Neurobehavioral effects of developmental toxicity

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INMA *(Environment and Childhood)* Spanish Birth cohort

The INMA cohort: **4,000 mother-child pairs**. Assessing the exposure to important environmental pollutants and analysed its **effects on child growth and development**.

Analyses of pesticides-neurodevelopment effects were performed in **Granada** subcohort.

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

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NEUROTOXIC PESTICIDES ALLOWED IN EU (2021)

Insecticides
Organophosphates: **chlorpyrifos**, dimethoate, fenamiphos, phosmet
Carbamates: pirimicarb, methomyl
Pyrethroids: **permethrin**, **cypermethrin**, **deltapermethrin**
Others: nicotine
Herbicides
Bipyridyl herbicides: diquat dibromide
Chlorophenoxy herbicides: 2,4-D
Fungicides
Dithiocarbamates: **maneb**, thiram
Rodenticides: warfarin
Fumigants: phosphides (Zn, Mg, Al), sulfuryl fluoride

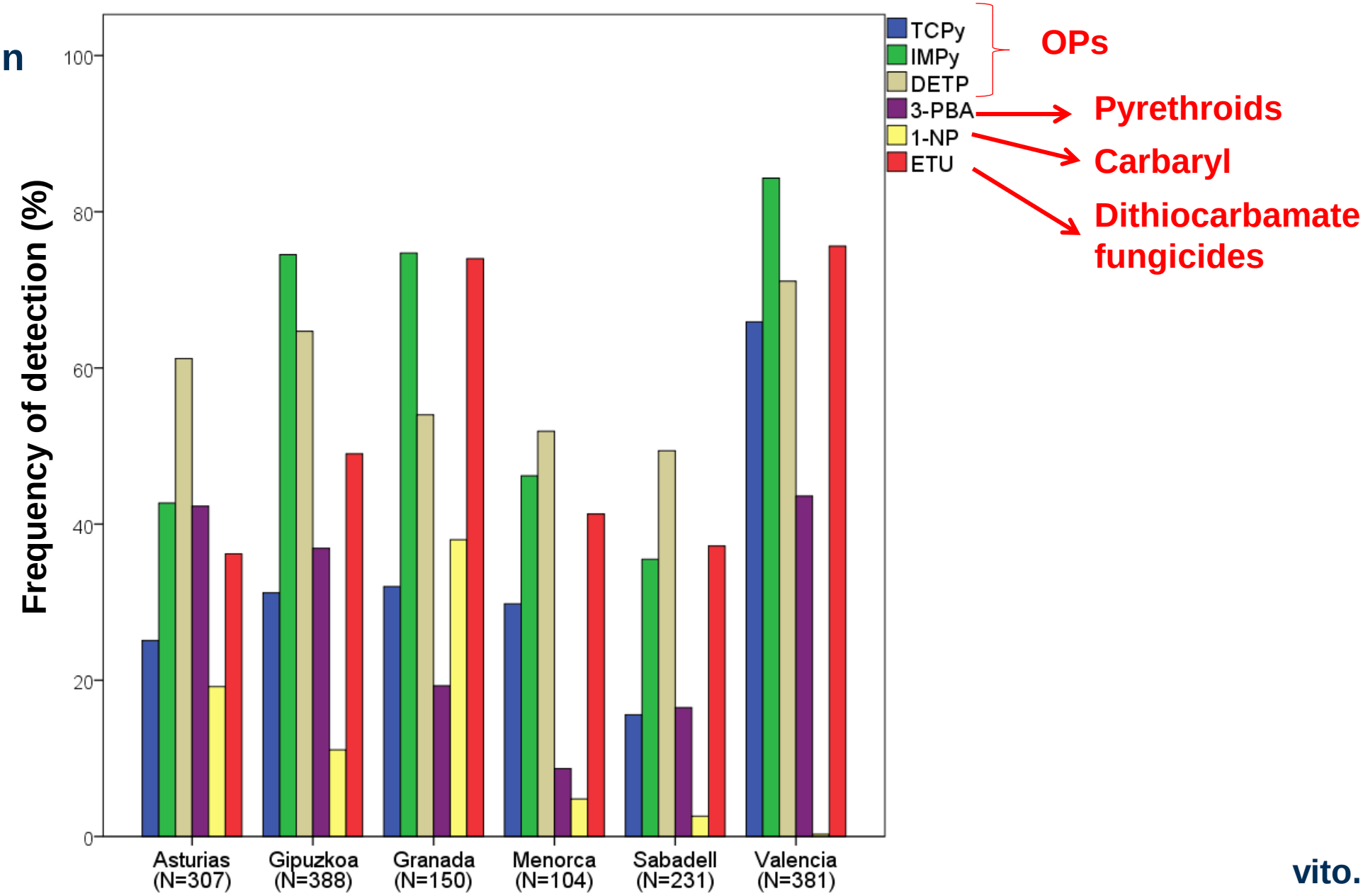
Selection of pesticides: biomarkers of exposure

