Pesticide Exposures and Children's Health

Konstantinos C. Makris, PhD

water and health [laboratory]
Cyprus University of Technology | Cyprus International Institute for Environmental and Public Health

Declaration

No Conflict of Interest of any kind

Synopsis

- Accumulating evidence of pesticides health effects on children's health: emphasis on neurodevelopment and cognition
- Gaps in exposure assessment of key pesticides in vulnerable populations
- Associations of urinary biomarkers of exposure to pesticides and oxidative damage
- Take home messages - Future steps

Makris et al., 2022; Environ. Res., https://www.sciencedirect.com/science/article/pii/S0013935122006430?via%3Dihub

Pesticides and Children's Health

Over the years, increasing use of **pesticide mixtures** and overall increasing **global pesticide use**

Scientific evidence on the human toxicity of **glyphosate (GLY) and its primary metabolite**, aminomethylphosphonic acid (AMPA) is limited, particularly for children.

GLY data is available for only about 500 children worldwide; urinary levels of **GLY in children > adults**, in studies with data for both (Gillezeau et al., 2020).

Le Monde

Une étude met en évidence les bénéfices du bio sur la santé

Ce régime permettrait de réduire les marqueurs de stress oxydatif, un phénomène impliqué dans différentes pathologies chroniques

En octobre 2018, des chercheurs français publiaient la première étude épidémiologique suggérant un risque diminué de certains cancers chez les consommateurs d'aliments issus de l'agriculture biologique. Trois ans plus tard, une équipe de scientifiques chypriotes a mis en évidence, au niveau moléculaire, un effet biologique susceptible d'expliquer ces résultats.

Leurs travaux, publiés dans l'édition de janvier de la revue *Environment International*, associent pour la première fois l'alimentation bio avec une réduction significative des marqueurs de stress oxydatif – un phénomène impliqué dans différentes pathologies chroniques, en particulier certains cancers, maladies neurodégénératives, ou encore le diabète. Pour les auteurs de ces travaux, cet effet est le plus probablement lié à la présence de résidus de pesticides de synthèse dans l'alimentation conventionnelle.

Pour parvenir à ces conclusions, les chercheurs de l'Institut pour l'environnement et la santé publique de l'université technique de Chypre ont enrôlé 191 enfants de plusieurs écoles primaires de la grande île méditerranéenne. Les enfants du premier groupe ont mangé exclusivement bio pendant quarante jours, avant de revenir à leur régime habituel, tandis qu'un second groupe a alterné les deux régimes dans l'ordre inverse.

Des centaines d'échantillons d'urine ont été collectées tout au long de l'expérience. Les auteurs ont ensuite procédé à leur analyse dite métabolomique. Cette méthode analytique consiste à relever la présence de dizaine de petites molécules (ou métabolites) qui sont le reflet du fonctionnement de l'organisme, c'est-à-dire la manière dont celui-ci utilise les nutriments, les graisses, les sucres, etc., et dont il réagit aux différents stress auxquels il est soumis.

Certains métabolites sont ainsi caractéristiques d'une réaction de l'organisme à des substances capables d'altérer l'ADN. *« Ce que nous observons dans les deux groupes d'enfants est que le régime bio est associé à une réduction des marqueurs de dommages oxydatifs, fortement corrélée à une augmentation des marqueurs d'exposition aux pesticides*, explique le professeur Konstantinos Makris, spécialiste de santé environnementale et coordinateur de ces travaux. *On constate aussi que cet effet se renforce au cours de la période où les enfants s'alimentent en bio, pour devenir significatif au bout d'environ quarante jours. »*

« Etude très précise »

Coautrice de travaux récents ayant montré une association entre l'alimentation bio et un risque moindre de certains cancers ou encore de diabète, Emmanuelle Kesse-Guyot, chercheuse dans l'équipe de recherche en épidémiologie nutritionnelle (Inserm, Inrae, université Paris-XIII), juge l'étude et l'analyse *« de haut niveau »*. *« Leur échantillon est petit, mais le schéma de l'étude est très précis donc ils détectent beaucoup de choses »*, ajoute la chercheuse.

Ces résultats, commente de son côté Denis Lairon, chercheur émérite à l'Institut national de la santé et de la recherche médicale (Inserm), et auteur de nombreux travaux sur les liens entre nutrition, santé et environnement, suggèrent que *« les réductions des marqueurs de l'inflammation et du stress oxydant sont originales et suggèrent en effet des modifications métaboliques »*. M. Lairon juge toutefois que les résultats obtenus grâce à l'approche métabolomique *« sont un peu difficiles à interpréter pour ce qui est des relations entre les changements métaboliques et les impacts sur la santé »*.

« Si les personnes qui mangent bio sont en meilleure santé, c'est souvent aussi dû à une meilleure hygiène de vie »

ROBIN MESNAGE
toxicologue

« La réalisation d'études interventionnelles comme celle-ci est très importante », juge le toxicologue Robin Mesnage (Clinic Buchinger Wilhelmi-King's College de Londres) et spécialiste de métabolomique. En effet, ajoute-t-il, les études épidémiologiques ne suffisent pas à parvenir à la certitude d'un effet sanitaire bénéfique intrinsèque de l'alimentation biologique : *« Il est clair depuis longtemps que les personnes qui mangent bio sont en meilleure santé que celles qui mangent de la nourriture non bio, et on sait aussi que c'est souvent parce que ces personnes ont une meilleure hygiène de vie. »* Même si les études épidémiologiques utilisent généralement des techniques d'analyse pour corriger des effets de ces biais, il ne peut être certain que ceux-ci soient intégralement pris en compte.

M. Mesnage confirme que les travaux de M. Makris et de ses coauteurs *« suggèrent clairement que la nourriture bio donnée dans cette étude est plus saine »*. Mais, ajoute le toxicologue, le protocole mis en œuvre *« ne permet pas de savoir si les effets mesurés viennent d'une diminution de l'exposition aux pesticides ou de différences de qualité nutritionnelles, ou même peut être de changements de comportements alimentaires »*.

