Relation between exposure to non-persistent pesticides and developmental neurotoxicity

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Infancia y Medio Ambiente

INMA Spanish birth cohort

Population-based cohorts

3 pre-existing cohorts

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

4 *de novo* cohorts

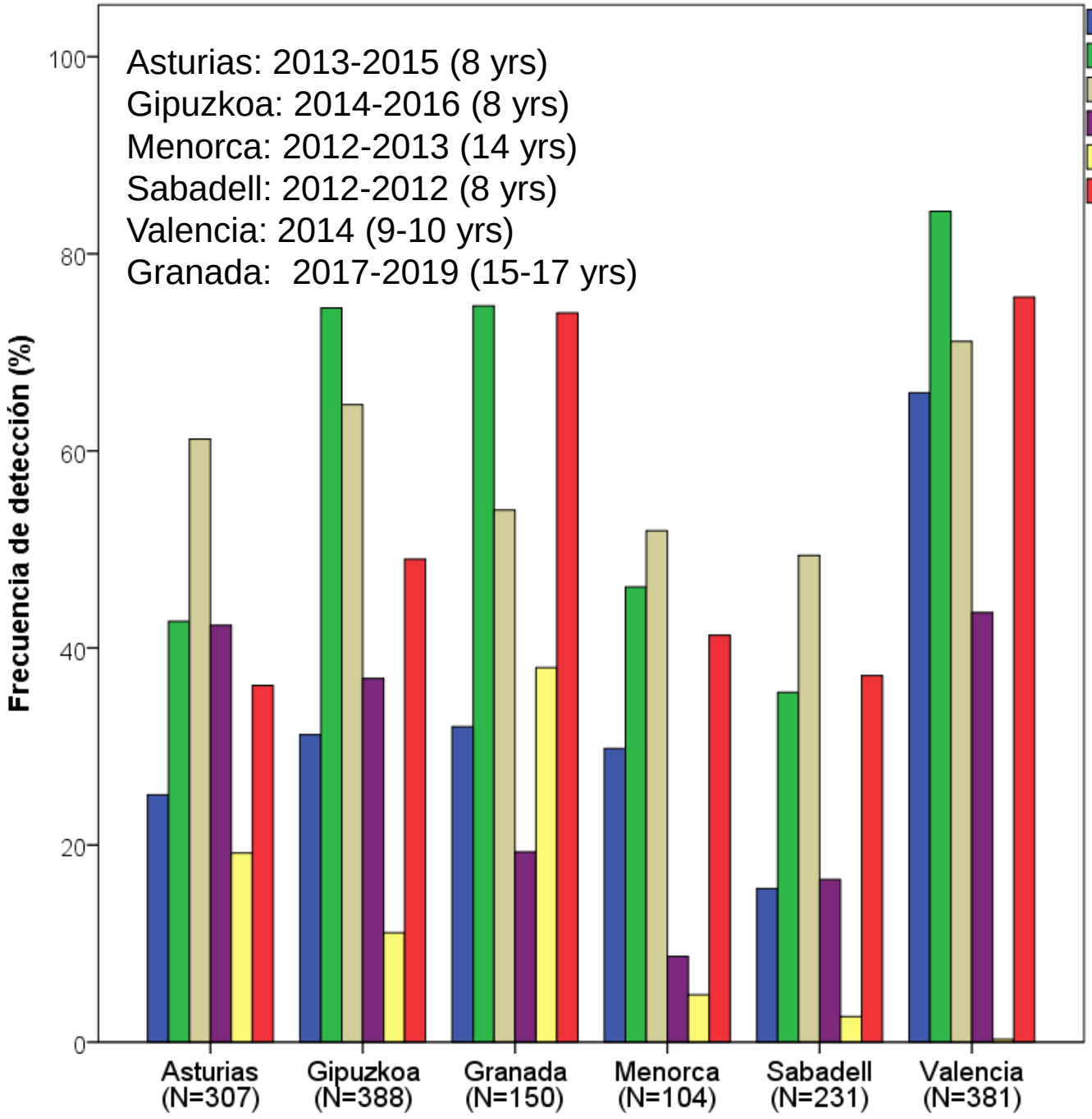
✓ Follow up from the 1st trimester of pregnancy: Sabadell, Valencia, Gipuzkoa y Asturias

The INMA cohort that includes almost **4,000 mother-child pairs**, has studied the exposure to some of the most important environmental pollutants present in air, water and diet of pregnant women and their children, and analysed the **effects of this exposure on child growth and development**.

