

Etablierung und Validierung von durchsatzfähigen Methoden zur detaillierten Analyse von Allergenprofilen in Lebensmitteln und zur individuellen Charakterisierung deren allergener Wirkung in Patienten

***Outcome and lessons learnt from
the ALLERGEN-PRO project***

ALLERGEN-PRO

Project title: **Establishment and validation of throughput-capable methods for the detailed analysis of allergen profiles in foods and for the individual characterization of their allergenic effect in patients**

Funder: Bundesministerium für Ernährung und Landwirtschaft

Call title: *Promotion of innovations for solutions for the reduction and detection of allergens and intolerance-triggering substances in food, consumer goods and cosmetic products*

Official duration: 3 years, from **1.9.2020** to **31.8.2023**

Partners (other than BfR):

- Signatope GmbH, Reutlingen
- HOT Screen GmbH, Reutlingen
- Hochschule Albstadt-Sigmaringen
- Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen, Reutlingen
- Charité Universitätsmedizin, Berlin
- Société des Produits Nestlé, Vevey, Switzerland



ALLERGEN-PRO: goals of the project

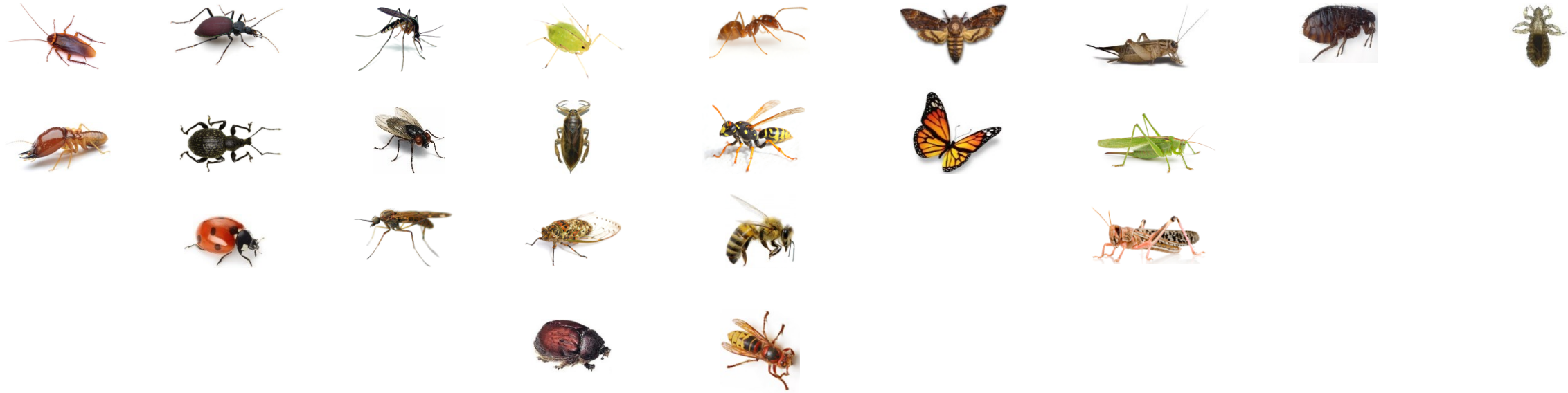


Provide **improved analytical methods** (DNA and/or protein-based) for the reliable detection of allergenic components derived from **insects** in different food matrices. Improve the safety for allergy sufferers and food manufacturers through the development of i) a novel ***in vitro* detection system** of allergenic IgE / IgG epitopes, and ii) a new ***in vitro* diagnostic system** of antigen-specific reactions of immune cells from allergy sufferers to certain allergens.

Allergies to insects

INSECTA

Blattodea Coleoptera Diptera Hemiptera Hymenoptera Lepidoptera Orthoptera Phthiraptera Siphonaptera



Inhalation Inhalation, ingestion Bite Inhalation, ingestion Sting Inhalation, ingestion Inhalation, ingestion Bite Bite

Food allergies to insects and by-products



grasshoppers and locusts



lentil pest



cicadas



ant eggs



mealworms



honey



silkworm pupae



Sago worms



Mopane worms



Bee larvae



cochineal red dye

Reported allergic reactions after insect consumption

- **silkworms** *Cheng et al., 1987; Ji et al., 2008; Gautreau et al., 2017; Chomchai et al., 2020*
- **mealworms** *Freye, 1996; Beaumont et al., 2019*
- **cicadas** *Piatt, 2005*
- **lentil pest (*Bruchus lentis*)** *Amentia et al., 2006*
- **caterpillars** *Inal et al., 2006; MacKinnon et al., 2015; Chomchai et al., 2020*
- **cochineal red dye** *Kotobuki et al., 2007*
- **grasshoppers** *Jirapongsananuruk et al., 2007; Piromrat et al., 2008; Ji et al., 2009; Chomchai et al., 2020*
- **bee pupae, bee larvae and moths** *Ji et al., 2009*
- **mopane worms (*Gonimbrasia belina*)** *Okezie et al., 2010; Kung et al., 2011*
- **sago worms (*R. ferrugineus*)** *Yew and Kok, 2012*
- **ant eggs** *Chansakulporn and Charoenying, 2012*
- **honey** *Veziir et al., 2014*
- **stink bugs** *Barennes et al., 2015*
- **psocids** *Bebaie and Vadas 2020*
- **crickets** *Chomchai et al., 2020; de las Marinas et al., 2021*

