Non-persistent pesticides and developmental neurotoxicity

Prof. Mariana F. Fernández

European pesticide market

Sales of pesticides, by country, 2011 and 2019

(tonnes)

	Fungicides and bactericides		Herbicides, haulm destructors and moss killers		Insecticides and acaricides		Molluscicides		Plant growth regulators		Other plant protection products	
	2011	2019	2011	2019	2011	2019	2011	2019	2011	2019	2011	2019
Belgium	2 452	2 449	2 611	2 328	695	359	14	11	269	297	885	682
Bulgaria	(c)	1 579	(c)	4 340	(c)	727	:	(c)	(c)	10	:	4
Czechia	1 627	1 651	3 473	2 399	291	307	13	3	1 183	435	462	258
Denmark	633	436	3 692	2 026	45	57	4	2	173	131	3	9
Germany	10 473	10 217	17 955	13 941	11 832	18 665	255	59	3 123	2 089	219	204
Estonia	51	105	357	531	19	33	(c)	(c)	32	76	(c)	(c)
Ireland	620	922	2 812	1 845	48	23	4	8	188	157	20	17
Greece	2 256	1 756	1 455	1 830	109	965	(c)	2	21	134	733	181
Spain	31 343	34 073	13 835	17 023	8 062	7 636	229	88	223	145	19 421	16 225
France	24 496	24 484	29 252	22 484	2 190	4 367	331	279	2 532	1 786	2 461	905
Croatia	:	[illegible]	:	[illegible]	:	122	:	2	:	[illegible]	:	4
Italy	43 574	24 286	8 327	8 524	2 494	1 683	97	41	390	455	15 443	13 417
Cyprus	250	867	170	168	179	135	2	2	3	0	6	58
Latvia	148	295	722	972	34	39	:	5	164	321	6	18
Lithuania	362	575	1 773	1 199	26	76	0	(c)	403	468	(c)	(c)
Luxembourg	92	(c)	102	54	(c)	(c)	1	0	(c)	8	(c)	(c)
Hungary	2 997	2 796	3 668	3 906	522	690	2	1	224	179	1 135	243
Malta	95	70	6	2	4	3	1	1	0	0	(c)	(c)
Netherlands	4 250	3 897	3 025	2 739	1 898	1 959	20	14	206	557	1 532	96
Austria	1 544	2 068	1 505	1 151	248	1 613	33	5	59	63	58	55
Poland	6 081	6 867	12 408	11 705	991	2 724	12	24	1 593	2 353	689	579
Portugal	9 975	5 767	1 996	2 222	878	812	13	14	4	5	1 158	1 045
Romania	3 482	4 021	6 771	4 013	808	809	1	4	335	68	30	132
Slovenia	797	752	264	172	38	36	1	2	1	7	20	4
Slovakia	541	653	1 080	1 160	64	149	0	(c)	113	322	9	70
Finland	165	2 832	1 452	1 107	31	23	(c)	0	59	56	1 311	16
Sweden	218	164	2 136	1 544	29	45	1	0	21	34	11	13
Iceland	:	0	:	1	:	0	:	0	:	0	:	0
Norway	107	77	679	479	5	8	1	2	38	37	0	9
Switzerland	933	954	919	509	261	294	38	21	33	33	91	110
Turkey	:	19 333	:	7 159	:	12 086	:	264	:	956	:	11 393

Note: Definition differs for the 2011 values of the following countries: Estonia, Greece, Spain, Latvia, Luxembourg, Hungary, Poland, Portugal, Romania, Slovenia, Slovakia, Finland and Norway. See main article.

Note: Reference year 2018 data used as 2019 for Luxembourg.

Note: (c) confidential value

Note: (:) data not available

Source: Eurostat (online data code: aei_fm_salpest09)

eurostat

INMA Spanish birth cohort

<u>Population-based cohorts</u>

3 pre-existing cohorts

✓ Follow up since birth: Granada, Menorca y Ribera d'Ebre

4 *de novo* cohorts

✓ Follow up from the 1st trimester of pregnancy: Sabadell, Valencia, Gipuzkoa y Asturias

The INMA Project <u>study population</u> consists of almost **4,000 mother-child pairs** residing in one of the seven study areas.

INMA Spanish birth cohort

Objetive:

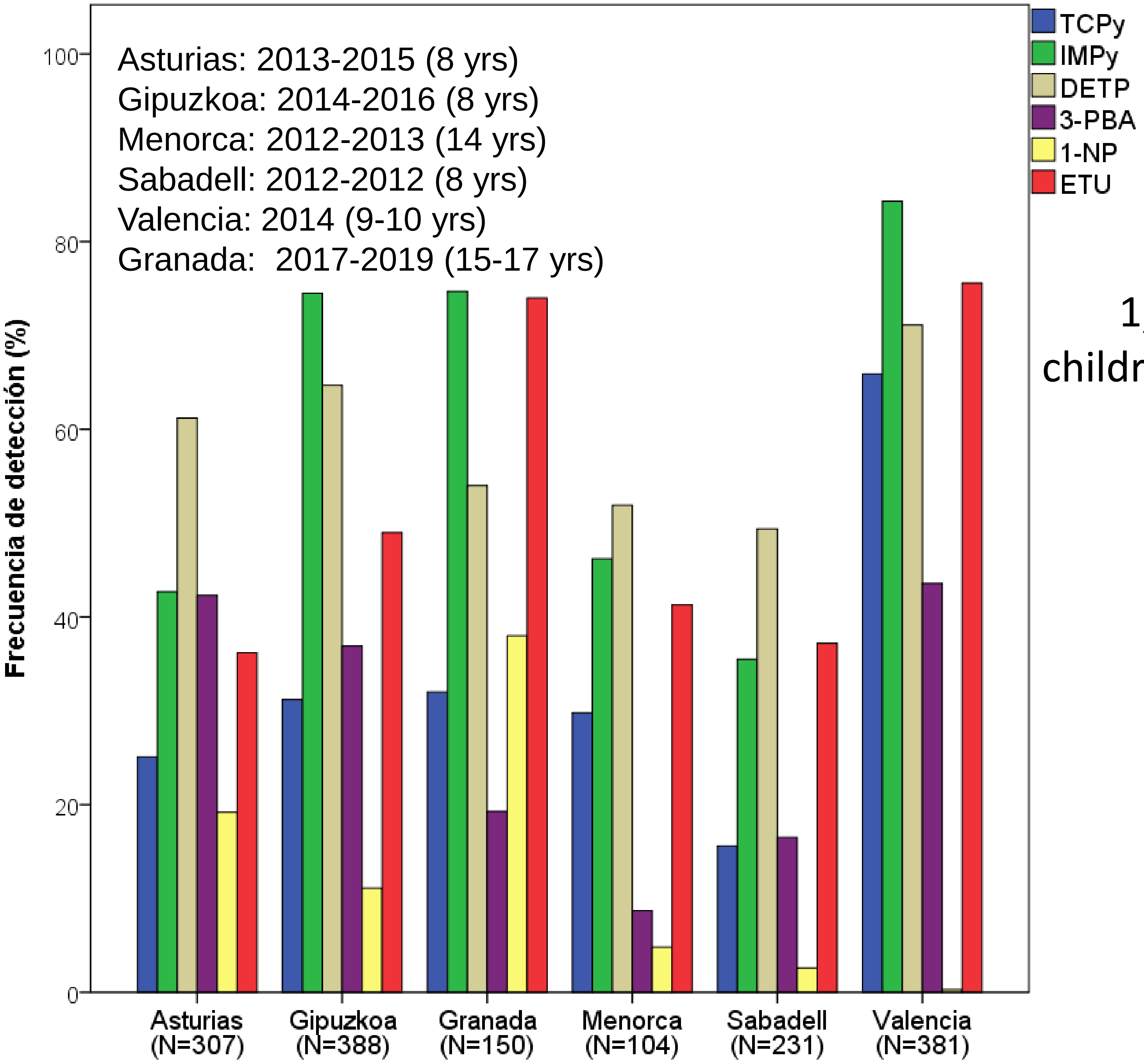
To study the role of the most important environmental pollutants in air, water and diet during pregnancy and early life, and their effects on child growth and development.

