samples, but far below the legal limit of 50 µg/Kg for the sum of DDT. Dioxins, PCBs and PBDEs were below the limit of quantification of the assay.

Identification and analysis of active ingredients

Relevant active ingredients were identified from the literature in the 6 categories of food supplements.

These active ingredients are listed below.

St John's wort	Ginkgo biloba	Soy isoflavones	Black Radish	Garlic	Maca
Hypericin	Ginkgolide A	Genistein	L-sulforaphane	Garlic oil	lepidilin A
Hyperforin	Ginkgolide B	Daidzein	DL-sulforaphane	S-allyl cysteine	Lepidlin B
	Ginkgolide C	Glycetein	Glucoraphanin	Allicin	Macaridin
	Ginkgolide J				MTCA
	bilobalide				
	Isorhamnetin				
	kaempferol				
	Quercetin				

These active ingredients are being analyzed using chemical methods (HPLC or LC-MS) in order to determine the real content of the chosen food supplements in these active ingredients.

Black radish and maca active ingredients have been analyzed so far.

The aim of the project is to determine the effects of these active ingredients on some physiological functions, using *in vitro* assays.

In order to perform these *in vitro* tests at realistic concentration in active ingredients, we calculated what we call "working concentrations", which correspond to plausible concentration of active ingredients at the human intestinal level, taking into account of the active ingredient content of FS and of the recommended daily intake of the FS.

So far, these realistic concentrations were calculated for St John's Wort, Gingko biloba and soy isoflavones.

3. Biological *in vitro* assays

The active ingredients (AI) were analyzed using several *in vitro* assays, in order to study :

- the general toxicity of the AI, on bacteria and eukaryotic cells (HepG2 and Caco2)
- the effect on targeted genes involved in oxidative damage, membrane damage, cellular stress and DNA damage
- the hormonal and dioxin-like activity using luciferase reporter gene assays
- the effect on human CYP1A and CYP3A4 activity in intestinal Caco2 cells

These analyses are ongoing and will be continued in the phase 2 of the project.

Detailed results are given in the text.

For example, we have evidenced that some AI of gingko biloba (flavonoids such as isorhamnetin, kaempferol and quercetin) are able to inhibit the dioxin activation of the Aryl Hydrocarbon receptor (Ahr), in hepatoma cells, and can also inhibit the BaP induced CYP1A1 activity in intestinal cells.

This kind of activity be may be considered as positive regarding detoxification of carcinogenic food contaminants such as PAHs.

Complete conclusions on the possible effects on physiological functions of active ingredients, at realistic concentrations, will be given at the end of phase II of the FOODINTER project, when a complete picture of all the *in vitro* effects measured with both pure standards of active ingredients and food supplements extracts will be available.