*Biomarkers of **exposure** assessment*

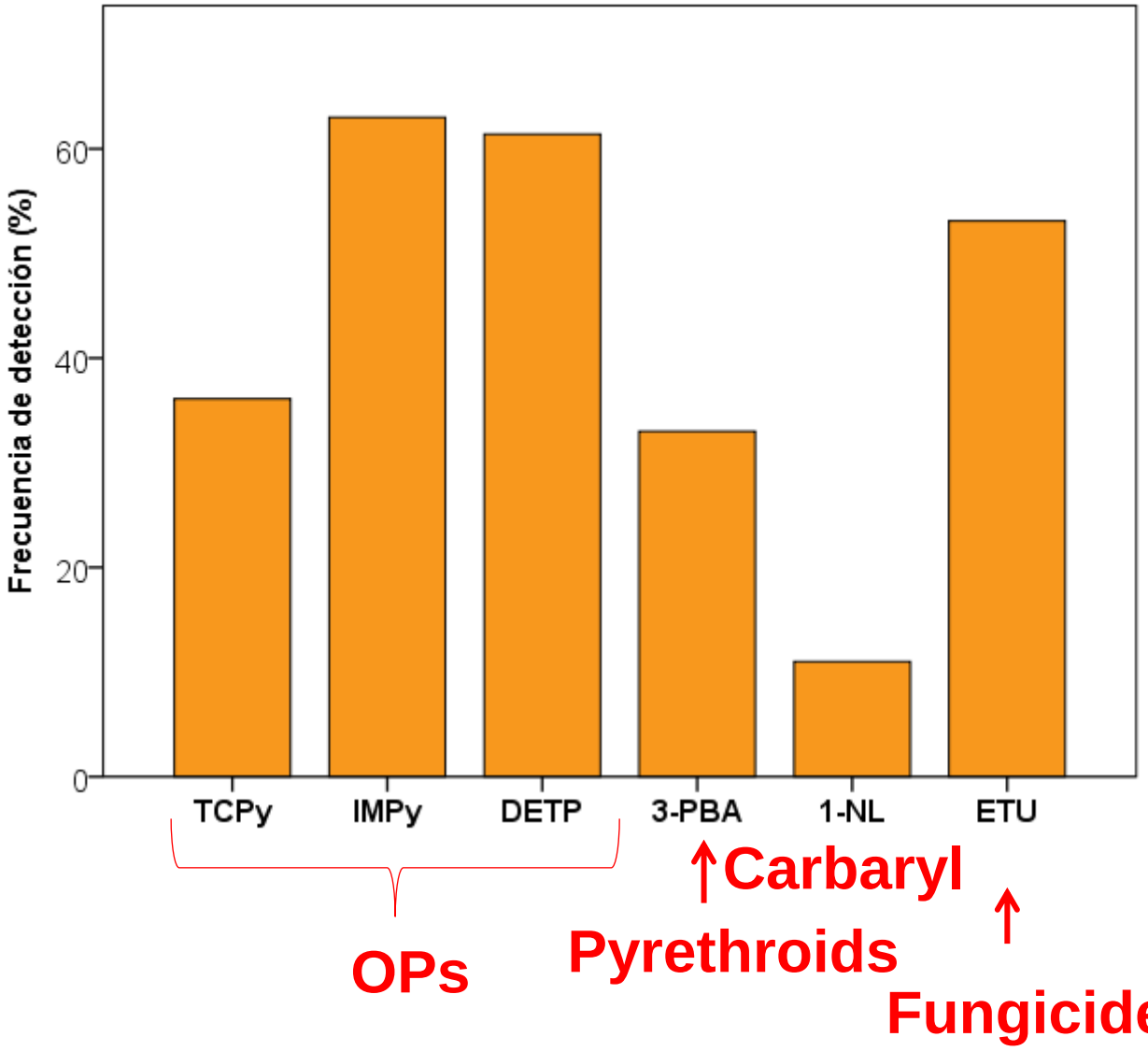
Prioritized HBM4EU chemicals → **Chlorpyriphos** → **Pyrethroids**