ADOPTED: 5 October 2015

PUBLISHED: 8 October 2015

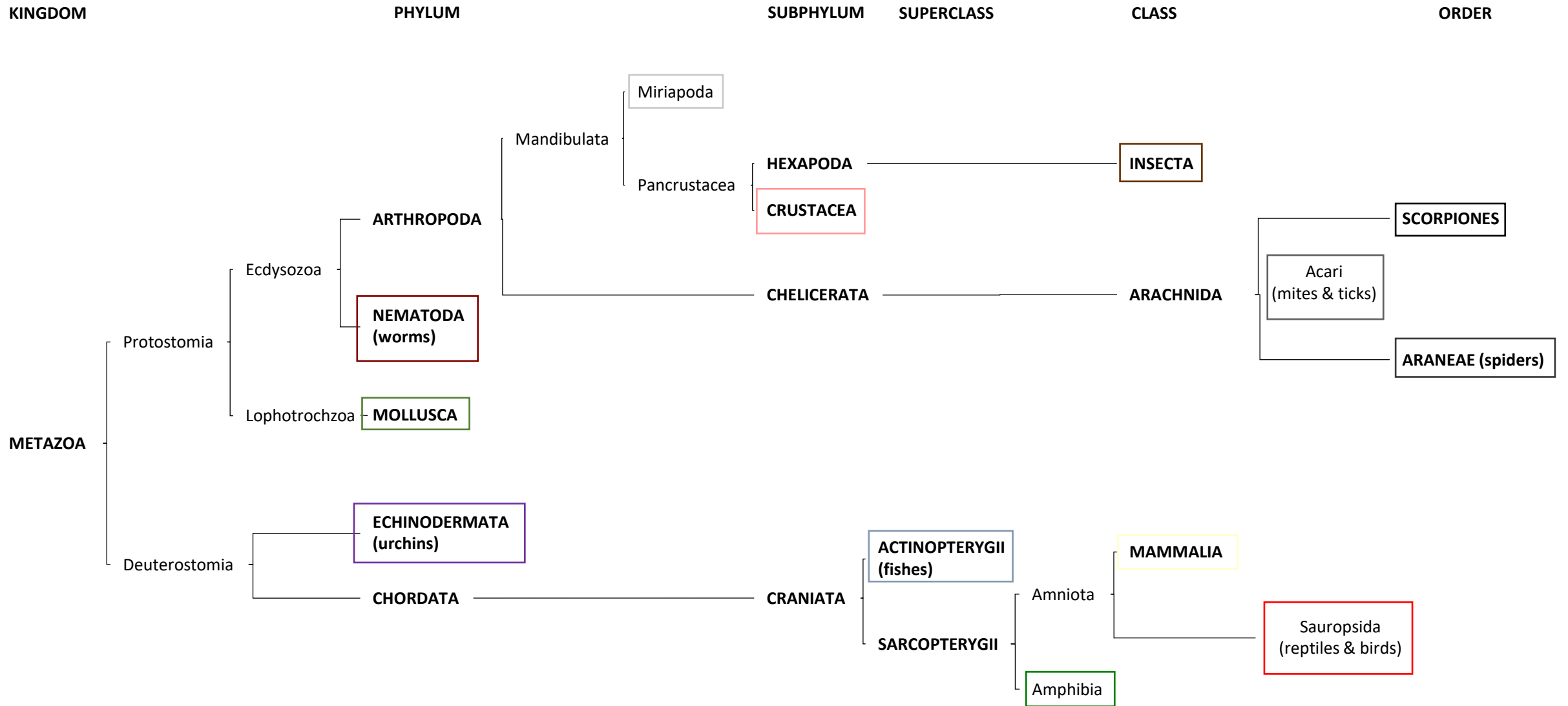
doi:10.2903/j.efsa.2015.4257

Risk profile related to production and consumption of insects as food and feed

EFSA Scientific Committee

*The risk of allergies to insects in the case of insects as a source of food or feed proteins is **plausible**, and may be based on the existence of **common allergens** (pan-allergens) of arthropods such as arachnids, **crustaceans** (lobster, shrimp, crab), myriapods and insects. Similarly, allergens of **molluscs** and helminths are often very similar to those of insects and may lead to cross-allergies. The more or less close **phylogenetic relationships** between the different classes of arthropods may explain **sequence homologies** and similarities in structure constituting B cell epitopes in common allergens (pan-allergen), responsible for possible **cross allergy** between **edible insects** and other arthropods, **mites** (arachnids), **crustaceans** and non-edible insects (**cockroaches**). Insect consumption by individuals allergic to e.g. dust mites or shrimp could therefore well trigger allergic reactions associated with this **cross-reactivity**.*

A taxonomic view



European point prevalence of **food challenge-verified** allergy to shellfish 0.1% (CI 95% 0.0–0.2)¹



¹ Spolidoro, Ali, Amera, Nyassi, Lisik, Ioannidou, Rovner, Khaleva, Venter, van Ree, Worm, Vlieg-Boerstra, Sheikh, Muraro, Roberts, Nwaru. Prevalence estimates of eight big food allergies in Europe: Updated systematic review and meta-analysis. *Allergy* 2023;00:1–57.

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R. Schmitz · M. Thamm

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Prävalenz von Sensibilisierungen gegen Inhalations- und Nahrungsmittelallergene

Ergebnisse der Studie zur Gesundheit
Erwachsener in Deutschland (DEGS1)

*Across all age groups and both sexes, the frequency of sensitization to *D. pt. (d1)* was 15.9% (95% confidence interval [CI] 14.8–17.1) of the population. [...]. Approximately 11 million adults in Germany are sensitized to house dust mites¹.*

¹Bergmann, *Allergo J Int* (2022) 31:279–283



Cross-reaction to shrimp allergens

15 individuals with shrimp allergy were included in a DBPCFC. Most had inhalant allergies to HDM (11 of 15) and pollen (11 of 15), and 9 patients had 1 or more other food allergies. 13 out of 15 had subjective (21.6 mg mealworm protein) or objective (216 mg) clinical symptoms

Patient	Sex (Male/Female)	Age (years)	0.01 g	0.1 g	1 g	3 g	10 g	30 g	60 g	Mealworm challenge Muller
			(2,16 mg)	(21,6 mg)	(216 mg)	(648 mg)	(2,16 g)	(6,48 g)	(12,96 g)	
A	F	46							OA, S, GI	2
B	F	23					OA, S, R	OA, S, GI	OA, S, GI, R	3
C	M	69			OA	OA			OA	0
D	M	45					S, GI			2
E	F	27		OA			OA	OA, S		1
F	M	19				S	GI			2
G	F	60				S			S	1
H	M	30						GI		2
I	M	27								Neg
J	F	47		S	S, R	S, GI, R				3
K	F	52								Neg
L	M	26						S, GI		2
M	M	34					OA	OA	OA	0
N	F	23			OA, S	OA	OA	OA, S		1
O	M	46		OA	OA		OA	OA, GI		2