- To describe the degree of exposure to environmental pollutants during gestation, birth and childhood in Spain.
- To evaluate the impact of pre- and postnatal exposure to different environmental pollutants on the growth, health and development of children, from the fetal stage to adolescence.
- To evaluate how genetic and nutritional factors may modify the effects of environmental pollutants on child growth.

*Biomarkers of **exposure** assessment*

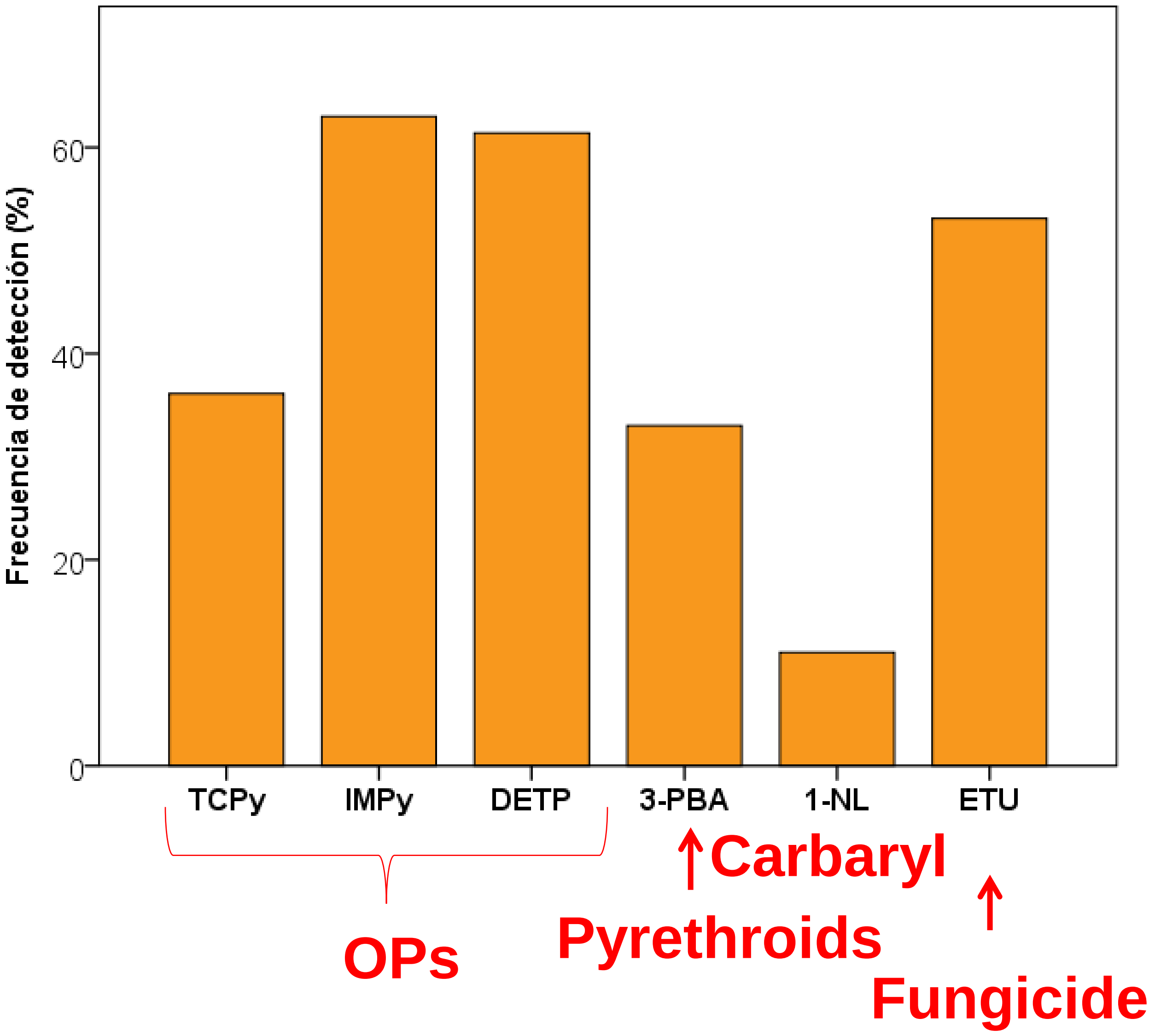
Prioritized HBM4EU chemicals → **Chlorpyriphos** → **Pyrethroids**

- **Organophosphate (OP) insecticide metabolites:** 3,5,6-trichloro-2-pyridinol (TCPy), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPy), malathion diacid (MDA), and diethyl thiophosphate (DETP) **+ΣOPs**
- **Pyrethroids (PYR) metabolites:** 3-phenoxybenzoic acid (3-PBA) and dimethylcyclopropane carboxylic acid (DCCA) **+ΣPYR**
- **Carbaryl metabolite:** 1-naphthol (1N)
- **Ethylene-bis-dithiocarbamate fungicides (EBDC):** ethylene thiourea (ETU)