Les chercheurs chypriotes reconnaissent que le régime bio administré aux enfants contenait légèrement plus de fruits et légumes que le régime conventionnel, mais ils estiment que cette différence ne peut expliquer la majeure part de l'effet relevé dans leur étude. *« Nous avons testé cette hypothèse en consultant les essais contrôlés randomisés disponibles dans la littérature scientifique, cherchant les effets d'une consommation systématique de fruits et légumes*, explique M. Makris. *Or, en particulier sur une période aussi courte que quarante jours, ces essais ne montrent pas de baisse de plusieurs des marqueurs de stress oxydatif qui sont diminués par l'alimentation bio. Nous attribuons donc plutôt l'effet bénéfique global de celle-ci à la quantité moindre de résidus de pesticides. »* ■

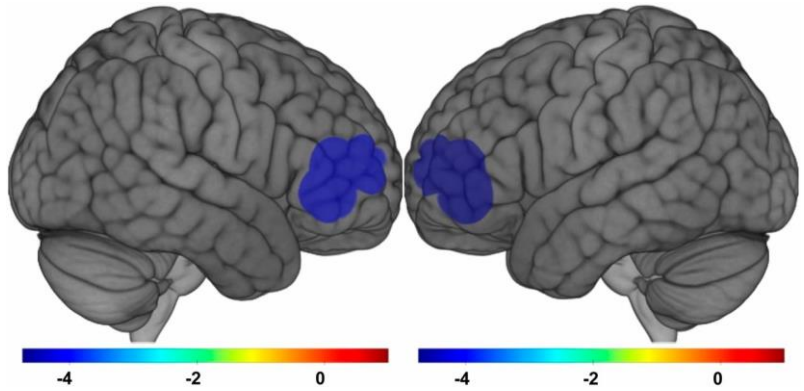
STÉPHANE FOUCART

CHAMACOS cohort studies in the USA

Prof. Eskenazi's CHAMACOS children cohort studies in the California, USA, were instrumental in phasing out organophosphates (OPs) in the USA, and later, globally.

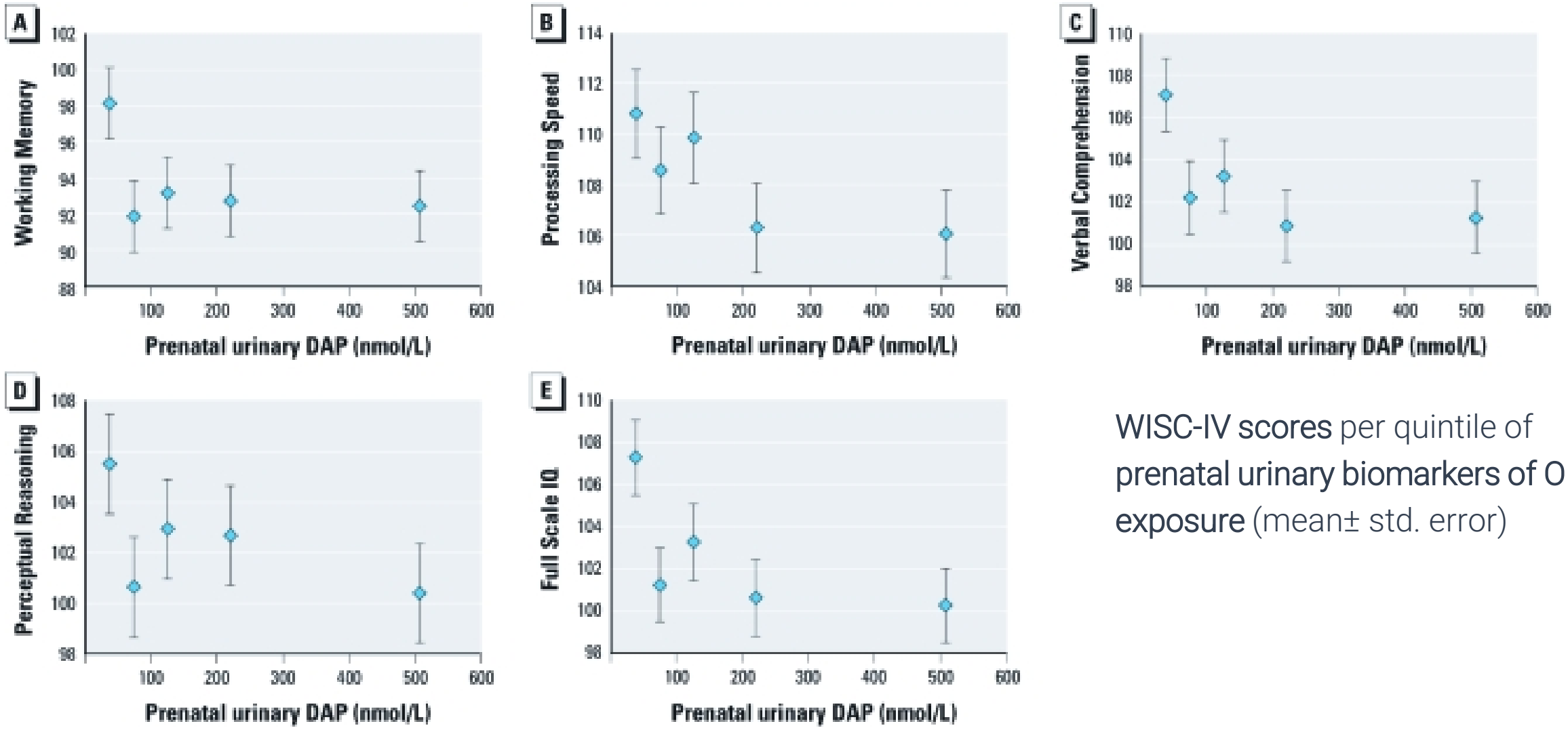
Higher OP pesticide exposures during pregnancy resulted in:

- ✓ shorter pregnancies
- ✓ Abnormal reflexes in newborns
- ✓ Poorer verbal abilities in preschool children
- ✓ Problems of attention at 5 years of age
- ✓ Poorer social skills at 7
- ✓ Poorer working memory at age 12
- ✓ Traits similar to autism at age 2 and 14



Eskenazi et al., 2004, EHP; Young et al., 2005, Neurotoxicology; Eskenazi et al., 2007, EHP; Marks et al., 2010, EHP; Bouchard et al., 2011, EHP; Sagiv et al., 2018, EHP; Sagiv et al., 2019, PN

Policy-influencing children's health studies: CHAMACOS study



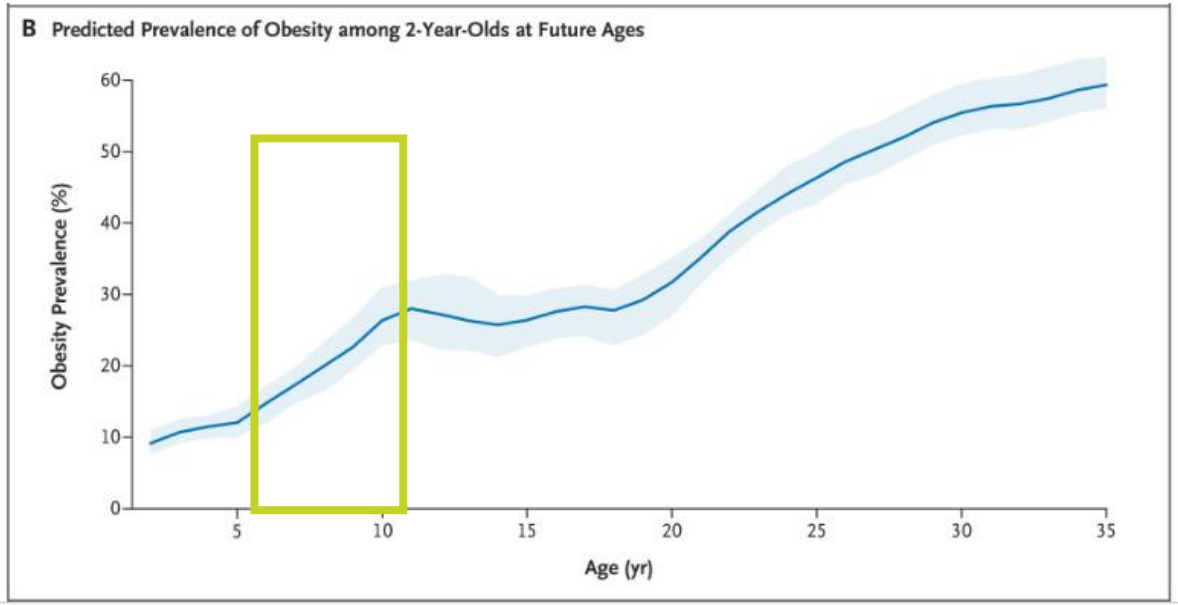
WISC-IV scores per quintile of prenatal urinary biomarkers of OP exposure (mean± std. error)

Bouchard et al., 2011, EHP

Critical Life Stage: Childhood

- **Barker hypothesis** (Barker, 2004)
  - organism is "plastic" or "sensitive" to its environment during specific developmental periods
  - programming: when stimuli are applied during early development, permanent changes are generated that persist throughout life
    - not just limited to the in-utero environment, extends into childhood
- **Increased susceptibility to environmental exposures**
  - rapid development
  - differences in behaviors and metabolism capacity of stressors
  - parents' environmental exposures leading to passive exposures
- **Lung function risk trajectories**
  - contributed to 75% of COPD cases
  - **associated with childhood factors,** like asthma, bronchitis, pneumonia, allergic rhinitis, eczema

- **Obesity risk trajectory in 5-11 years old:**
  - **Steep increase in obesity prevalence**, based on CHOICES simulation model



Projected prevalence of obesity at future ages among 2-year-olds in 2016

Children's health effects associated with exposure to HBM4EU priority pesticides

Target organ of the body	Effects	Relevant substances	Adults (men)	Adults (women)	Infants/foetuses
Brain/Neurological system	Disturbance of neurodevelopment e.g. cognitive deficits	Pyrethroids	X	X	Suspected
		Glyphosate-based herbicides	X	X	More evidence needed
		Organophosphates (Chlorpyrifos/Dimethoate)	X	X	Strong evidence
	Behavioural disorders	Pyrethroids	X	X	Strong evidence
		Organophosphates (as a group)	X	X	Suspected

Key:

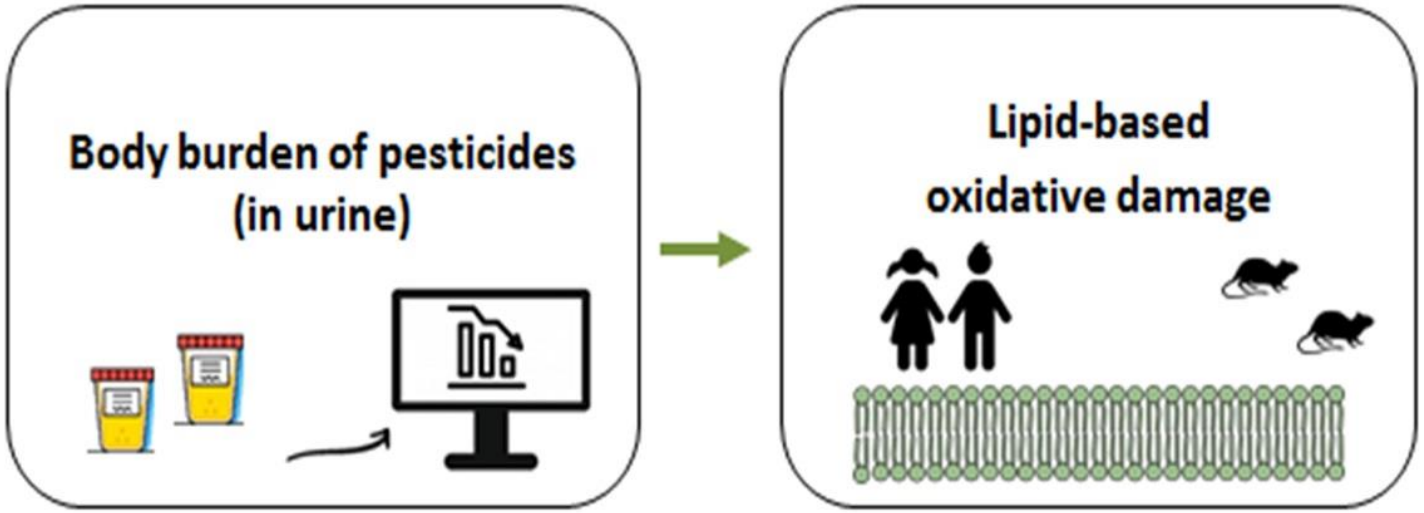
- Strong evidence
- Suspected
- More evidence needed
- X Not applicable

Scientific evidence

- <u>Strongest:</u> Neurodevelopmental effects of pesticides on children adversely affect their normal development and growth
- <u>Suspected:</u> effects of pesticides on childhood leukemia, other cancer sites, and on the endocrine system.

How pesticides impact human health and ecosystems in Europe — European Environment Agency (europa.eu)

Study Objective

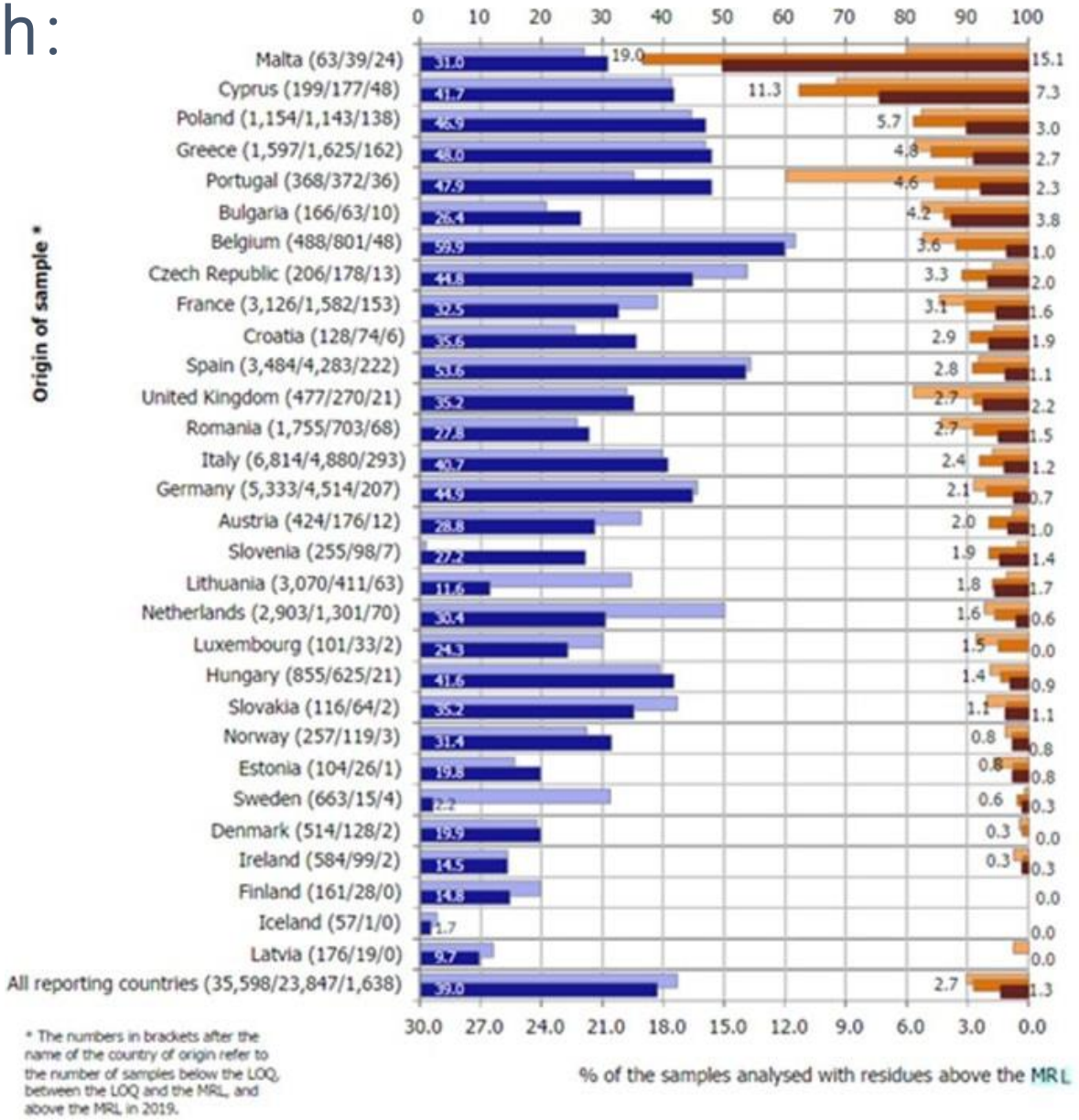


Determine the association between the urinary pesticides and the biomarkers of DNA and lipid oxidative damage in primary school children.

Pesticides and Children's Health: Why Cyprus?