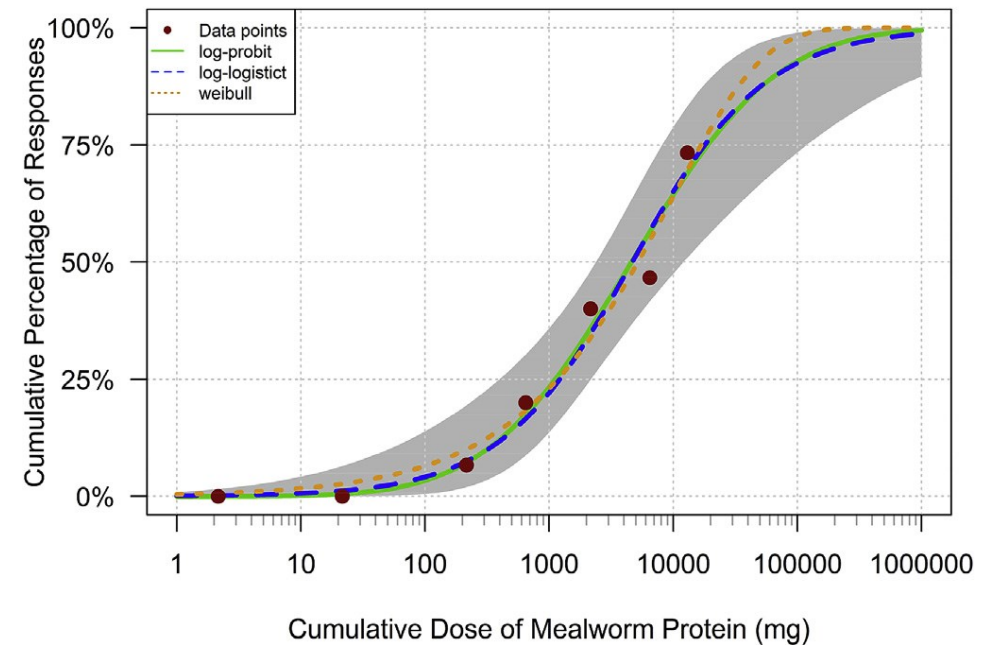
OA Oral allergy, local S Skin/mucosa GI Gastrointestinal R Respiratory C Cardiovascular Dose not given

Majority of shrimp-allergic patients are allergic to mealworm

Henrike Broekman, MD • Kitty C. Verhoeckx, PhD • Constance F. den Hartog Jager, BSc • ...
Carla A. Buijnzeel-Koomen, MD, PhD • Geert F. Houben, PhD • André C. Knulst, MD, PhD •
[Show all authors](#) • [Show footnotes](#)

Published: March 01, 2016 • DOI: <https://doi.org/10.1016/j.jaci.2016.01.005>

Check for updates



Predicted log-logistic, log-probit and Weibull distribution models of allergic response to mealworm (expressed as cumulative mg mealworm protein) intake. The gray area represents the confidence band for the log-probit model¹.

¹Garino et al. Food and Chemical Toxicology 142 (2020) 111460.

Cross-reaction to HDM allergens



Contents lists available at [ScienceDirect](#)

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Influence of processing and *in vitro* digestion on the allergic cross-reactivity of three mealworm species

Sarah van Broekhoven ^{a,*,1}, Shanna Bastiaan-Net ^{b,1}, Nicolette W. de Jong ^c, Harry J. Wichers ^b

10 out of 11 sera of HDM allergic patients without clinical history of crustacean allergy showed *in vitro* medium to high response to several extracts of raw, lyophilized, boiled or fried mealworms



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REVUE FRANÇAISE
D'**Allergologie**

Revue française d'allergologie 59 (2019) 389–393

Case report

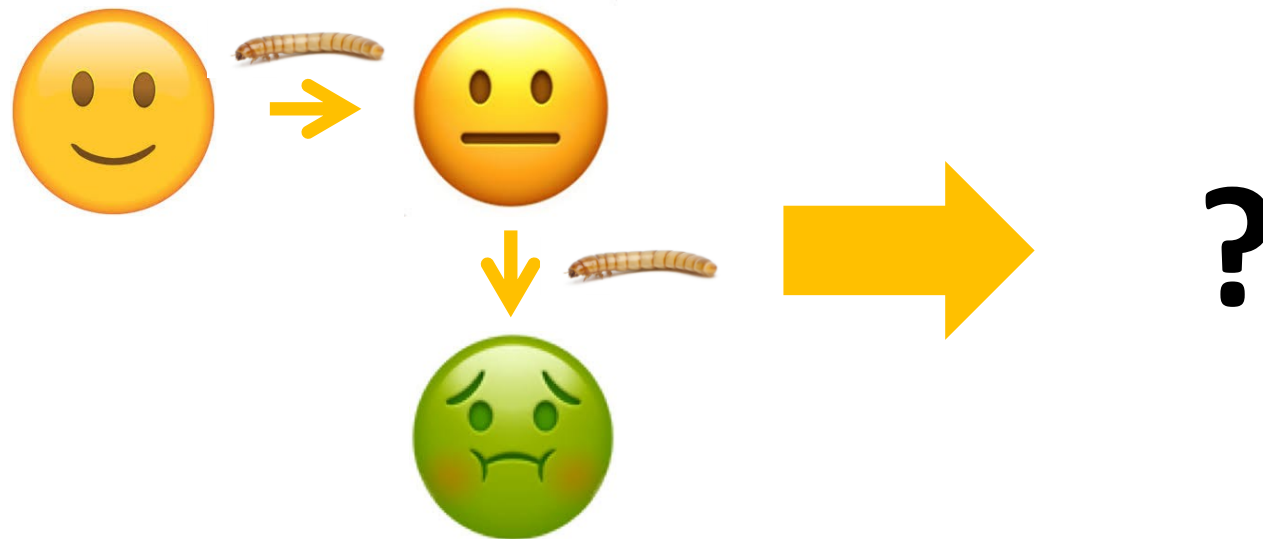
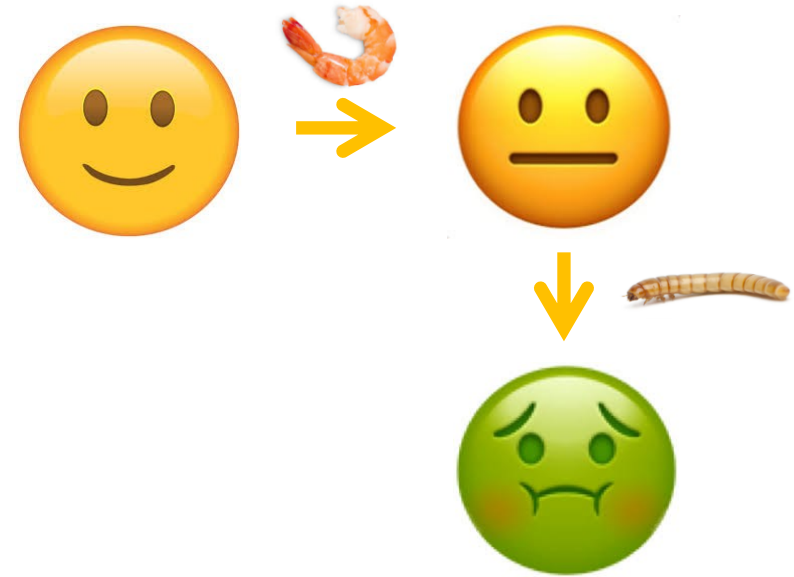
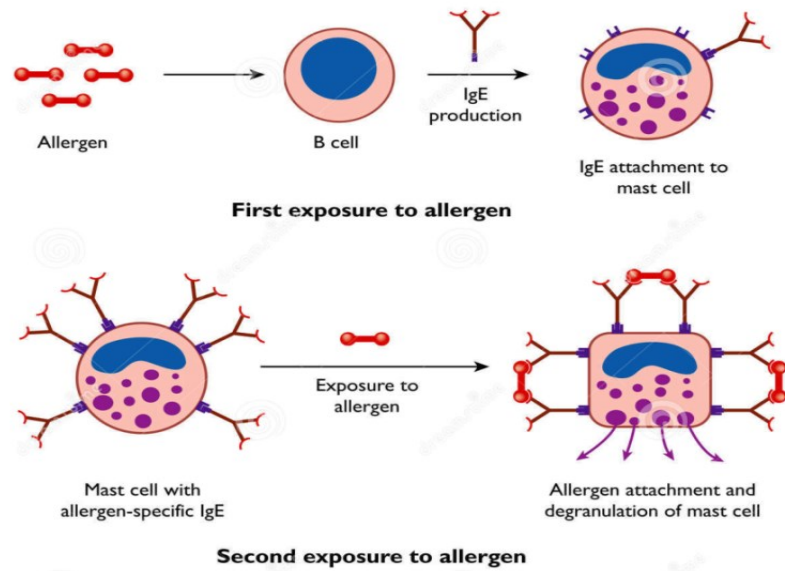
Food-induced anaphylaxis to *Tenebrio molitor* and allergens implicated