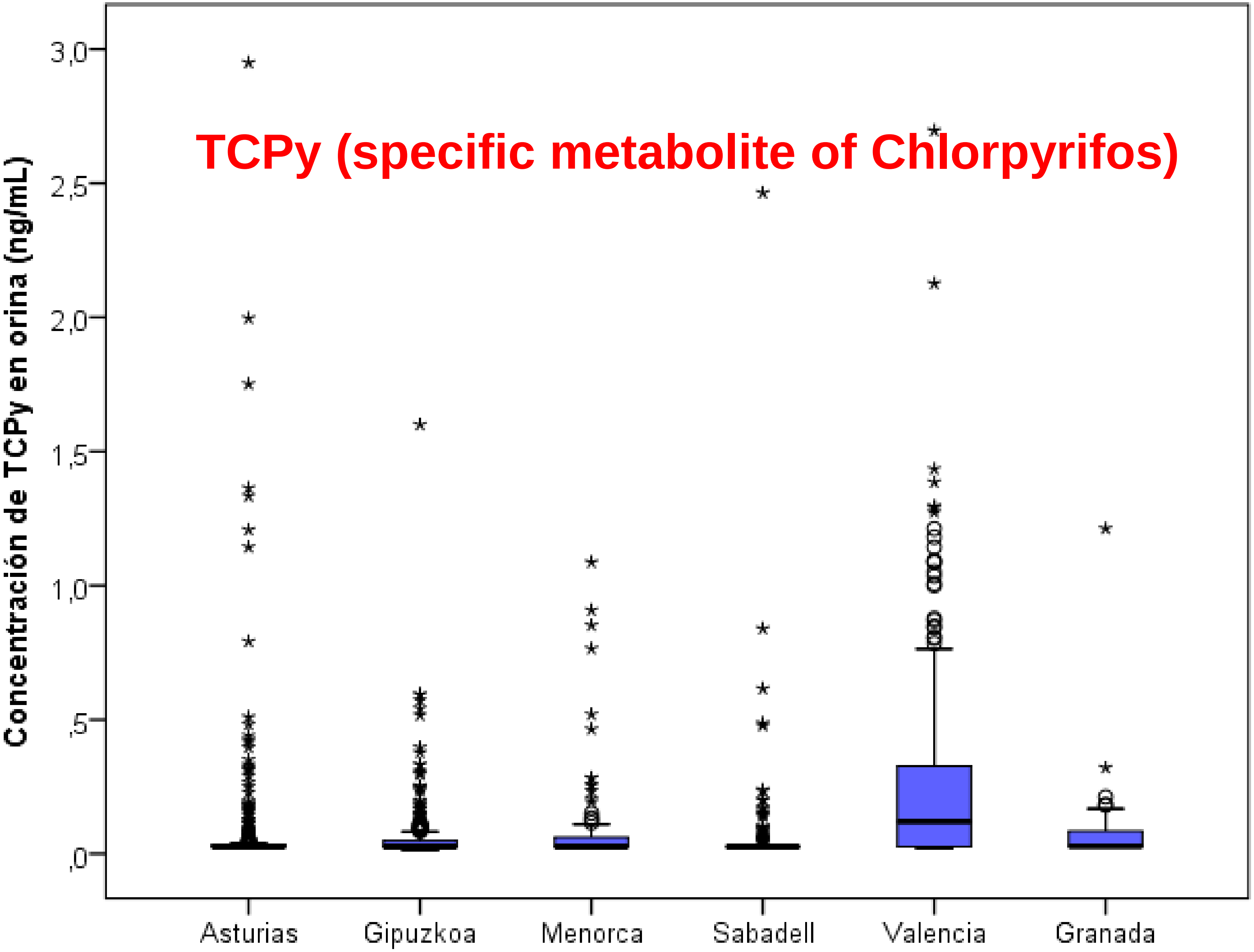


1,561 Spanish children & adolescents 2012-2019

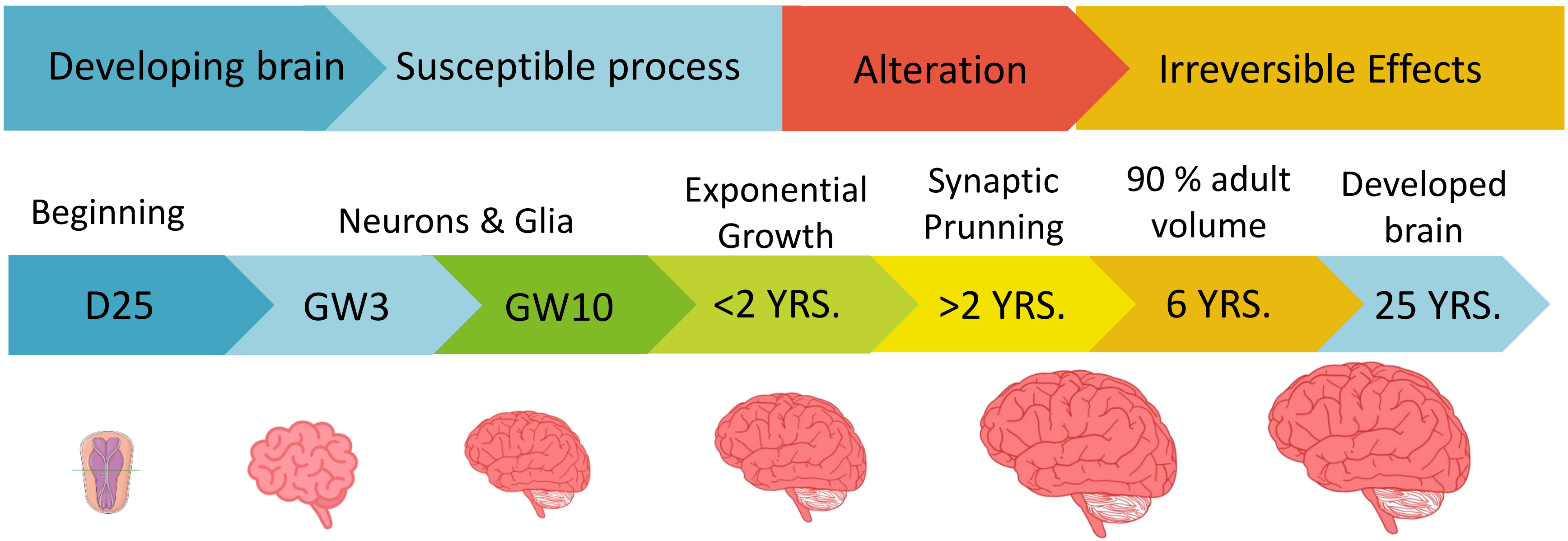
Non-persistent pesticide residues in the urine of 1,561 Spanish children (INMA cohort)



TCPy (specific metabolite of Chlorpyrifos)