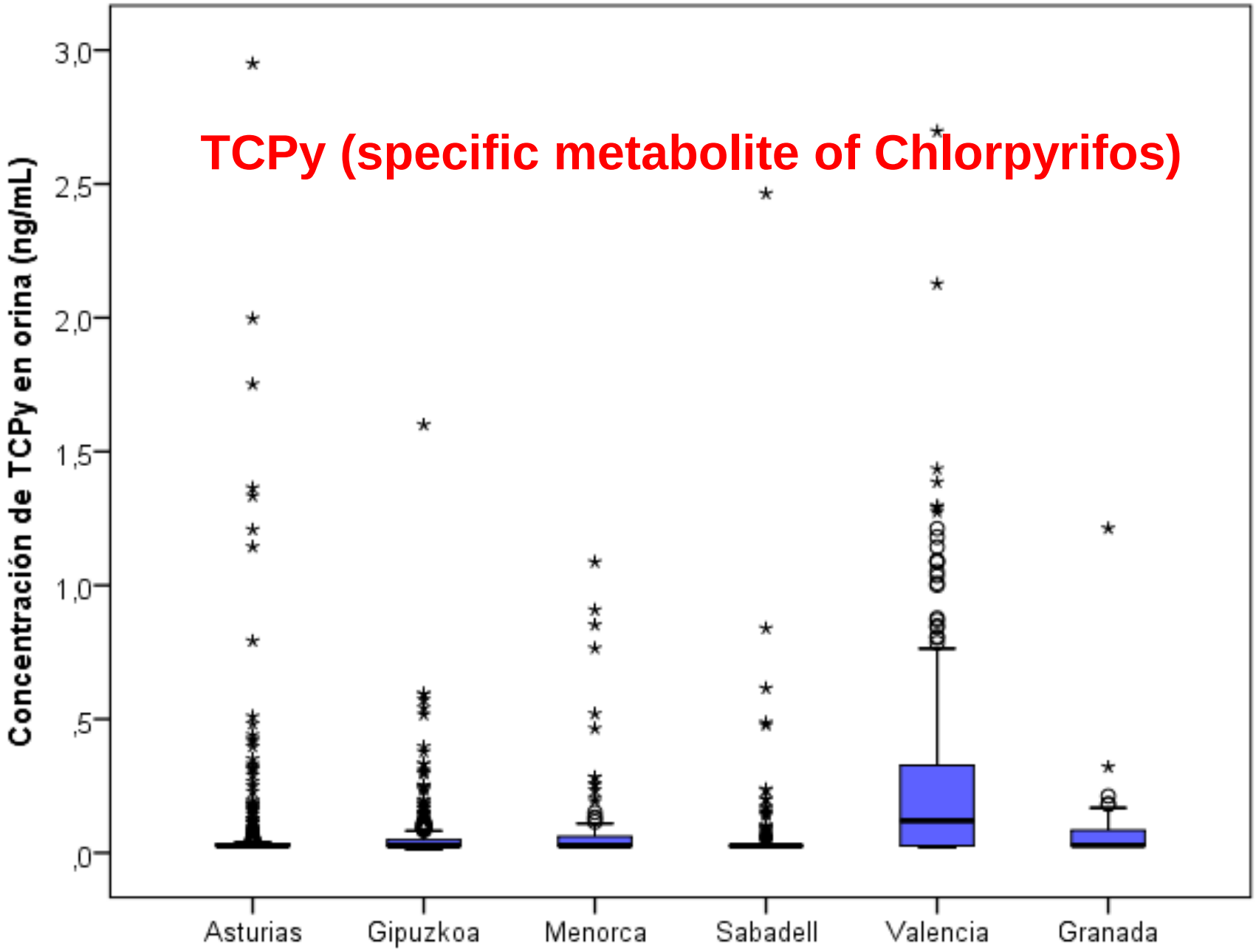
- **Organophosphate (OP) insecticide metabolites:** 3,5,6-trichloro-2-pyridinol (TCPy), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPy), malathion diacid (MDA), and diethyl thiophosphate (DETP) **+ΣOPs**
- **Pyrethroids (PYR) metabolites:** 3-phenoxybenzoic acid (3-PBA) and dimethylcyclopropane carboxylic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