Cyprus:

- Top 3 of EU countries with the highest % pesticide residues in foods exceeding permissible limits (MRL)
    - Light orange: 2018 residues > MRL
    - Orange: 2019 residues > MRL
- This is a historic and persistent trend (10 yrs or more)
- *EU Farm to Fork* strategy will help in reducing pesticide use to the benefit of the environment and human health



water and health [laboratory]

Cyprus University of Technology | Cyprus International Institute for Environmental and Public Health

Methods

Methods

A cross-sectional study of the baseline dataset of the ORGANIKO LIFE + trial (ClinicalTrials.gov number: NCT02998203) (Makris et al., 2019).

In 2020, ORGANIKO was approved for alignment in the frame of the European Human Biomonitoring Initiative, HBM4EU (Gilles et al., 2021) with the aim to collect harmonized HBM data of children's exposure to prioritized pesticides with EU wide coverage.

Out of 191 children recruited through their primary schools located in the Limassol urban area of Cyprus, a total of 179 children, aged 10–11 years were included in this analysis.

Cyprus National Bioethics Committee, ΕΕΒΚ/ΕΠ/2016/25

Children's HBM4EU aligned study

Questionnaires

- Sociodemographic variables, diet, pesticide use at home

Urine samples

- Biomarkers of exposure to pesticides, biomarkers of oxidative damage

Biomarkers of exposure

HBM4EU-accredited labs using mass spectrometry:

i) the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Germany

ii) the Wageningen Food Safety Research Laboratory, the Netherlands.

The following pesticides were measured:

- glyphosate (GLY)
- aminomethylphosphonic acid (AMPA)
- 3,5,6-trichloro-2-pyridinol (TCPy)
- cis-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (cis-DBCA)
- cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis-DCCA)
- trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (trans-DCCA)
- 3-phenoxybenzoic acid (3-PBA)
- 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA)
- cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid (CIF3CA or CFMP)

Biomarkers of effect (lipid damage and DNA damage)

Competitive ELISA kits:

- 8-iso-PGF2α (catalog no: STA-337; Cell Biolabs, Inc, California, USA)
    - Lipid damage biomarker
- 8-OHdG (catalog no: ABIN2964843; antibodies-online, Aachen, Germany)
    - DNA oxidation biomarker

Results

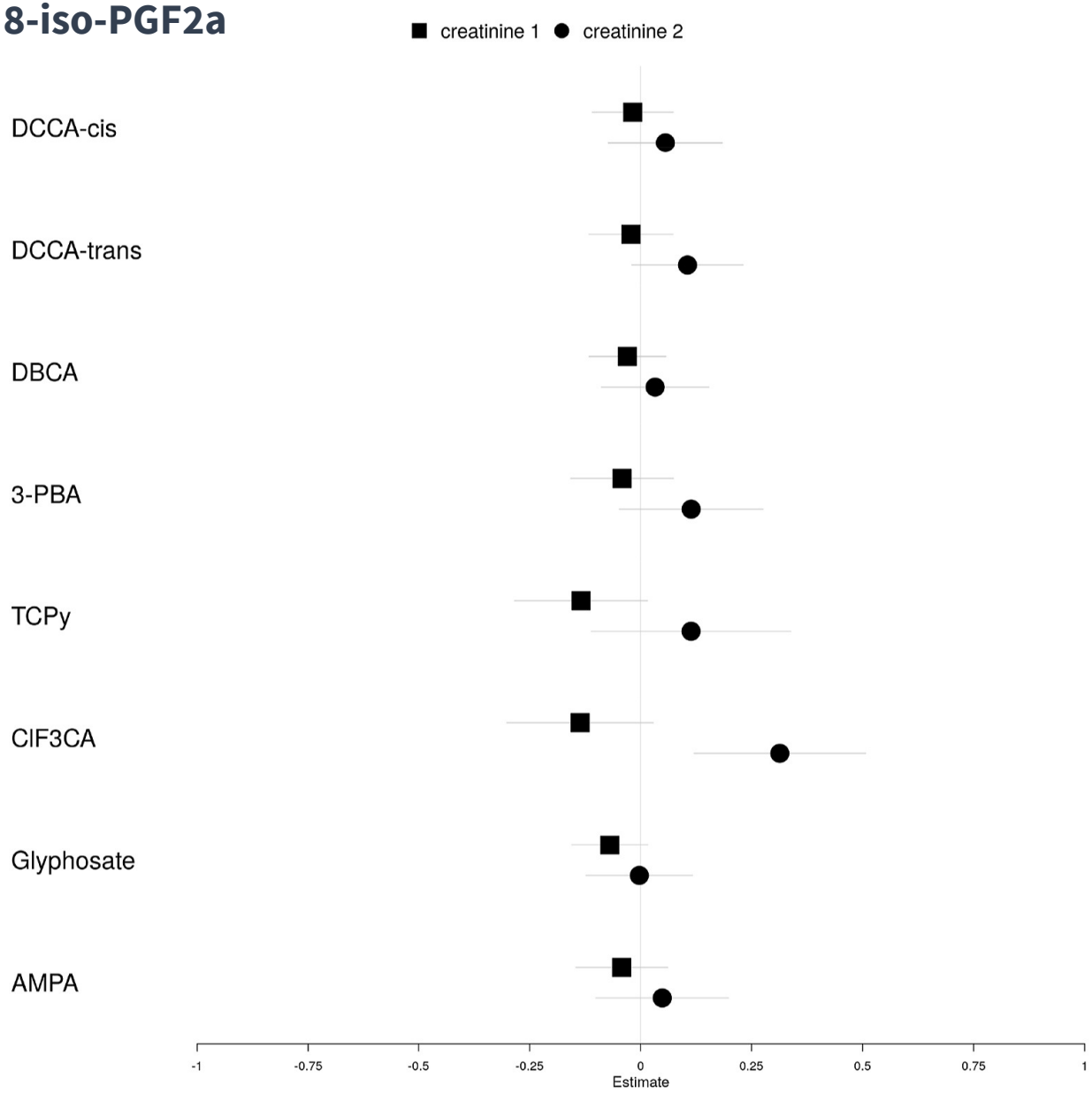
	Overall		Male		Female		p-value^
	median [IQR]	n (%)	median [IQR]	n %)	median [IQR]	n (%)	
n		177		93		84	
Age (years)	10.97 [10.53,11.52]		10.97 [10.49,11.48]		10.97 [10.53,11.55]		0.879
Mother's education							0.945
Primary/Secondary		32 (18)		16 (17)		16 (19)	
University/college		100 (57)		54 (58)		46 (55)	
Master/PhD		44 (25)		23 (25)		21 (25)	
Father's education							0.482
Primary/Secondary		53 (31)		29 (32)		24 (30)	
University/college		72 (42)		41 (45)		31(39)	
Master/PhD		46 (27)		21 (23)		25 (31)	
Weight status*							0.125
Underweight		4 (2)		1 (1)		3 (4)	
Normal Weight		111 (66)		54 (61)		57 (71)	
Overweight		32 (19)		18 (20)		14 (18)	
Obese		22 (13)		16 (18)		6 (8)	
Physical activity (hr/wk)	3.5 [2,6]		3 [0,5.5]		4 [2,6]		0.117
Sedentary (hr/wk)	20 [13,28.5]		21 [14,29]		16 [11.75,27.25]		0.055
Vegetables (portions/wk)	4.5 [2.81,8]		3.5 [2,7]		6 [3.5,8]		0.001