Anaphylaxie alimentaire à Tenebrio molitor et allergènes en cause

P. Beaumont ^{a,b,*}, J. Courtois ^{c,d}, X. Van der Brempt ^{a,e}, S. Tollenaere ^c

A man without food allergy, and whose history consisted solely of HDM allergy, had severe food anaphylaxis after eating cooked mealworm (*Tenebrio molitor*) larvae

Primary sensitization vs cross-reactivity



Insect primary sensitization



Four Dutch mealworm farmers were sensitized to **mealworm**, confirmed by skin prick test (SPT), immunoblot and basophil activation test (BAT). Only one patient had an allergy to **house dust mites** (HDM). They underwent a double blind placebo controlled food challenge (DBPCFC) with **mealworm snacks and shrimps**. 2/4 subjects (50%) reported a history of food allergic symptoms to mealworm, which was confirmed in the DBPCFC, starting at a dose of 0.1 g of mealworm. **None of the subjects reacted to shrimp**. Mealworm exposure is a **risk** for developing food allergy to mealworm



Letter to the Editor

Primary respiratory and food allergy to mealworm

[Henrike C.H.P. Broekman MD](#)^{a,d} ✉, [André C. Knulst MD, PhD](#)^{a,d},
[Constance F. den Hartog Jager BSc](#)^{a,d}, [Jolanda H.M. van Bilsen PhD](#)^{b,d},
[Florine M.L. Raymakers RN](#)^a, [Astrid G. Kruijzinga PhD](#)^{b,d}, [Marco Gaspari PhD](#)^c,
[Caterine Gabriele PhD](#)^c, [Carla A.F.M. Bruijnzeel-Koomen MD, PhD](#)^{a,d}, [Geert F. Houben PhD](#)^{a,b,d},
[Kitty C.M. Verhoeckx PhD](#)^{a,b,d}



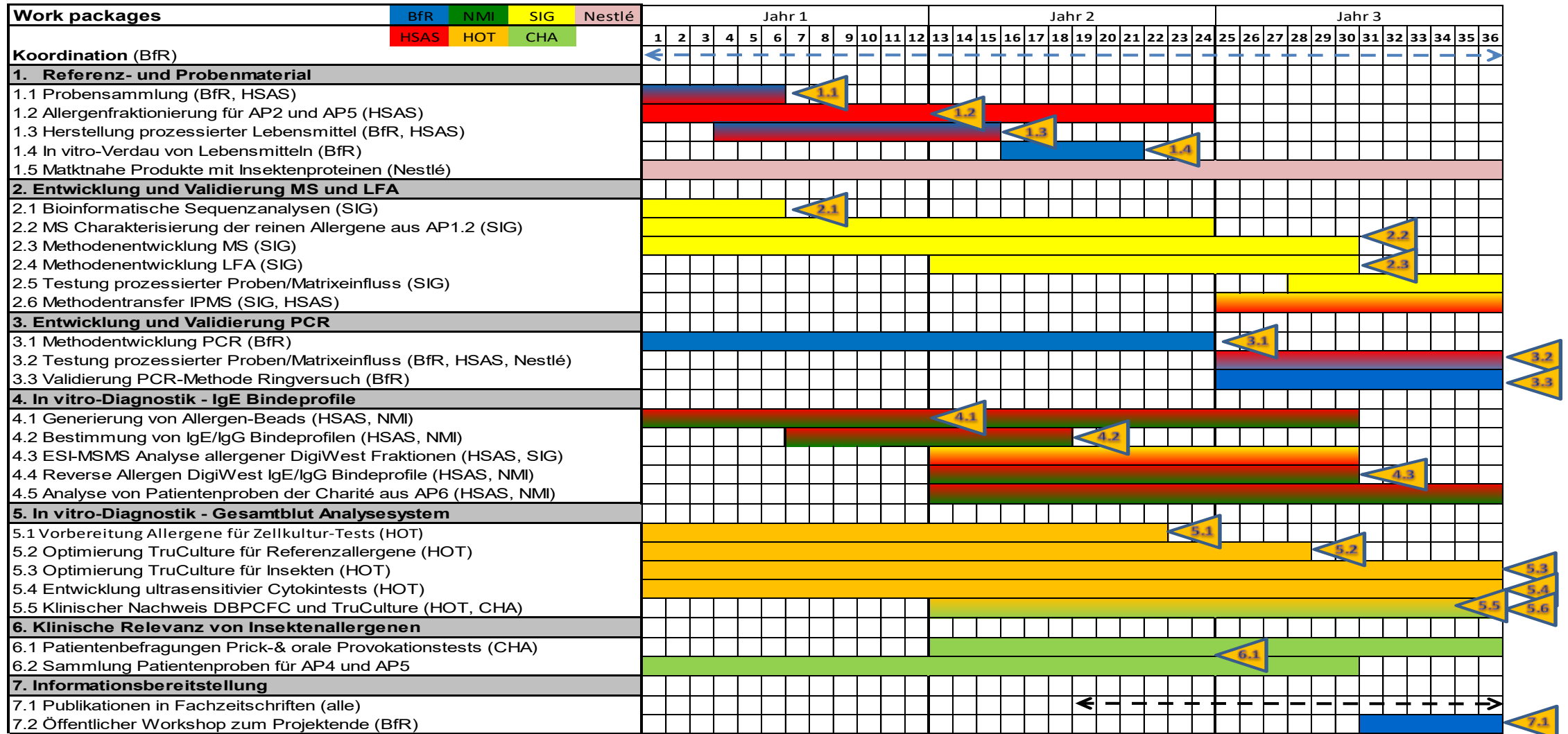
Exposure to larvae of *Tenebrio molitor* can lead to sensitization and subsequent development of allergic symptoms after ingestion of mealworms