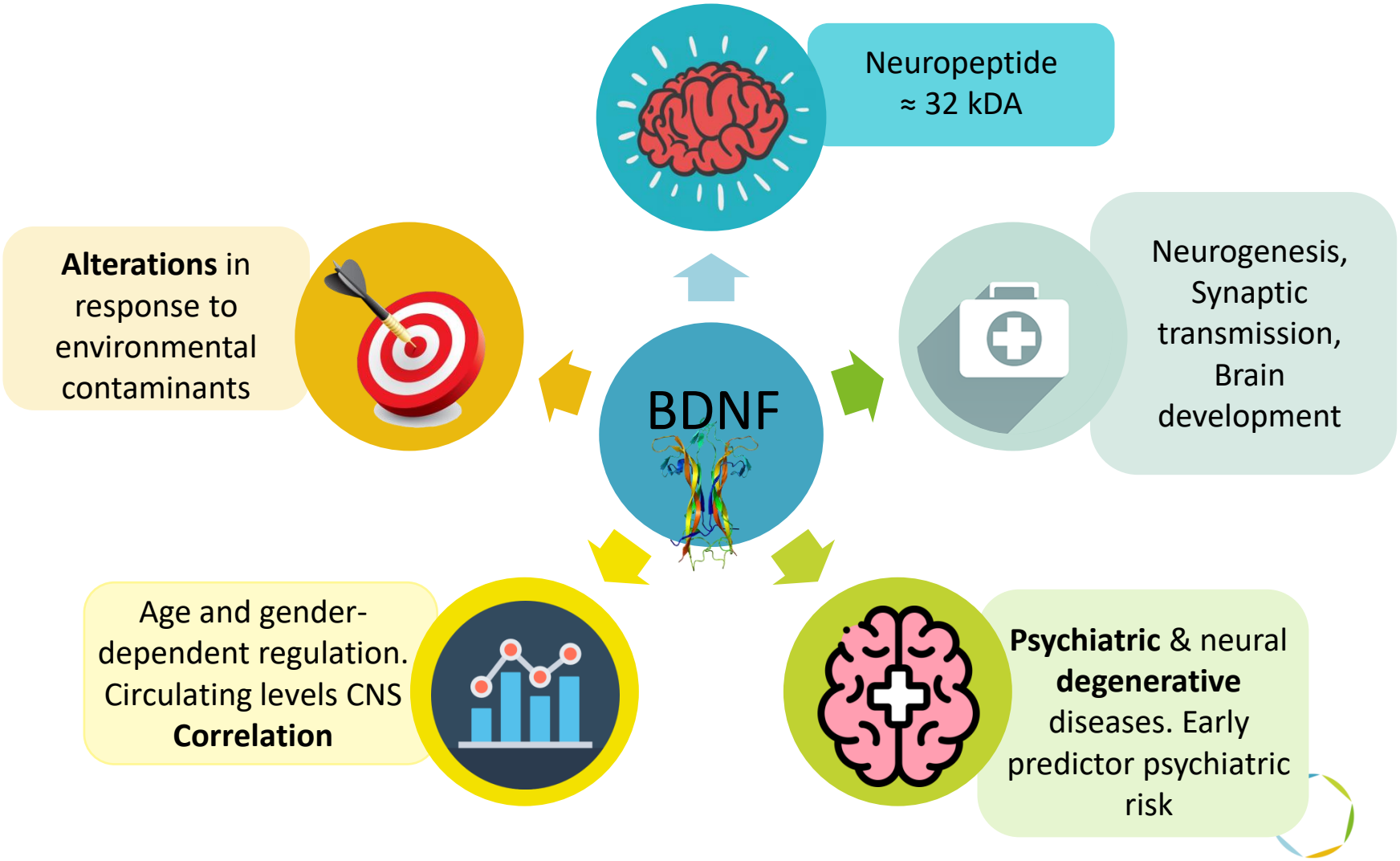
1,561 Spanish children & adolescents 2012-2019