Higher pesticide exposures in Cyprus and Spain

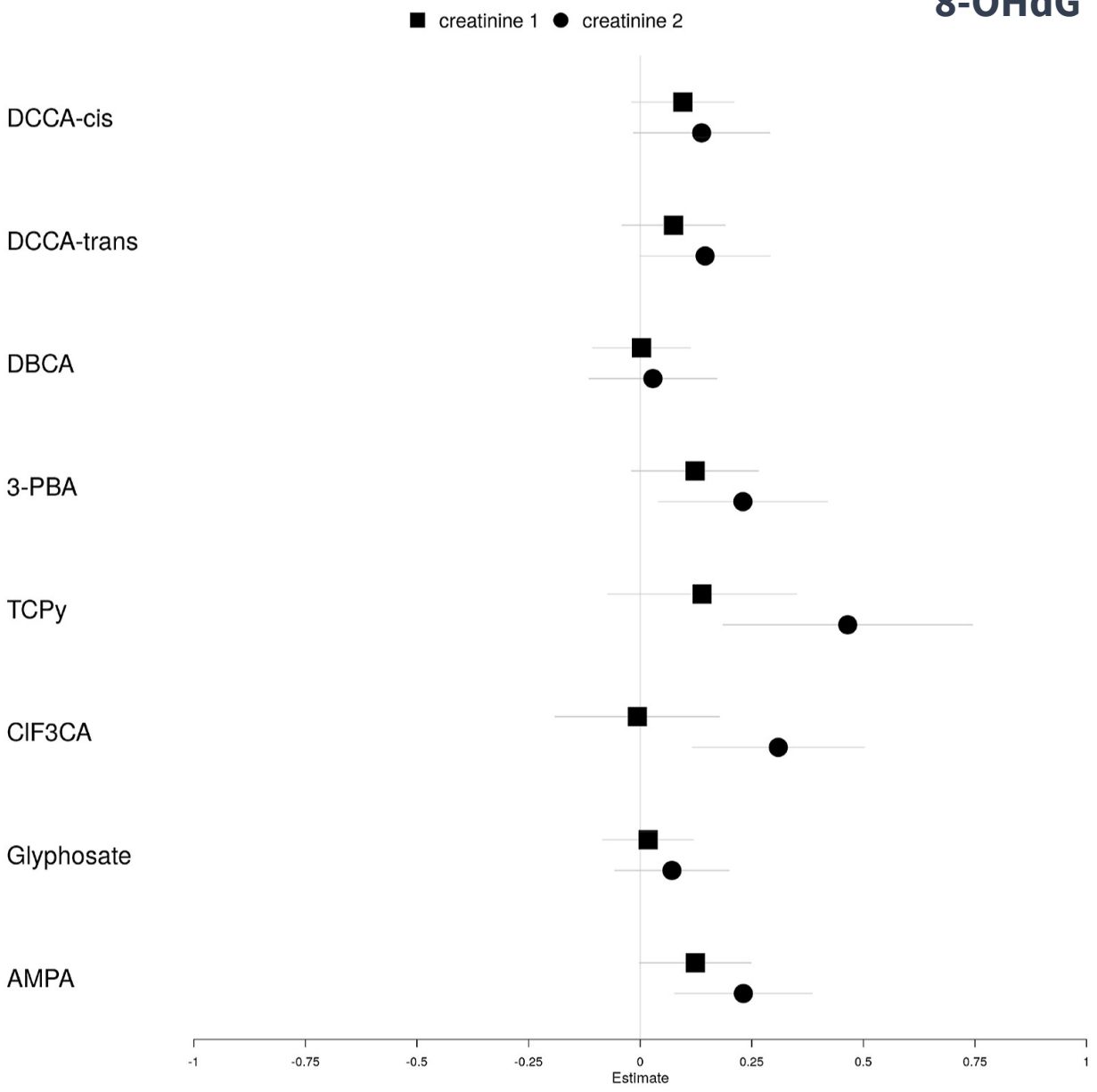
Population, year	Sample size, age range	3-PBA (ug/L)	TCPy (ug/L)
Cyprus, 2017 (this study)	177, 10-12 years	1.93	6.72
Spain, 2016[13]	568, 5-12 years	1.63	1.13
Italy, 2014-2015[9]	199, 7 years	0.56	0.36
USA, 2009-2010	383 & 386, 6-11 years	0.48	1.46
Canada, 2016-2017 (3-PBA), 2014-2015 (TCPY)[10]	3-PBA: 534, 6-11 years TCPY:489, 6-11 years	0.38	1.4
Costa Rica, 2007[7]	140, 6-9 years	0.8	1.4
Thailand, 2003[11]	207, 12-13 years	0.07	2.64
China, 2014[6]	406, 3-6 years	<LOD	0.63*
Chile, 2016-2017[12]	48, 5-13 years	1.29^	1.96^

Association of oxidative tissue damage with pesticides

8-iso-PGF2a



8-OHdG



Key study Findings

This is the first children's health study, globally, exploring the association between glyphosate (GLY)/AMPA and oxidative damage, being aligned with the methodology and tools used in the HBM4EU project.