Novel food applications submitted to EFSA



Question Number	Subject	Status	Last Updated	Reception Date	Applicant	Country	RA deadline
EFSA-Q-2023-00375	Dafatted Whole Cricket Powder (NF-2023-15560)	Intake	26/10/2023	30/5/2023	CRICKET ONE CO., LTD	Vietnam	
EFSA-Q-2023-00703	Application for authorisation of Dried defatted powder of <i>Hermetia illucens</i> larvae as a novel food (NF-2023-15216)	Intake	25/10/2023	24/10/2023	InnovaFeed	France	
EFSA-Q-2023-00374	<i>Acheta domesticus</i> (house cricket) spray-dried powder (NF-2022-5951)	Intake	13/10/2023	30/5/2023	Alia Insect Farm società agricola	Italy	
EFSA-Q-2022-00534	Vitamin D3 containing UV-treated mealworm oil (NF-2021-0039)	Ongoing Risk Assessment	16/10/2023	29/8/2022	Nutriearth	France	11/7/2024
EFSA-Q-2021-00105	Request for a scientific opinion on Protein-rich flour from fresh larvae of mealworm (<i>Tenebrio molitor</i>) as a novel food (NF 2020/1959)	Ongoing Risk Assessment	28/8/2023	24/2/2021	Ynsect NL B.V.	France	2/9/2023
EFSA-Q-2021-00262	Request for a scientific opinion on <i>Acheta domesticus</i> Flour as a novel food (NF 2020/1860)	Ongoing Risk Assessment	25/9/2024	11/5/2021	Italian Cricket Farm S.r.l	Italy	22/2/2024
EFSA-Q-2019-00690	Request for a scientific opinion on Protein powders from the <i>Alphitobius diaperinus</i> larva as a novel food (NF 2019/1292)	Ongoing Risk Assessment	29/9/2023	30/10/2019	Protifarm Holding N.V.	Netherlands	13/12/2023
EFSA-Q-2019-00589	Request for a scientific opinion on defatted whole cricket (<i>Acheta domesticus</i>) powder as a novel food (NF 2019/1227)	Finished and approved	13/5/2022	10/9/2019	CRICKET ONE CO., LTD	Vietnam	
EFSA-Q-2019-00748	Request for a scientific opinion on UV-treated powder of whole yellow mealworm (<i>Tenebrio molitor</i>) larvae (NF 2019/1142)	Published	19/7/2023	21/11/2019	Nutriearth	France	
EFSA-Q-2019-00121	Request for a scientific opinion on Whole and ground crickets (<i>Acheta domesticus</i>) as a novel food (NF 2018/0804)	Finished and approved	7/7/2021	27/2/2019	Fair Insects BV (A Protix Company)	Netherlands	
EFSA-Q-2019-00115	Request for a scientific opinion on Whole and ground Grasshoppers (<i>Locusta migratoria</i>) as a novel food (NF 2018/0803)	Finished and approved	25/5/2021	22/2/2019	Fair Insects BV (A Protix Company)	Netherlands	
EFSA-Q-2019-00101	Request for a scientific opinion on whole and ground mealworms (<i>Tenebrio molitor</i>) larvae as a novel food (NF 2018/0802)	Finished and approved	7/7/2021	15/2/2019	Fair Insects BV (A Protix Company)	Netherlands	
EFSA-Q-2019-00046	Request for a scientific opinion on <i>Hermetia illucens</i> meal as a novel food (NF 2018/0765)	Ongoing Risk Assessment	29/3/2022	28/1/2019	Enorm Biofactory A/S	Denmark	27/9/2023
EFSA-Q-2019-00201	Request for a scientific opinion on <i>Apis mellifera</i> male pupae as a novel food (NF 2018/0754)	Ongoing Risk Assessment	22/9/2023	22/3/2019	The Finnish Beekeepers' Association	Finland	11/1/2024
EFSA-Q-2020-00748	Request for a scientific opinion on Dried <i>Acheta domesticus</i> as a novel food (NF 2018/0623)	Intake	7/5/2021	17/11/2020	National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives	Thailand	
EFSA-Q-2018-00746	Request for a scientific opinion on mealworm (<i>Tenebrio molitor</i>) as a novel food (NF 2018/0396)	Ongoing Risk Assessment	16/10/2023	28/9/2018	Belgium Insect Industry Federation (BiiF)	Belgium	29/3/2024
EFSA-Q-2018-00513	Request for a scientific opinion on <i>Locusta migratoria</i> as a novel food (NF 2018/0395)	Intake	7/5/2021	20/6/2018	Belgian Insect Industry Federation (BiiF)	Belgium	
EFSA-Q-2018-00263	Request for a scientific opinion on dried crickets (<i>Grylodes sigillatus</i>) as a novel food (NF 2018/0260)	Withdrawn	22/4/2022	21/3/2018	SAS EAP Group - Micronutris	France	
EFSA-Q-2018-00262	Request for a scientific opinion on dried mealworm (<i>Tenebrio molitor</i>) as a novel food (NF 2018/0241)	Finished and approved		21/3/2018	SAS EAP Group - Micronutris	France	
EFSA-Q-2018-00543	Request for a scientific opinion on <i>Acheta domesticus</i> as a novel food (NF 2018/0128)	Ongoing Risk Assessment	28/8/2023	3/7/2018	Belgian Insect Industry Federation (BiiF)	Belgium	17/4/2024
EFSA-Q-2018-00282	Request for a scientific opinion on whole and grinded lesser mealworm (<i>Alphitobius diaperinus</i>) larvae products as a novel food (NF 2018/0125)	Finished and approved	4/7/2022	10/4/2018	Ynsect NL B.V.	Netherlands	