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

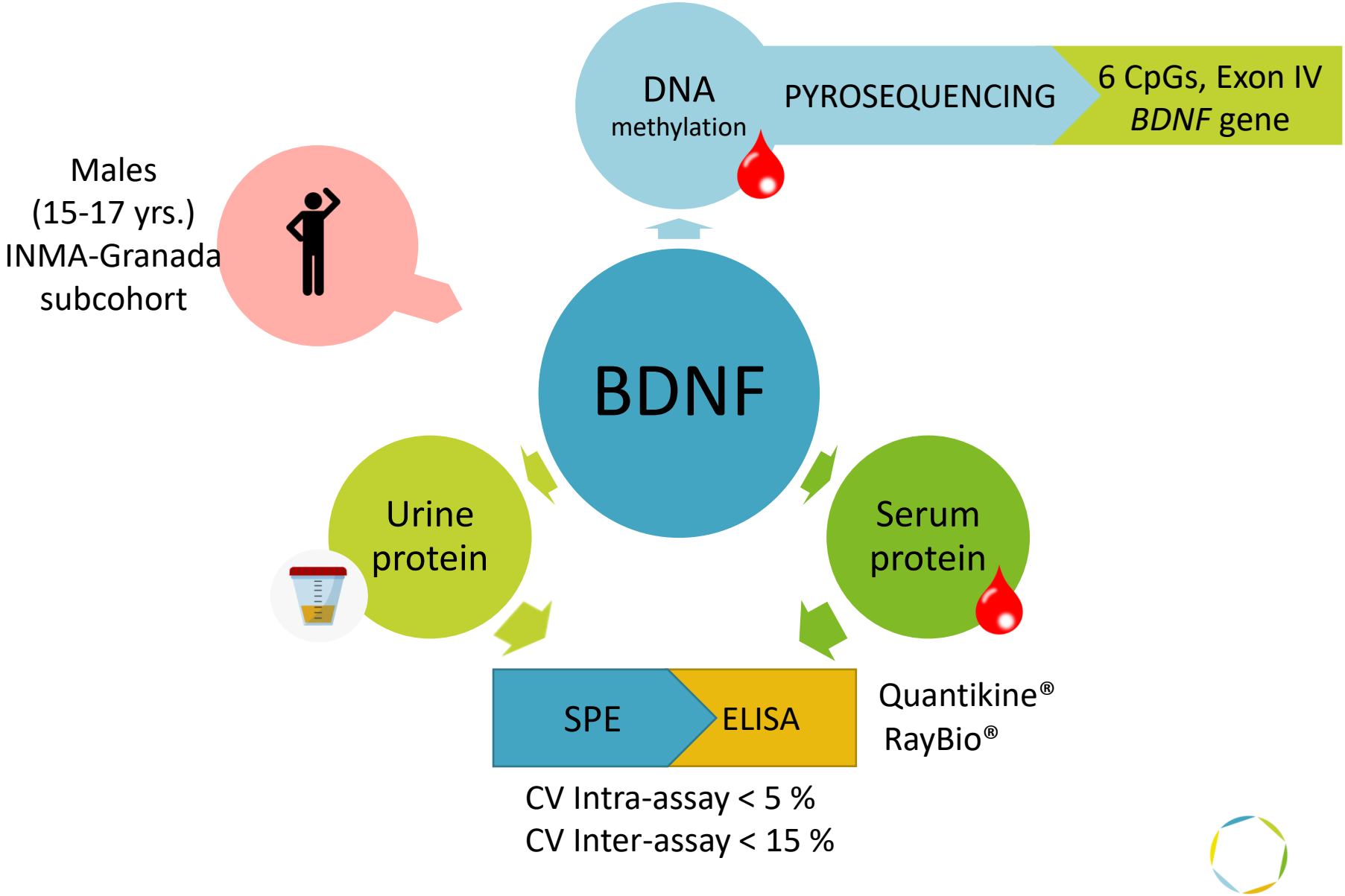



TCPy (specific metabolite of Chlorpyrifos)
Concentración de TCPy en orina (ng/mL)
3,0
2,5
2,0
1,5
1,0
,5
,0
Asturias
Gipuzkoa
Menorca
Sabadell
Valencia
Granada

*Brain-Derived Neurotrophic Factor (BDNF), as a **effect biomarker***



*Biomarkers of **effect** assessment*



Neuropsychological assessment

Materials & Methods

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