Non-persistent pesticide metabolites selected for measurement in the INMA cohort

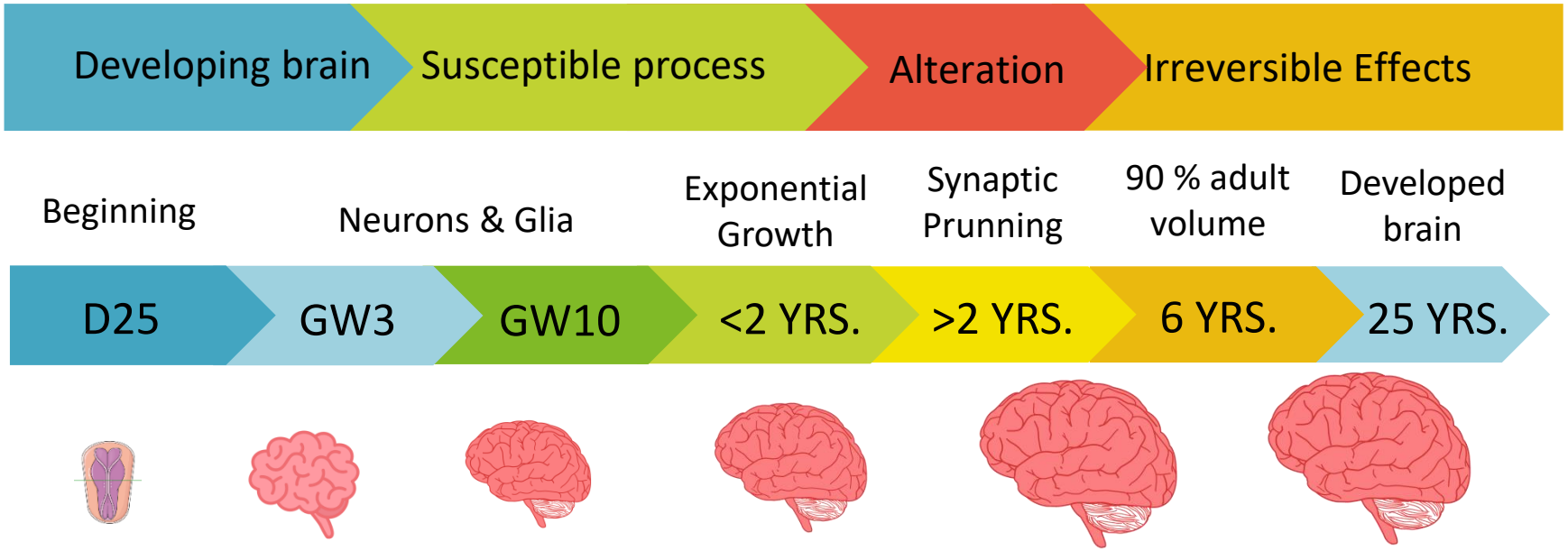
Prioritized chemicals → **Chlorpyriphos** → **Pyrethroids**

- **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)

N= 1,561 children & adolescents



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

PESTICIDES AND NEURODEVELOPMENTAL DISORDERS IN CHILDREN.