ALLERGEN-PRO



Legende und Abkürzungen:



Balkenfarbe = Hauptanteil des Verbundpartners am Arbeitspaket



Meilenstein

BfR Bundesinstitut für Risikobewertung

NMI Naturwissenschaftliches und Medizinisches Institut

SIG Signatope

HSAS Hochschule Albstadt-Sigmaringen

HOT Hot Screen

CHA Charité

WP 1 Provision of reference and sample material



Main activities: sample procurement, allergen fractionation, production of processed foods, *in vitro* digestion

2 model foods

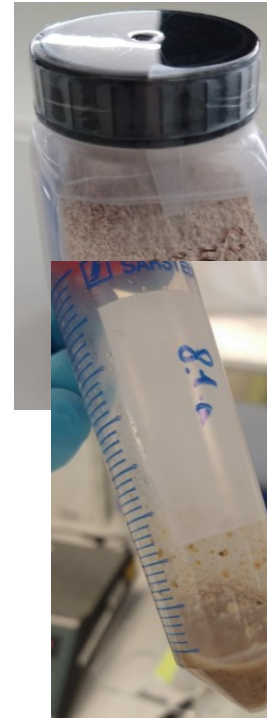
Experimental cookies:

- 3 levels of incurrence: 100 ppm, 20 ppm, 5 ppm
- 3 baking protocols: 180°C, 10' - 180°C, 20' - 210°C, 10'

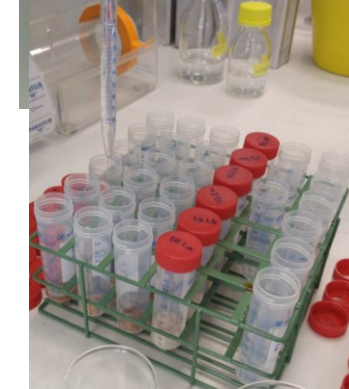


Experimental canned meat:

- 3 levels of incurrence: 100 ppm, 20 ppm, 5 ppm
- 3 cooking protocols: 70-80°C (pasteurization), ~100°C (cooking), 121°C (autoclaving).



INFOGEST static *in vitro* simulation of gastrointestinal food digestion



WP 1.4. *In vitro* digestion of food. 10 different food samples containing different amounts of *Tenebrio molitor* (0-47%) underwent *in vitro* digestion following the INFOGEST 2.0 protocol. Aliquots from each digestion step, as well as non-digested, were distributed to the Partners involved in the follow up analyses

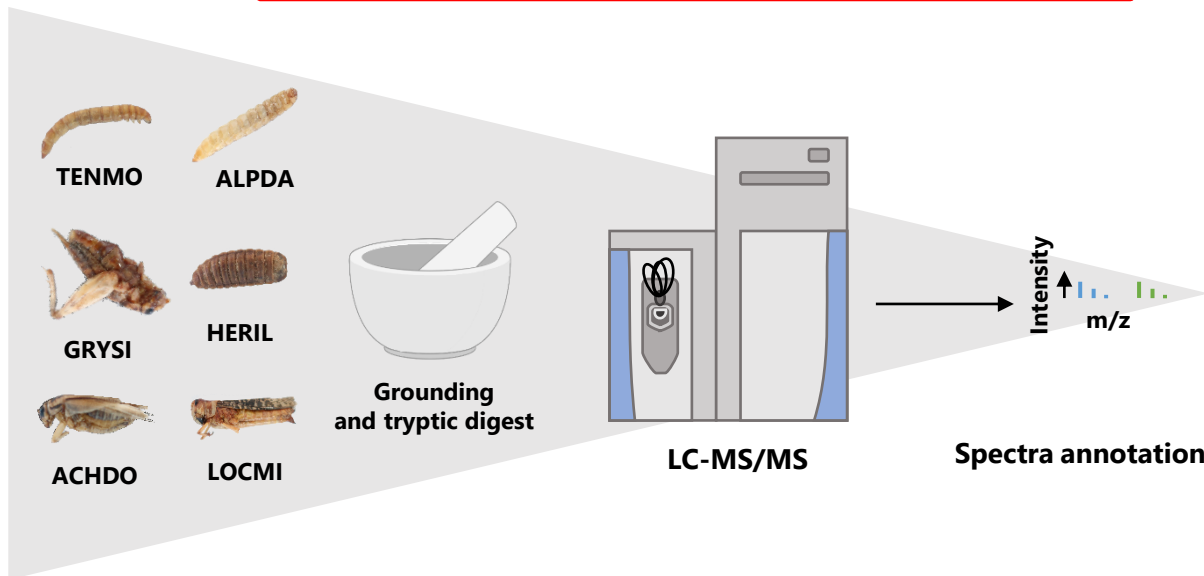
WP 2 Development and validation of mass spectrometry-based immunoassays and a peptide-centric lateral flow assay