Health outcome of interest: ***Neurodevelopment***



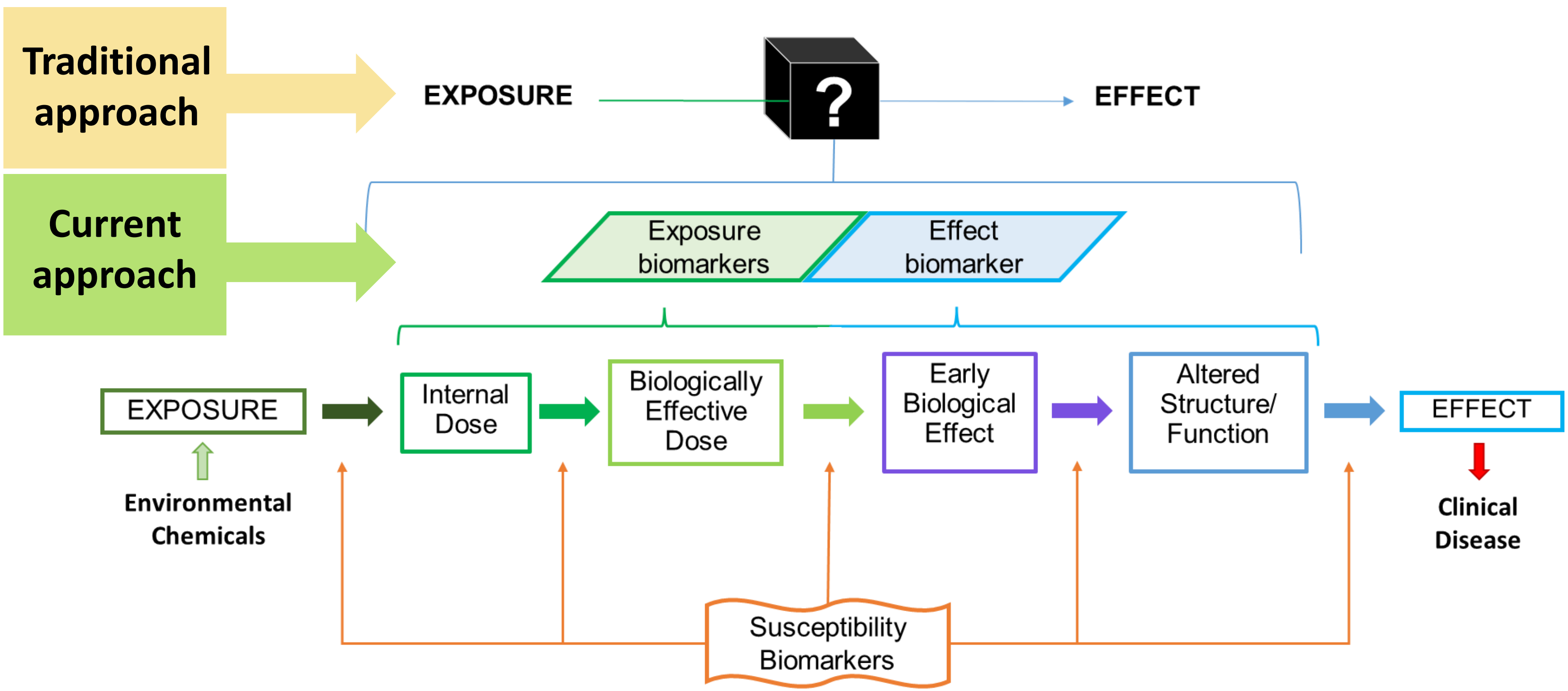
Disorders on neurobehavioral development (Autism spectrum disorders and attention deficit hyperactivity disorders) are increasing word-wide. **Subclinical decrease** in brain functioning with deleterious **consequences** for individuals and the entire society

<70% environmental factors

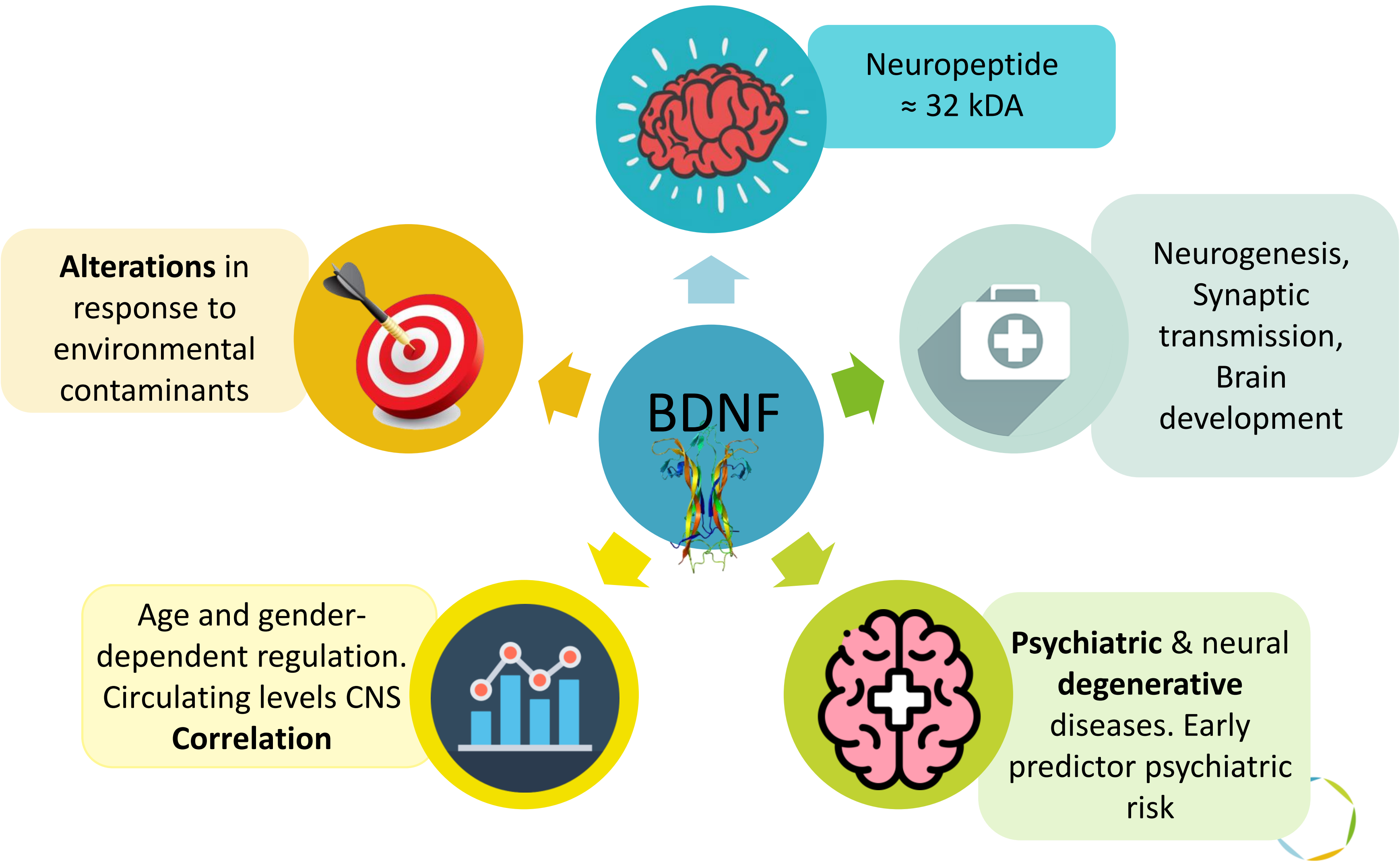
Introduction

HBM can establish exposure-health associations, filling of gaps in knowledge, and new research hypothesis using biomarkers

Biomarkers can connect environmental chemical exposure with a potentially adverse effect on health and eventually, a disease (Studies, 2006).