Key messages from the scientific literature

The prevention of neurodevelopmental disorders is a public health priority

The causes of these disorders are unclear, and interacting genetic, environmental and social factors are likely determinants of abnormal brain development

Many pesticides target the nervous system

Scarce or non-existing scientific evidence on the developmental neurotoxicity of many of the pesticides in current use

Exposure to residues in food and other exposures should be prevented regarding neurotoxic pesticides

Neuropsychological assessment: Behavioral function

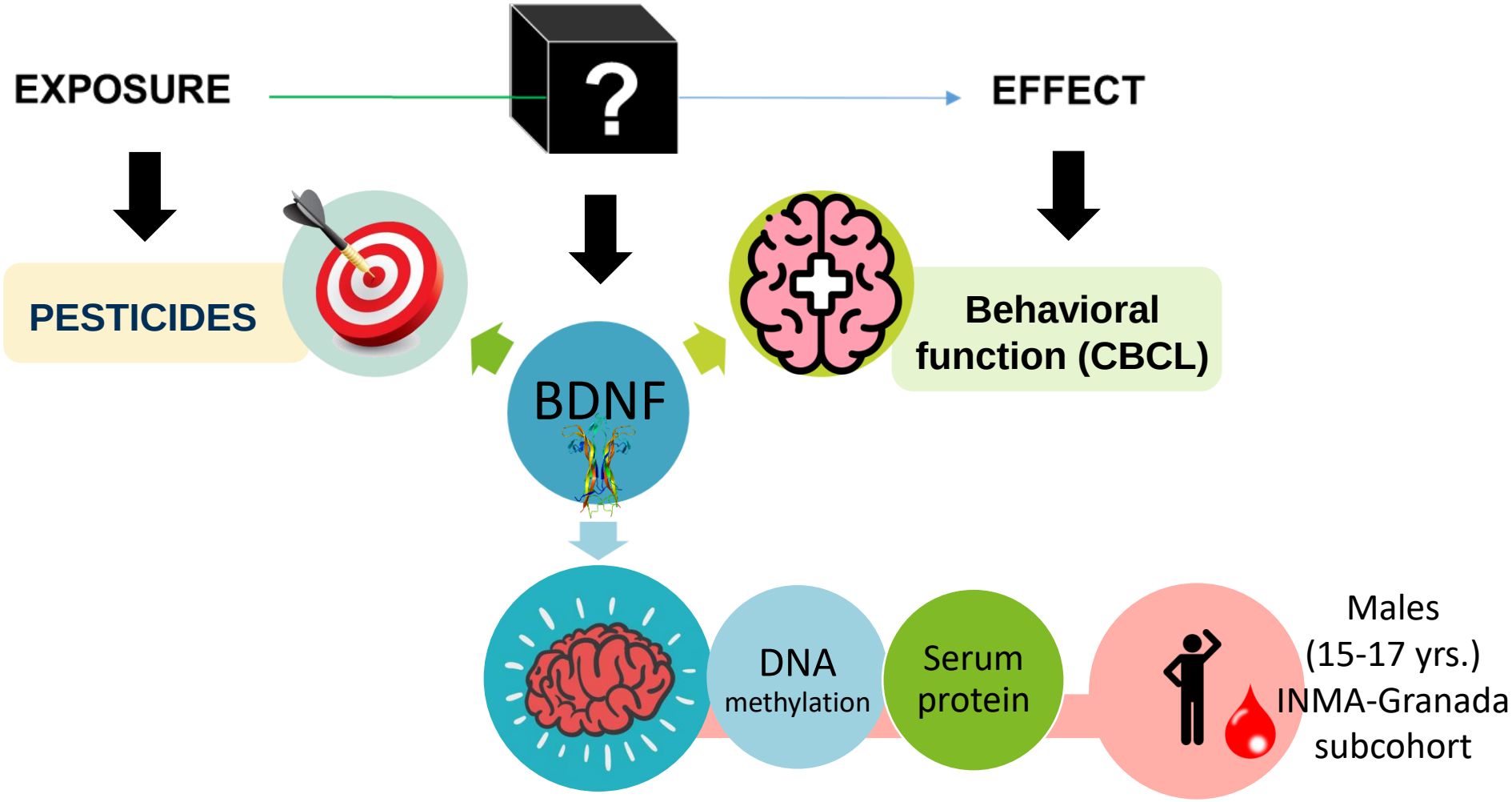
Child-Behavior Check list 6/18 (CBCL)

- **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.