Participants:  SIGNATOPE



Main activities: MS method development, LFA method development, method transfer

**Results presented at the IMSC
2022 Conference in Maastricht**

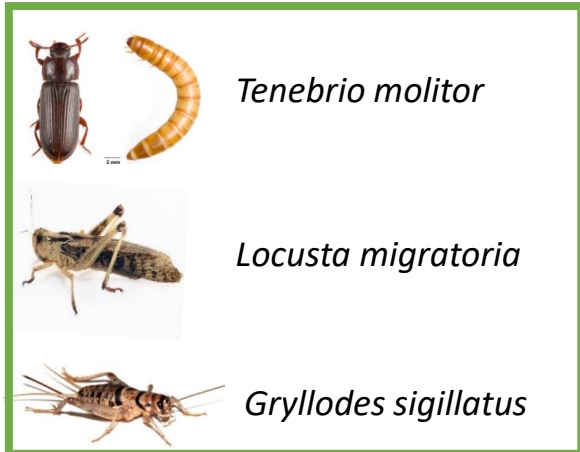


Species	UniProtKB # entries (NOV-2021)	# proteins found in this study
<i>T. molitor</i>	634	1,150
<i>A. diaperinus</i>	47	440
<i>G. sigillatus</i>	37	360
<i>H. illucens</i>	17,599	2,051
<i>A. domesticus</i>	159	450
<i>L. migratoria</i>	1,559	600

WP 3 Development and validation of DNA-based methods



Main activities: Method development, method validation



Debode et al. 2017, Food Additives & Contaminants: Part A
 Köppel et al. 2019, European Food Research and Technology

Single copy gene, declared LOD 10 pg, unable to detect low ppm levels in processed foods

Multicopy gene, performance under evaluation in processed foods



Köppel et al. 2019, European Food Research and Technology

Multicopy gene, performant but not robust when used at 60°C

New method developed! (paper in preparation) based on
 Daniso et al. 2020, European Food Research and Technology

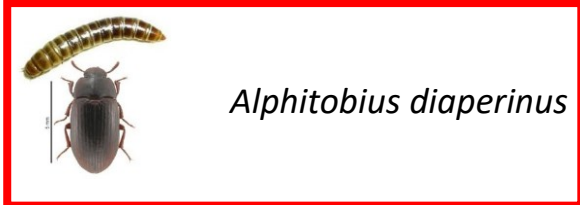
Multicopy gene, performance at low ppm levels under evaluation in processed foods

Zagon et al. 2018, Food Control

Multicopy gene, good performances at low ppm levels (20 ppm) in processed foods

Garino et al. 2021, Animal Feed Science and Technology

Multicopy gene, good performances at low ppm levels (20 ppm) in processed foods



New method developed!
 Garino et al. 2022, Food Control

Multicopy gene, good performances at low ppm levels (20 ppm) in processed foods

WP 3 Development and validation of DNA-based methods



Results presented at the Food Allergy Forum 2023

Main activities: Method development, method validation

Tab. 2: results of the detection of insects in commercial food products containing (pink) and non containing (blue) insects. Green and red cells: expected vs. unexpected results.



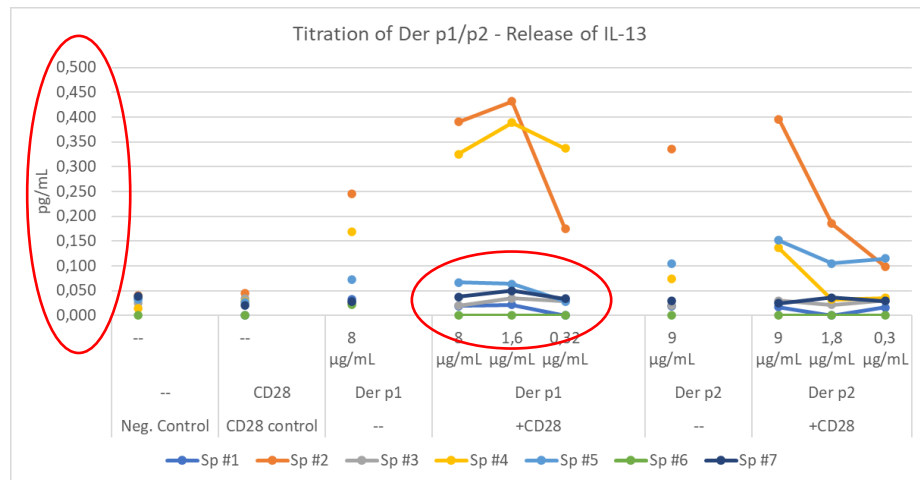
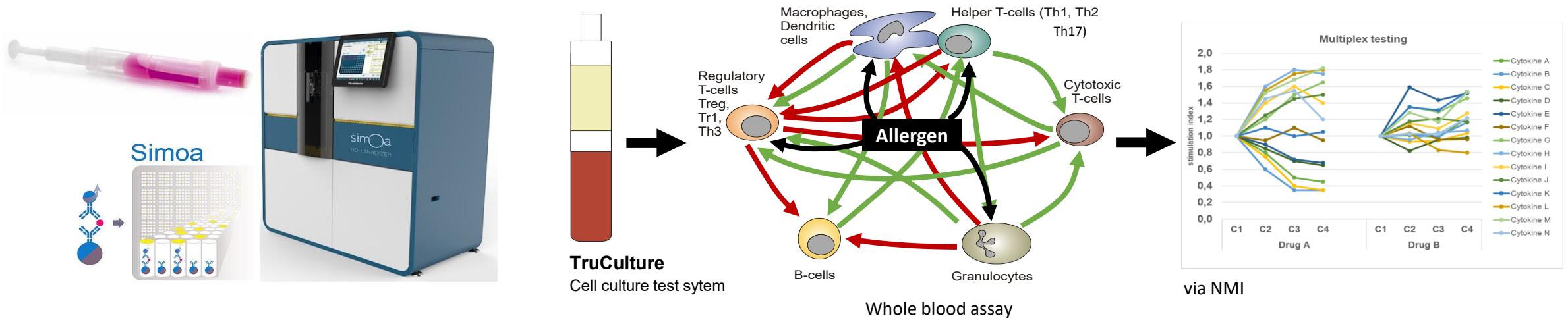
	description	insect declared	SYSTEMS							
			A.diaperinus	T.molitor	H.illucens	A.domesticus	G.sigillatus	L.migratoria	IC*	AC
Te	Protein bar 'SENS'	A.domesticus	+	-	+	+	-	-	+	+
	Protein bar 'SENS'	A.domesticus	-	-	-	+	-	-	+	+
Lo	Protein bar 'Jiminis'	A.domesticus	+	-	-	+	-	-	+	+
	Insect burger 'BugFoundation'	A. diaperinus	+	-	+	+	-	-	+	+
C	Pasta 'Plumento Foods'	A. diaperinus	+	-	-	+	-	+	+	ista migratoria
	Protein bar 'Isaac Nutrition'	A. diaperinus	+	-	-	-	-	-	+	+
C	Crunchy Müsli 'Entomos'	T. molitor	-	+	-	-	-	-	+	+
	Tortillas 'Entomos'	T. molitor	-	+	-	-	-	-	+	+
H	Crispies 'Entomos'	T. molitor	-	+	-	+	-	-	+	+
	Protein bar 'Insectafood'	crickets and T.molitor	+	+	-	+	-	-	+	+
H	Burger 'Essento'	T. molitor	-	+	-	-	-	-	+	+
	Cookies 'Entomos'	A. domesticus	-	-	-	+	-	+	+	+
A	Baking flour 'Cricket Flours'	Gryllodes sigillatus	+	-	-	-	+	-	+	+
	Pancake & Waffle mix 'Cricket Flours'	Gryllodes sigillatus	+	-	-	-	+	-	+	+
A	cookies 'Pavesi'		-	-	-	-	-	-	+	+
	pasta 'La Molisana'		-	-	-	-	-	-	+	+
	chicken burger		-	-	-	-	-	-	+	+
	müsli 'Dr. Oetker'		-	-	-	-	-	-	+	+
	protein bar 'Veganz'		-	-	-	-	-	-	+	+
Al	Toortillas chips 'Bio Zentrale'		-	-	-	-	-	-	+	+