Brain-Derived Neurotrophic Factor (BDNF) an adequate candidate



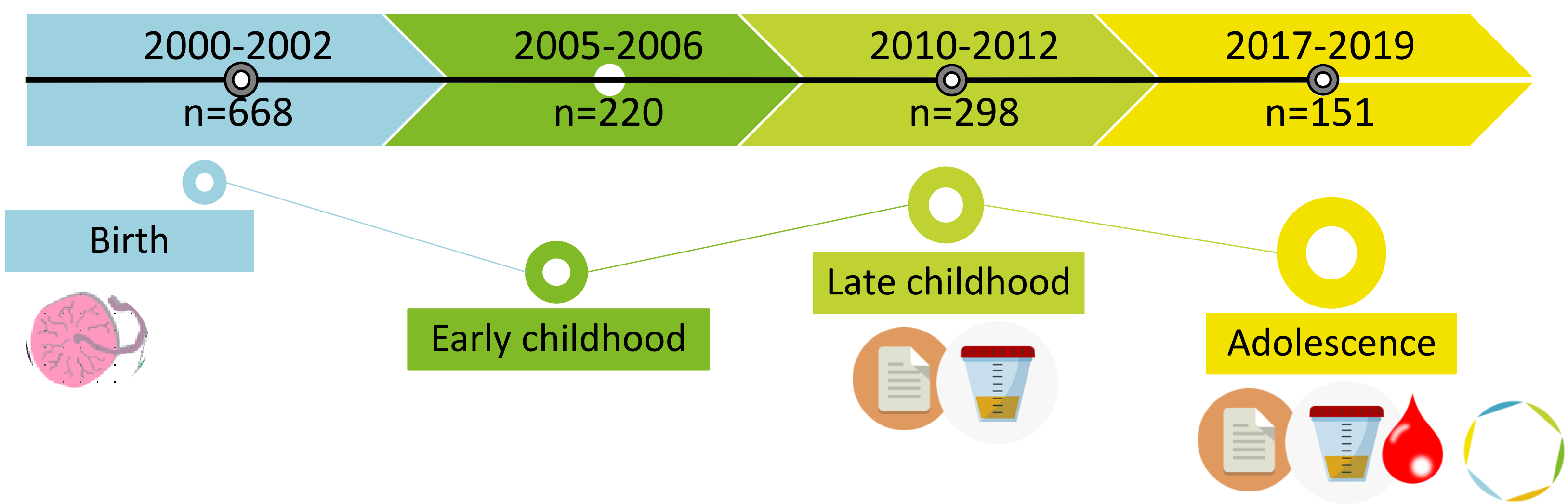
Main Objective

To investigate the relationship between exposure to various non-persistent pesticides, BDNF, and behavioral function among Spanish adolescent males from the INMA-Granada cohort.

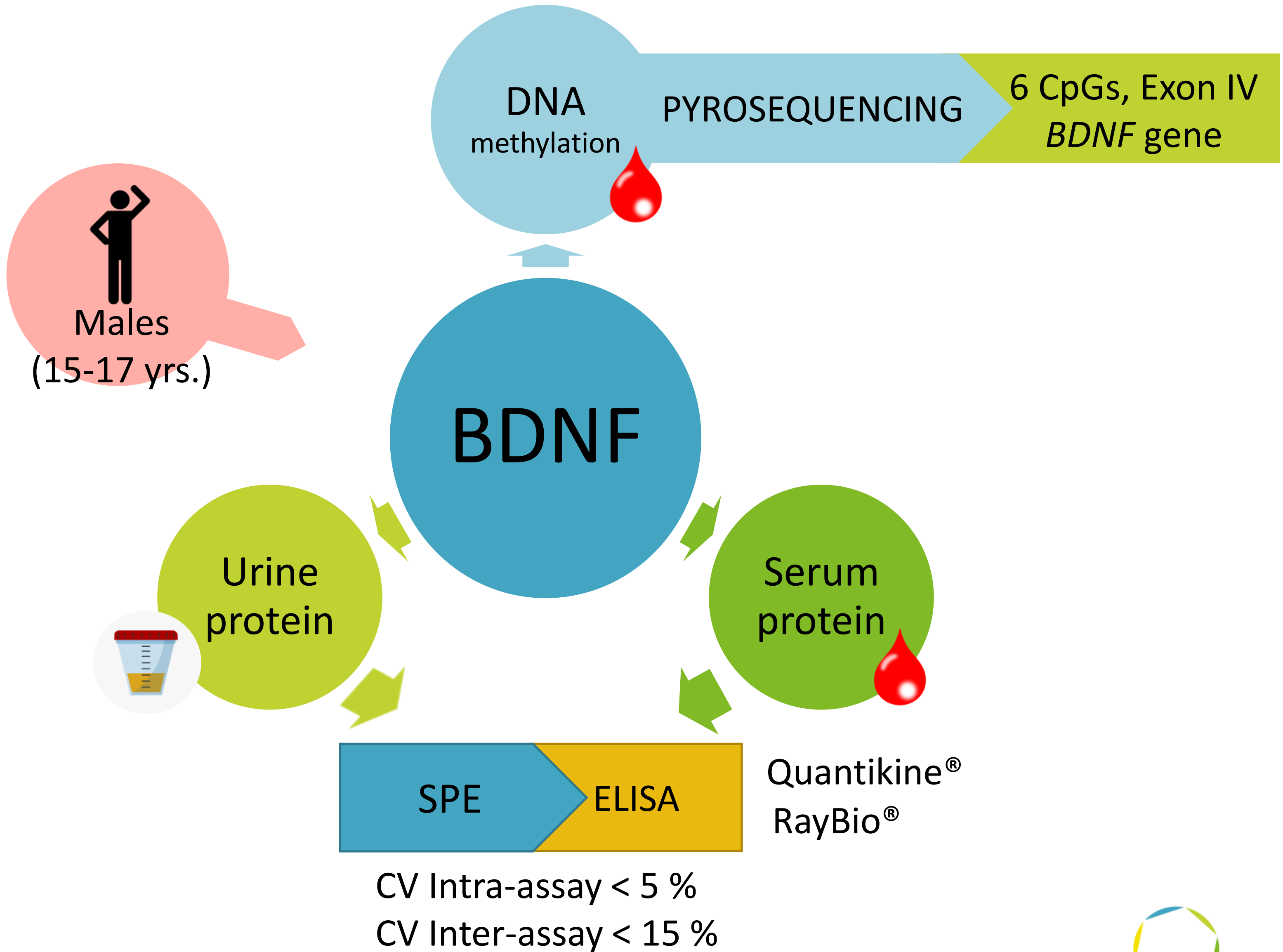
Rodríguez-Carrillo, A., et al. Environ Res. 2022;211:113115 (open accesss)

Study Population

The Environment and Childhood (INMA)-Granada Cohort.