Percentiles	25	0.08	0.14	<LOD	<LOD	0.67	0.12	<LOD	0.21	<LOD	0.05
	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

Results & Discussion

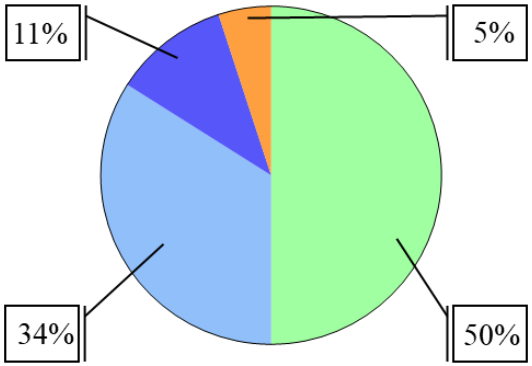
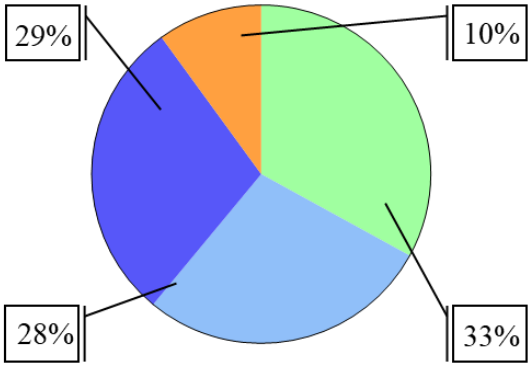
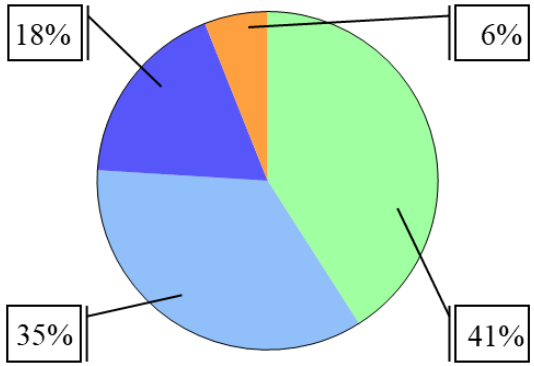
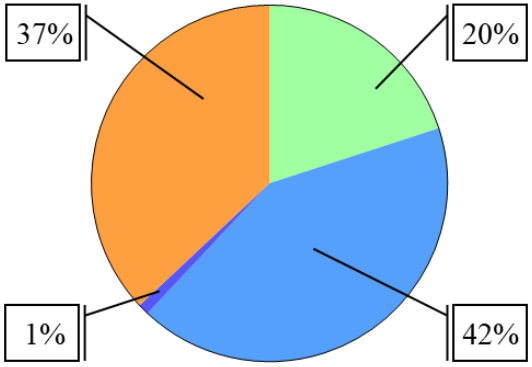
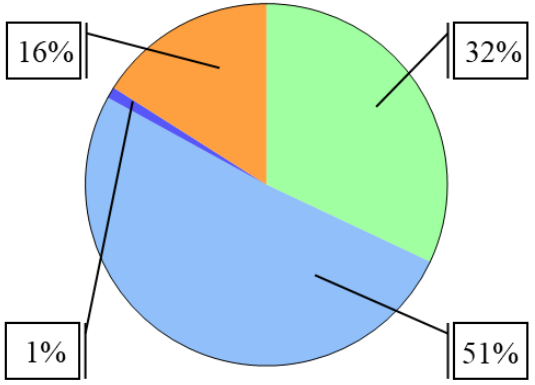
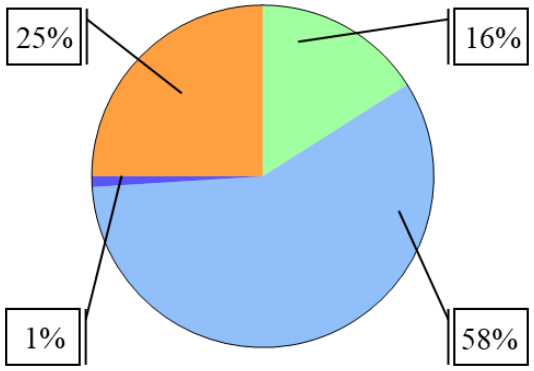
Table 3. Regression estimates change (β, 95% CI) of the associations between urinary pesticide metabolites concentrations and BDNF gene DNA methylation