This is the first children's health dataset that presents **evidence of AMPA oxidative stress toxicity**, albeit this was not the case for glyphosate.

Median 3-PBA and TCPy levels were higher compared to those in other children's populations; these **pesticides metabolites were associated with genotoxicity** marker (8-OHdG).

Although a significant association was seen between AMPA and the DNA oxidative stress marker in this children's population, these results need to be replicated in a larger study.

ELSEVIER

Environmental Research

Volume 212, Part B, September 2022, 113316

Oxidative stress of glyphosate, AMPA and metabolites of pyrethroids and chlorpyrifos pesticides among primary school children in Cyprus

Konstantinos C. Makris [a], Nikolaos Efthymiou [a], Corina Konstantinou [a], Elena Anastasi [b], Greet Schoeters [c], Marike Kolossa-Gehring [d], Andromachi Katsonouri [b]

[a] Cyprus International Institute for Environmental and Public Health, Cyprus University of Technology, Limassol, Cyprus

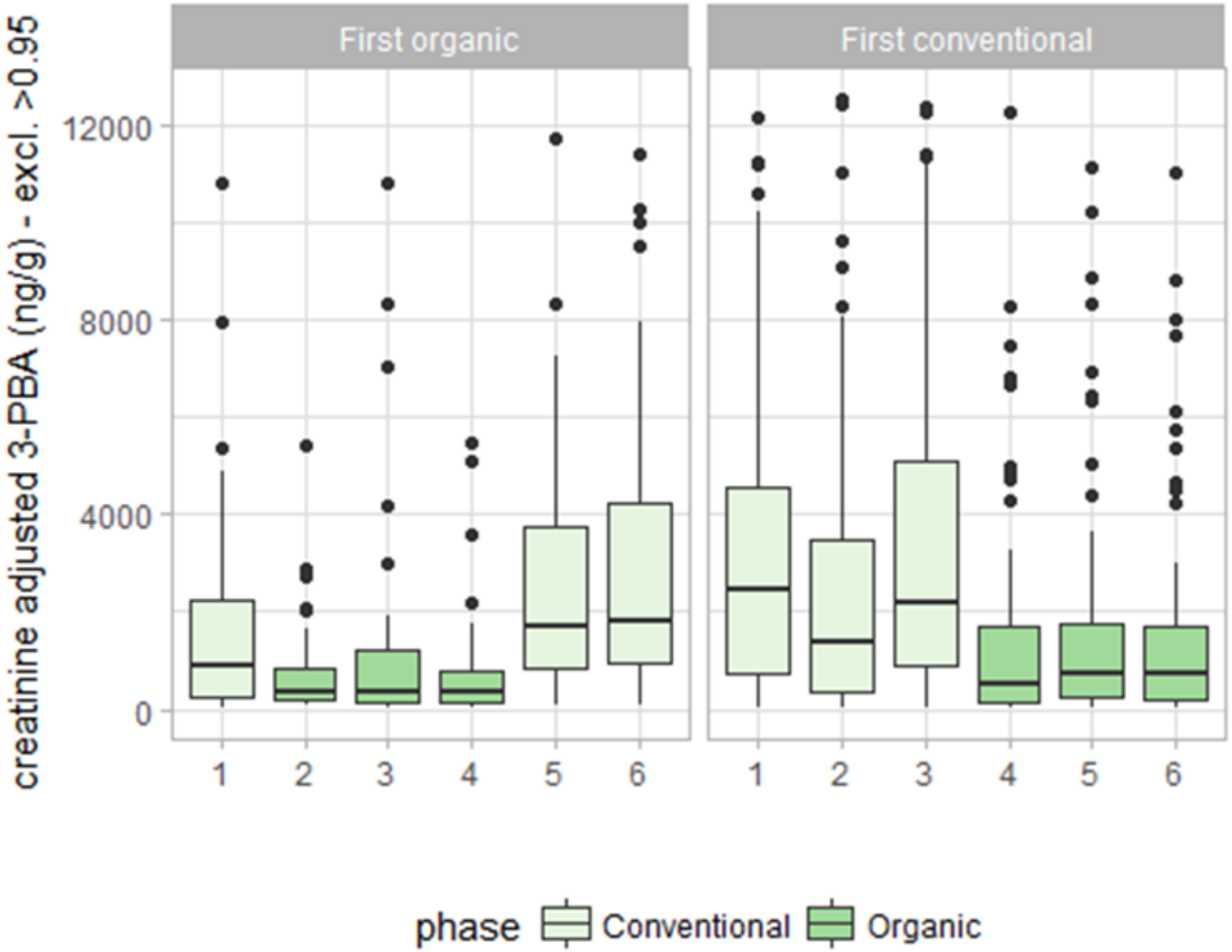
[b] Cyprus State General Laboratory, Ministry of Health, Nicosia, Cyprus