Biomarkers of ***effect*** assessment



Neuropsychological assessment

Cognitive

Intelligence: K-BIT
Language: K-BIT
Attention: CPT
Verbal memory: TAVEC I
Visual-motor coordination: TMT A
Processing speed: WISC-IV
Working memory: WISC-IV
Verbal fluency: FAS
Inhibition: Stroop Color&Word + Go/no go tests
Shifting: TMT B

Behavior

Behavior

Child-Behavior Check list 6/18 (CBCL)

- **Internalizing:** Anxiety, depression, thought problems, and somatic complaints
- **Externalizing:** social problems, attention problems, aggressive behavior, and rule-breaking problems
- **Total Problems**

9-11 yrs.

15-17 yrs.

Results & Discussion

Study design	Exposure	Outcome	Statistical Method	Covariates
Cross-sectional	Non-persistent pesticides metabolites (ng/mL)	Behavior (CBCL)	Multivariate linear regression models **Weighted quintile sum (WQS)** Mediation analysis	Age, BMI, alcohol consumption, season of urine collection, urine creatinine, maternal education

BDNF

n=**140** participants urinary **pesticides** & **CBCL** data

n=**130** participants **serum BDNF** protein levels, **pesticides** & **CBCL**

n=**118** participants **BDNF gene DNA** methylation, **pesticides** & **CBCL**

Non-persistent pesticide concentrations

Pesticide metabolites		**IMPy**	**MDA**	**TCPy**	**DETP**	**ΣOPs**	**DCCA**	**3-PBA**	**ΣPYR**	**1-N**	**ETU**
% Detection		74.8	83.0	32.5	54.3	-	100	19.9	-	38.0	74.2
	25	0.08	0.14	<LOD	<LOD	0.67	0.12	<LOD	0.21	<LOD	0.05
Percentiles	50	0.25	0.30	<LOD	0.25	1.29	1.06	<LOD	1.17	<LOD	0.26
	75	0.81	0.50	0.08	0.74	2.27	3.45	0.083	3.53	0.34	0.70

IMPy, MDA, DCCA, and ETU selected for WQS analysis

Table 1. Pesticide metabolites and CBCL behavior scoring (β, 95% CI)

		Syndrome Scores				Composite scores		
		Social problems	**Thought problems**	**Rule-breaking behavior**	Aggressive behavior	Internalizing problems	Externalizing problems	Total problems
IMPy	T2	1.47 (-1.19,4.13)	**2.33 (-0.24,4.90)**	0.76 (-1.90,3.43)	**2.47 (-0.20,5.13)**	2.19 (-1.83,6.21)	2.46 (-1.43,6.34)	2.54 (-1.34,6.42)
	T3	**3.34 (0.65,6.02)**	**2.56 (-0.04,5.16)**	**3.76 (1.06,6.45)**	**3.77 (1.07,6.46)**	1.13 (-2.93,5.20)	**5.50 (1.58,9.42)**	**4.60 (0.68,8.52)**
TCPy	D vs ND	**2.13 (-0.16,4.42)**	**2.48 (0.29,4.67)**	-0.61 (-2.95,1.74)	0.21 (-2.13,2.56)	-0.09 (-3.53,3.36)	-0.74 (-4.14,2.67)	0.58 (-2.80,3.95)
ΣOPs	T2	1.87 (-0.87,4.61)	1.62 (-1.04,4.27)	1.19 (-1.55,3.93)	1.42 (-1.35,4.19)	1.61 (-2.50,5.72)	2.44 (-1.56,6.45)	2.01 (-1.98,6.00)
	T3	2.25 (-0.49,4.99)	2.21 (-0.44,4.86)	**3.40 (0.67,6.14)**	**2.47 (-0.30,5.23)**	2.53 (-1.58,6.63)	**4.33 (0.33,8.33)**	**3.61 (-0.38,7.59)**
ETU	T2	**3.18 (0.64,5.71)**	1.59 (-1.25,4.44)	-0.56 (-3.18,2.07)	1.15 (-1.46,3.76)	-0.87 (-4.69,2.96)	0.10 (-3.69,3.89)	0.28 (-3.47,4.02)
	T3	0.48 (-2.12,3.07)	-0.15 (-3.06,2.77)	-1.16 (-3.85,1.53)	-0.78 (-3.45,1.89)	-3.00 (-6.91,0.92)	-2.60 (-6.48,1.27)	-2.75 (-6.58,1.09)

$p<0.05$; $p<0.10$
Model adjustment: Age, BMI, alcohol consumption, season of urine collection, urine creatinine, maternal education

Higher **IMPy, TCPy, and ΣOPs** concentration showed significant association with externalizing and internalizing problems

Table 2. Regression estimates change (β, 95% CI) of the associations between urinary pesticide metabolites concentrations and BDNF protein levels

		BDNF protein
IMPy	T2	-1.77 (-6.03,2.50)
	T3	**-4.29 (-8.33,-0.25)**
	p-trend	**0.04**
MDA	T2	-2.71 (-6.88,1.46)
	T3	**-6.74 (-11.38,-2.10)**
	p-trend	**<0.01**
DETP	T2	**-0.68 (-7.87,0.52)**
	T3	**-3.82 (-8.25,0.61)**
	p-trend	**0.09**
1-N	Detected vs undetected	**-3.91 (-7.35,-0.46)**
ETU	T2	-1.23(-5.43,2.97)
	T3	-3.27 (-7.36,0.82)
	p-trend	0.16
ΣOPs	T2	**-5.05 (-9.24,-0.85)**
	T3	**-7.88 (-12.09,-3.67)**
	p-trend	**<0.01**

p<0.05; p<0.10

Model adjustment: Age, BMI, alcohol consumption, season of urine collection, urine creatinine, maternal education

Higher **IMPy, MDA, DETP, 1N, ETU and ΣOPs** concentration showed association with decreasing serum BDNF protein levels

Table 3. Regression estimates change (β, 95% CI) of the associations between urinary pesticide metabolites concentrations and BDNF gene DNA methylation