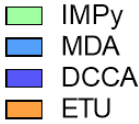
		CpG1	CpG2	CpG3	CpG4	CpG5	CpG6	ΣCpG
MDA	T2	0.21 (-0.15,0.57)	**0.26 (0.04,0.46)**	0.12 (-0.16,0.39)	0.04 (-0.57,0.65)	0.18 (-0.21,0.56)	-0.07 (-0.62,0.48)	0.12 (-0.17,0.42)
	T3	0.31 (-0.08,0.71)	**0.21 (-0.04,0.46)**	0.24 (-0.06,0.54)	0.25 (-0.41,0.91)	0.23 (-0.18,0.64)	0.05 (-0.54,0.65)	0.22 (-0.10,0.53)
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

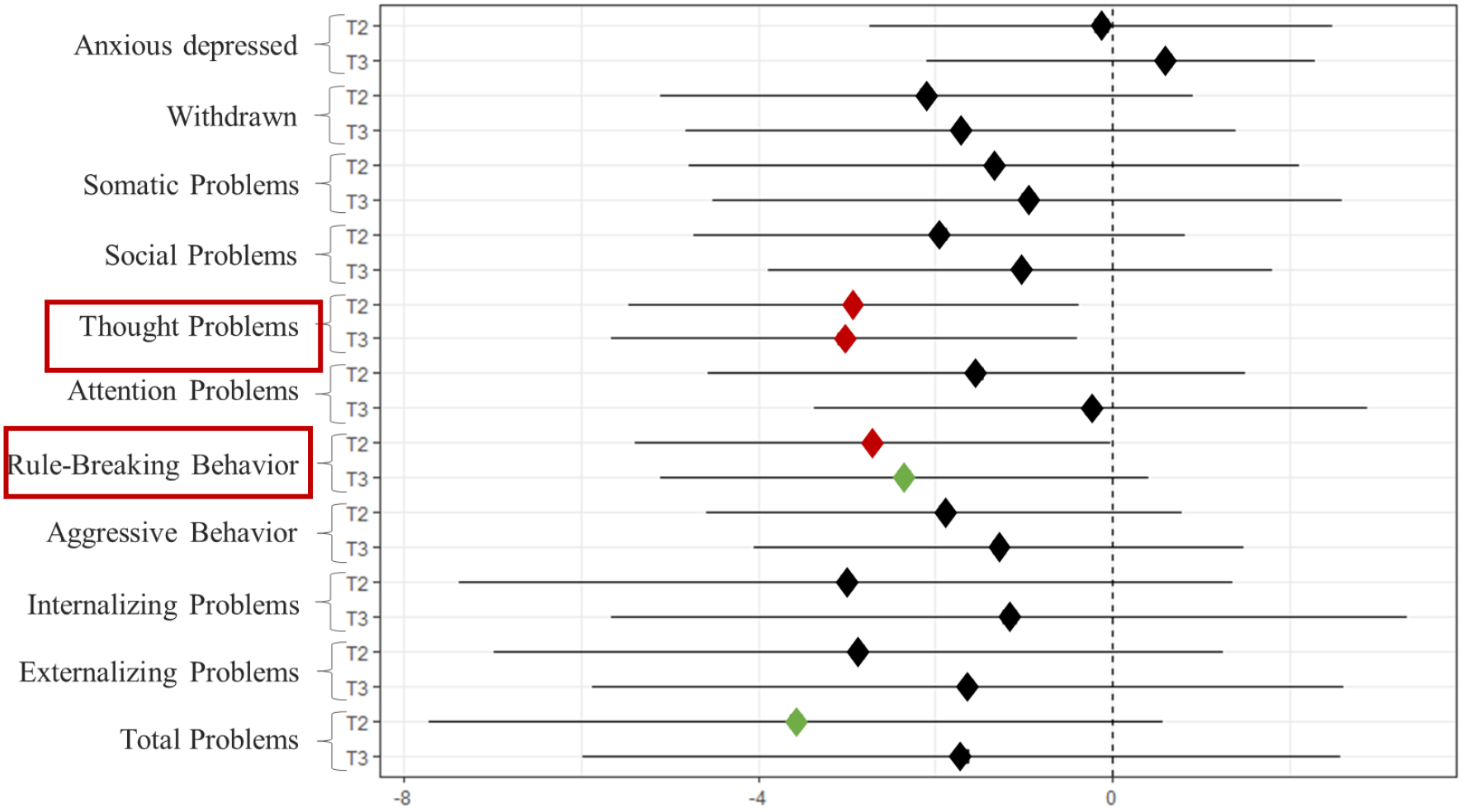
Figure 1. Mixture Effect analysis (WQS)

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**

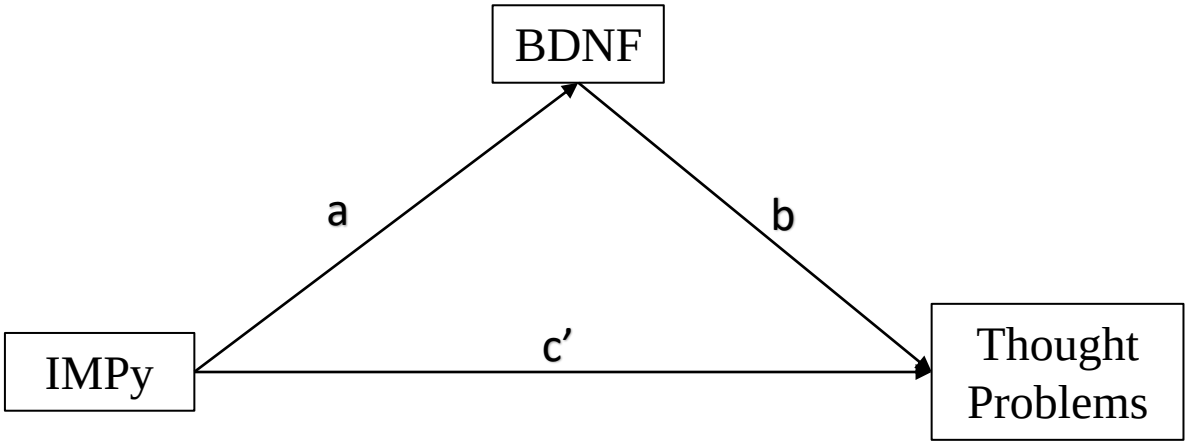
Model adjustment: Age, BMI, alcohol consumption, and maternal education

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

Results & Discussion

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