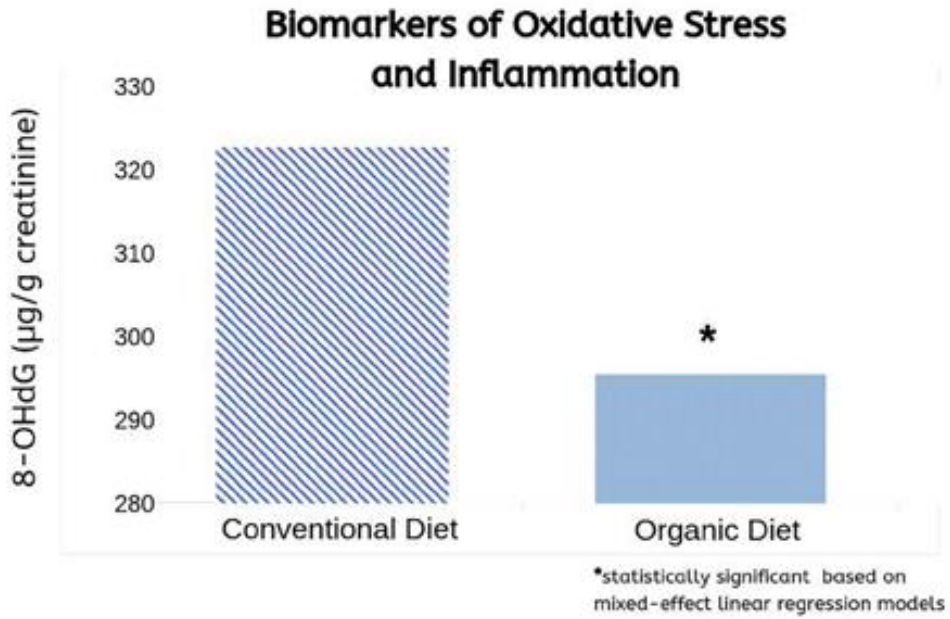
[c] The Flemish Institute for Technological Research (VITO) and the University of Antwerp, Belgium

[d] German Environment Agency (Umweltbundesamt), Berlin, Germany

Received 4 March 2022, Revised 11 April 2022, Accepted 12 April 2022, Available online 16 April 2022, Version of Record 22 April 2022.

Can this health risk be reversed/controlled in children?





Makris et al., 2019, PLOS ONE,
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0219420

Reducing children's exposure to pesticides by providing organic food in Cypriot schools

ORGANIKO LIFE study: Evidence of impact presented in the European Environment Agency (EEA) briefing

- ✓ Switching from a conventional to organic diet for **children's meals in schools** was demonstrated to be cost effective, supporting the broader implementation of such a measure (McKinney and Makris, 2019).

- ✓ Providing organic food in school was found to be **cost-effective** in Cyprus.

McKinney, A. and Makris, K., 2019, http://organikolife.com/wp-content/uploads/2019/10/Ecosystem-function-restoration-study.pdf

https://www.eea.europa.eu/publications/how-pesticides-impact-human-health/reducing-childrens-exposure-to-pesticides

Lowering pesticide content of foods could be implicated with children's health benefits: An EFSA health claim application

- ✓ I led the team that submitted a health claim dossier to EFSA for organic food (lower pesticide residues) for the protection of cells from oxidative damage, being relevant for children's health and development.
- ✓ This EFSA dossier preparation (causality inference assessment) was made possible thanks to our ORGANIKO trial results and those of other observational and animal studies around the globe.
- ✓ The application assembled a synthesis report on causality assessment for the proposed health benefits, abiding by EFSA internationally renowned standards

efsa JOURNAL OPEN ACCESS

Scientific Opinion | Open Access

Organic foods and contribution to the protection of body cells and molecules (lipids and DNA) from oxidative damage: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst ... See all authors

First published: 20 October 2021 | https://doi.org/10.2903/j.efsa.2021.6847

Find It Here

Requestor: Competent Authority of Cyprus following an application by Cyprus International Institute for Environmental and Public Health, Cyprus University of Technology
Question number: EFSA-Q-2021-00055
Panel members: Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.
Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.
Acknowledgements: EFSA wishes to acknowledge the contribution of the WG on Claims: Jean-Louis Bresson, Stefaan de Henauw, Alfonso Siani and Frank Thies to this opinion.
Adopted: 14 September 2021

Policy Implications

- ✓ Will European Union's pesticide policy follow that of the USA, by **banning a few** every 1-2 decades for which substantial scientific evidence have accumulated, and at the same time **continue introducing newer** active ingredients/formulations in the market?

- ✓ Observational studies coupled with randomized controlled trials of no or little pesticide residues (organic food) have shown that **pesticide use and human exposure can be controlled**; thus, facilitating the market uptake of such available options from *farm to fork*.

- ✓ The much needed **paradigm change for pesticides use** can be accomplished over time gradually, by offering incentives to farmers and by accounting for technical and socioeconomic constraints of related interventions.

Cyprus University of Technology | Cyprus International Institute for Environmental and Public Health

A Big Thank You!

CII Team

- Dr. Pantelis Charisiadis
- Dr. Xanthi Andrianou
- Corina Konstantinou
- Christina Xeni
- Samuel Abimbola
- Nicholas Efthymiou

Collaborators

A Katsonouri[1], E Anastasi[1], G Schoeters[2], M Kolossa-Gehring[3]

[1]Cyprus State General Laboratory, Ministry of Health, Nicosia, Cyprus

[2]The Flemish Institute for Technological Research (VITO) and the University of Antwerp, Belgium

[3]German Environment Agency (Umweltbundesamt), Berlin, Germany

HBM4EU science and policy for a healthy future

ΓΕΝΙΚΟ ΧΗΜΕΙΟ ΤΟΥ ΚΡΑΤΟΥΣ
ΚΥΠΡΙΑΚΗ ΔΗΜΟΚΡΑΤΙΑ

www web.cut.ac.cy/waterandhealth

@waterhealth_lab

www.facebook.com/waterandhealthlab.CII