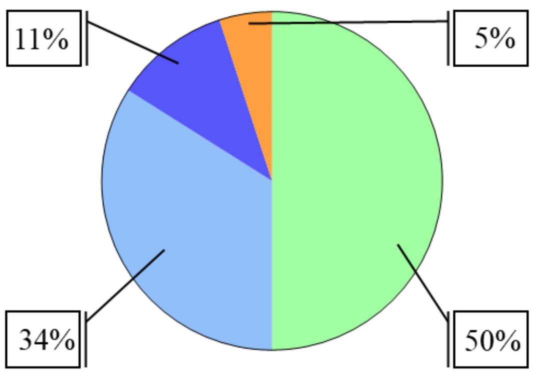
		CpG1	CpG2	CpG3	CpG4	CpG5	CpG6	ΣCpG
MDA	T2	0.21 (-0.15,0.57)	**0.26 (0.04,0.46)**	0.12 (-0.16,0.39)	0.04 (-0.57,0.65)	0.18 (-0.21,0.56)	-0.07 (-0.62,0.48)	0.12 (-0.17,0.42)
	T3	0.31 (-0.08,0.71)	**0.21 (-0.04,0.46)**	0.24 (-0.06,0.54)	0.25 (-0.41,0.91)	0.23 (-0.18,0.64)	0.05 (-0.54,0.65)	0.22 (-0.10,0.53)
3-PBA	D vs ND	0.01 (-0.37,0.39)	-0.00 (-0.24,0.24)	0.21 (-0.08,0.50)	**0.65 (0.03,1.26)**	**0.38 (-0.01,0.76)**	**0.57 (0.02,1.12)**	**0.30 (0.00,0.60)**
ETU	T2	0.20 (-0.16,0.57)	**0.23 (0.01,0.46)**	**0.27 (0.01,0.54)**	**0.68 (0.09,1.27)**	**0.36 (-0.02,0.73)**	0.40 (-0.14,0.93)	**0.36 (0.07,0.64)**
	T3	0.18 (-0.17,0.54)	**0.27 (0.05,0.49)**	**0.41 (0.15,0.67)**	**0.53 (-0.05,1.11)**	0.22 (-0.15,0.58)	0.32 (-0.21,0.84)	**0.32 (0.04,0.60)**

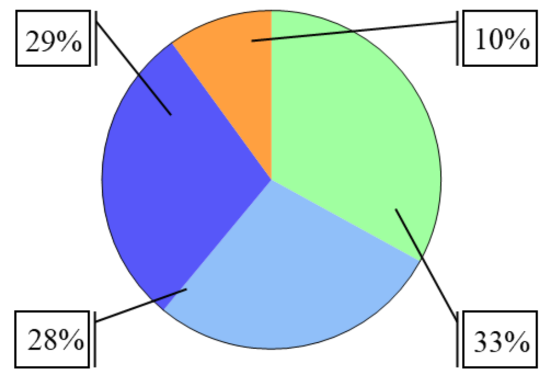
p<0.05; **p<0.10**

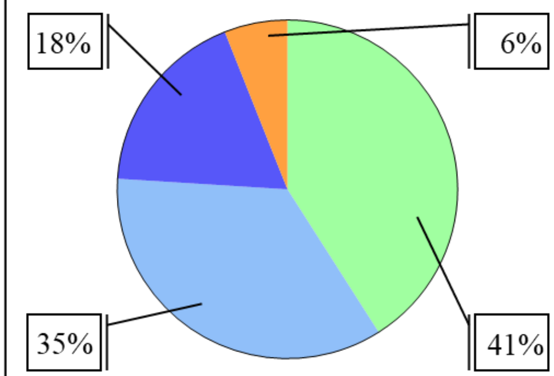
Model adjustment: Age, BMI, alcohol consumption, season of urine collection, urine creatinine, maternal education

Figure 1. Mixture Effect analysis (WQS)

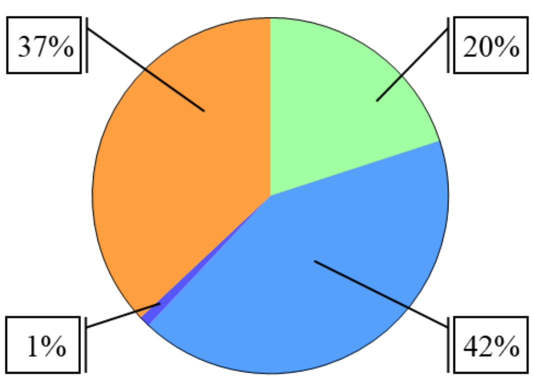
Withdrawn			Social Problems			Thought Problems		
Beta	Standard Error	p-value	Beta	Standard Error	p-value	Beta	Standard Error	p-value
2.71	1.38	0.05	4.27	1.33	<0.01	2.24	1.34	0.10

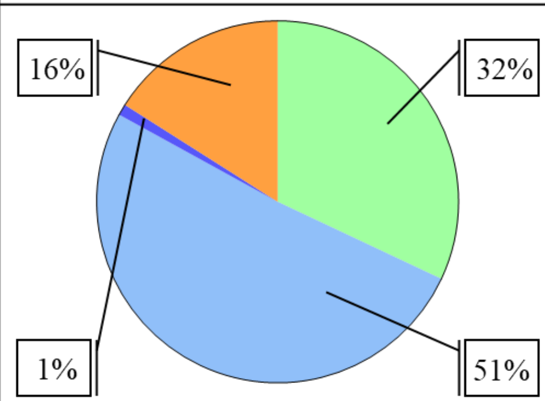


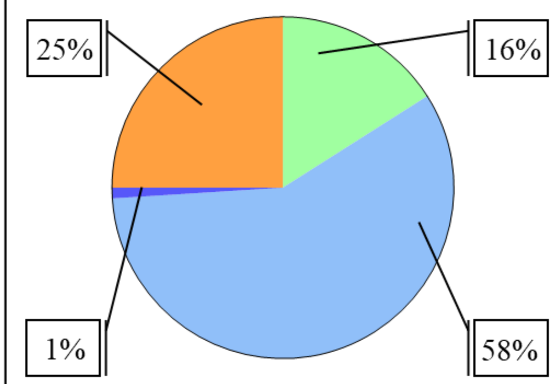


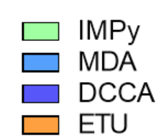


BDNF gene DNA Methylation at CpG3			BDNF gene DNA Methylation at CpG6			BDNF gene DNA Methylation at CpG total		
Beta	Standard Error	p-value	Beta	Standard Error	p-value	Beta	Standard Error	p-value
2.26	0.12	0.03	0.49	0.27	0.07	0.27	0.12	0.03