*+ means absence of inhibition, Ct values around 27

WP 5 *In vitro* diagnostics: innovative whole blood analysis system for food allergies

Participants:    

Main activities: Preparation of allergens for cell culture tests, optimization TrueCulture® system, clinical evidence of allergenic effects in whole blood



House dust mite allergens:

- Bad availability & immune response of donors with HDM
- High concentrations (µg/mL) of allergens needed
- Poor quality and high costs (250 µg; 700 Euro)

Peanut allergen:

- No detectable cytokine release
- Lacking of suitable donors
- Several donor recruitment approaches were unsuccessful

Both approaches were so far unsuccessful



WP 6 Assessing clinical relevance of insects as potential food allergens (*in vivo* testing)

Participants:  CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

Main activities: Collection of patient samples, *in vivo* assessment of the clinical relevance of insects

- Out of the 26 who performed the SPT, 2 (sensitized to HDM) showed sensitization to buffalo worm, 1 (sensitized to HDM and shellfish) showed sensitization to locust and cricket, and 1 (sensitized to peanut/hen's egg and HDM) showed sensitization to mealworm.
- No one accepted to undergo 'open food challenge' (OFC).
- Sera from 100 house dust mite sensitized patients will be analysed regarding sIgE to mealworm and eventually other edible insects with the ImmunoCAP

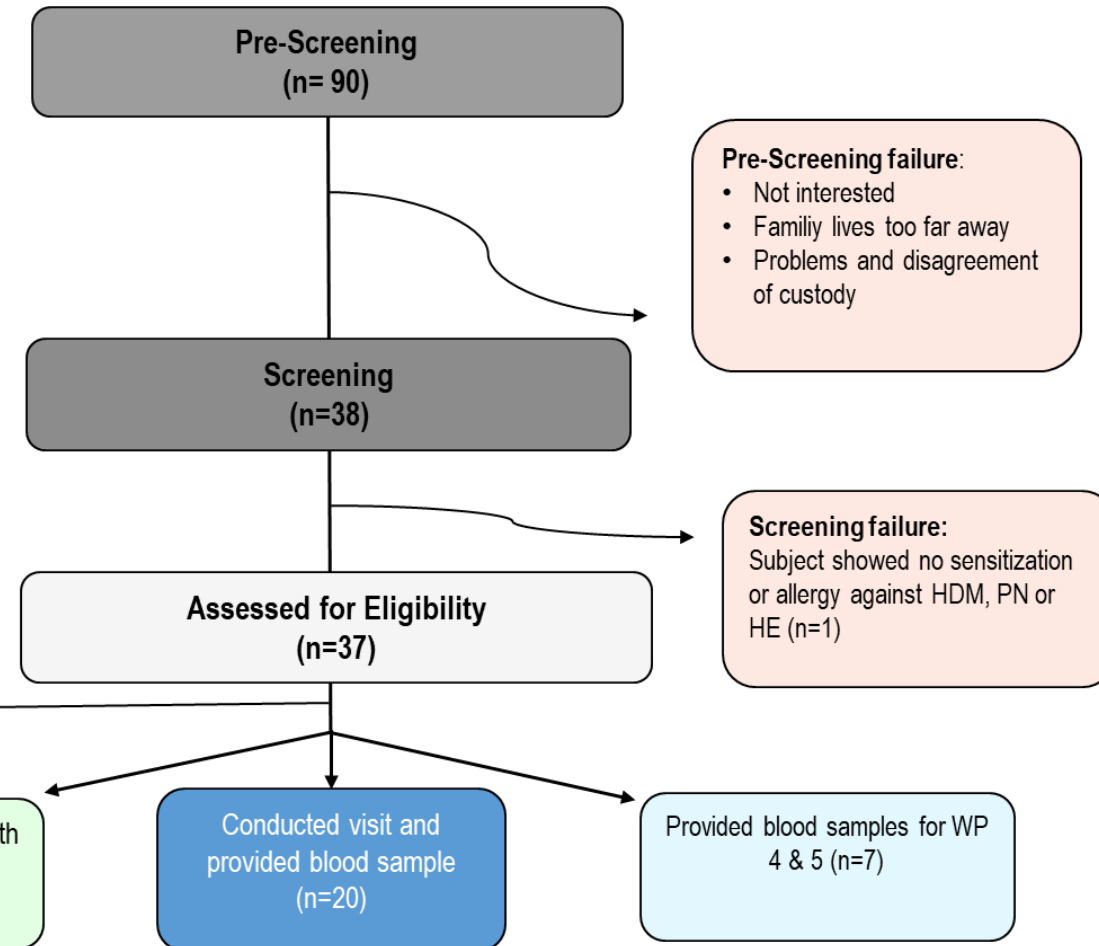


Questionnaires (n=1) and blood sample (n=1), but skin prick test not possible (n=2)

Only conducted study visit with skin prick test (n=6)

Conducted visit and provided blood sample (n=20)

Provided blood samples for WP 4 & 5 (n=7)



Danke für Ihre Aufmerksamkeit

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