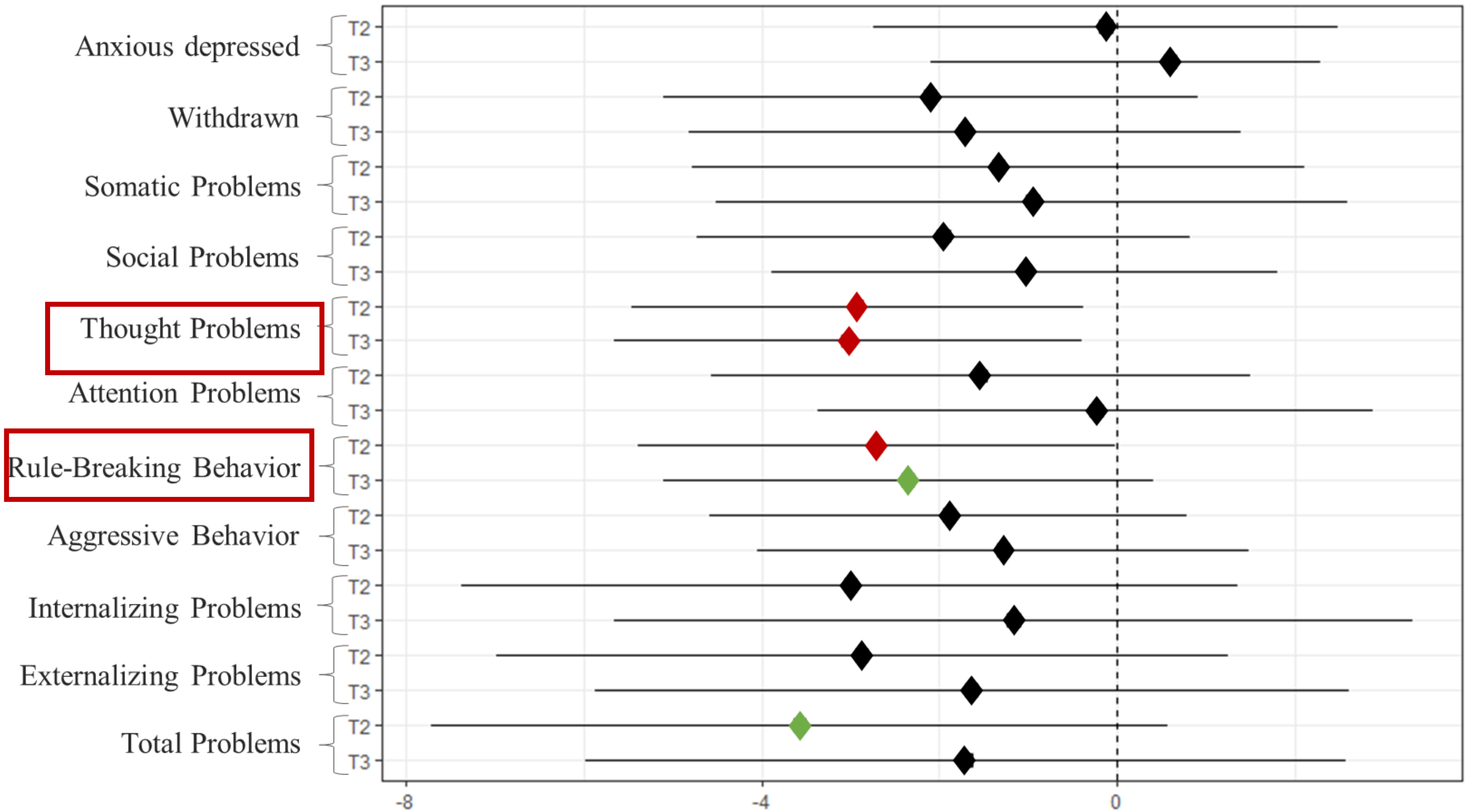






Model adjustment: Age, BMI, alcohol consumption, season of urine collection, urine creatinine, maternal education

Figure 2. Linear regression estimates of categorized serum BDNF and CBCL scores (95% CI)



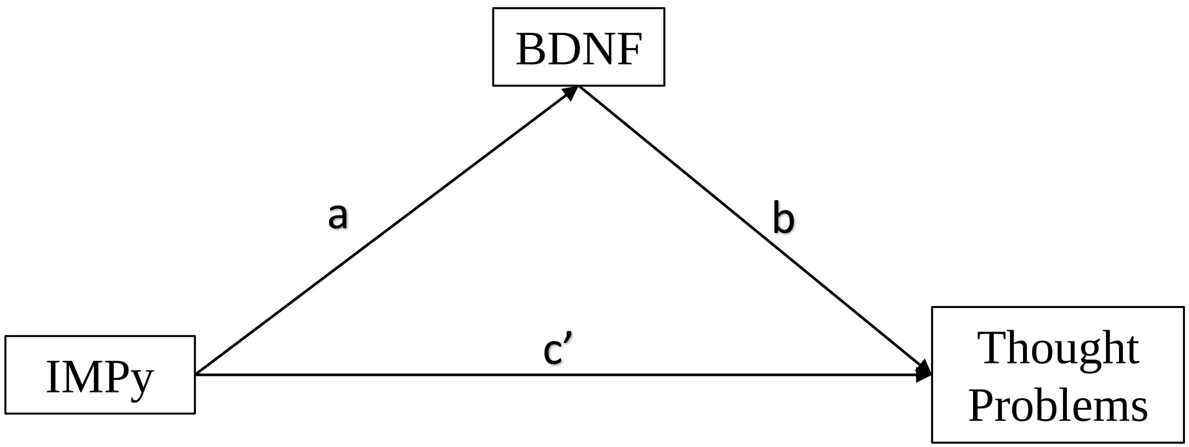
p<0.05; p<0.10

Model adjustment: Age, BMI, alcohol consumption, and maternal education

Higher BDNF protein levels were associated with lower thought and rule-breaking problems

Mediation analysis n=113

↑ IMPy ↑ Thought Problems | ↑ IMPy ↓ BDNF protein | ↑ BDNF protein ↓ Thought Problems



Total effect (C): β= 0.79, (-0.47,2.05)
Direct effect (c'): β= 0.62, (-0.64,1.88)
Indirect Effect (ab): β= 0.17; 95% CI= (-0.07,0.57)
Percentage of mediation= **21.5 %**

A suggested mediation effect of serum BDNF in the IMPy-Thought problems association was found

IN SUMMARY

Possible association IMPy, ΣOPs, and ETU levels with behavioral problems, partly explained by BDNF protein levels.

A possible **combined effect** for some pesticides with more withdrawn, social, and thought problems, CpG 3, and total CpGs DNA methylation.

Serum BDNF levels associated with more thought problems and rule-breaking behavior

IN SUMMARY

- **LIMITATIONS:**

Cross-sectional design

Small **sample** size;

One **spot** urine + metabolites of short-term = Risk of exposure **missclassification**

IMPy and 3-PBA were measured as conjuated: **underestimation**

- **STRENGHTS**

Novelty of non-persistent **pesticides** exposure during **adolescence**

BDNF: DNA methylation + protein levels

Mediation assessment

Combined effect

Consequences of non-persistent pesticides exposure

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Organophosphate pesticide exposure, hormone levels, and interaction with PON1 polymorphisms in male adolescents

Beatriz Suárez [a,b,c,1], Fernando Vela-Soria [a,1], Francesca Castiello [a,e], Alicia Olivas-Martinez [a,c], Dario Acuña-Castroviejo [a,c,d], José Gómez-Vida [e], Nicolás Olea [a,b,c,f], Mariana F. Fernández [a,b,c,f], Carmen Freire [a,b,c,*]

- Mediated by effect on thyroid hormones

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Urinary metabolites of non-persistent pesticides and serum hormones in Spanish adolescent males

Carmen Freire [a,b,c,*], Beatriz Suárez [a,b,c], Fernando Vela-Soria [a], Francesca Castiello [a,d], Iris Reina-Pérez [c,e], Helle R. Andersen [f], Nicolás Olea [a,b,c,e], Mariana F. Fernández [a,b,c,e]

- Mediated by effect on reproductive hormones

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