Neurodevelopment assessment: Brain-derived neurotrophic factor, BDNF



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

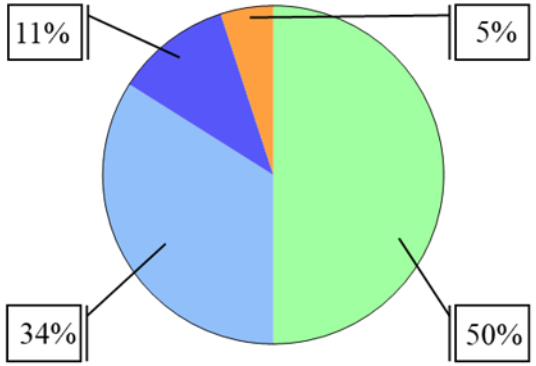
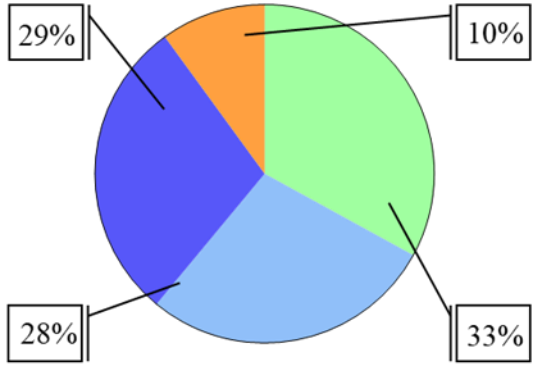
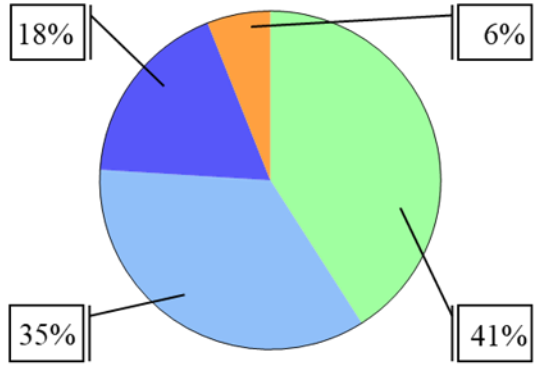
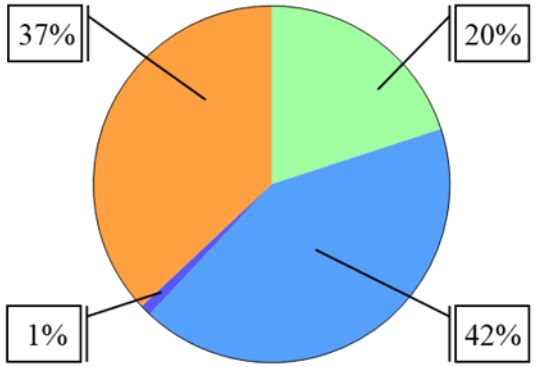
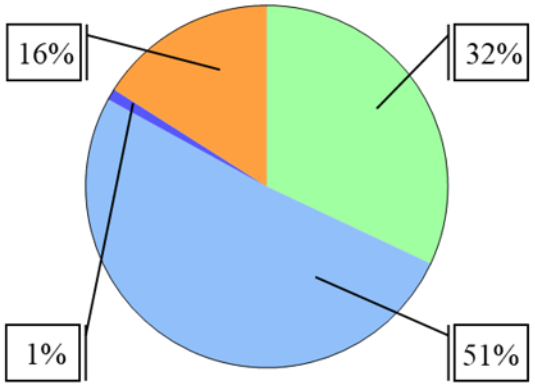
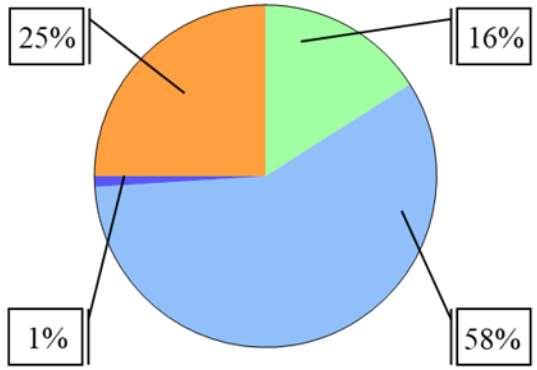
Table 3. Regression estimates change (β, 95% CI) of the associations between urinary pesticide metabolites concentrations and BDNF gene DNA methylation

		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

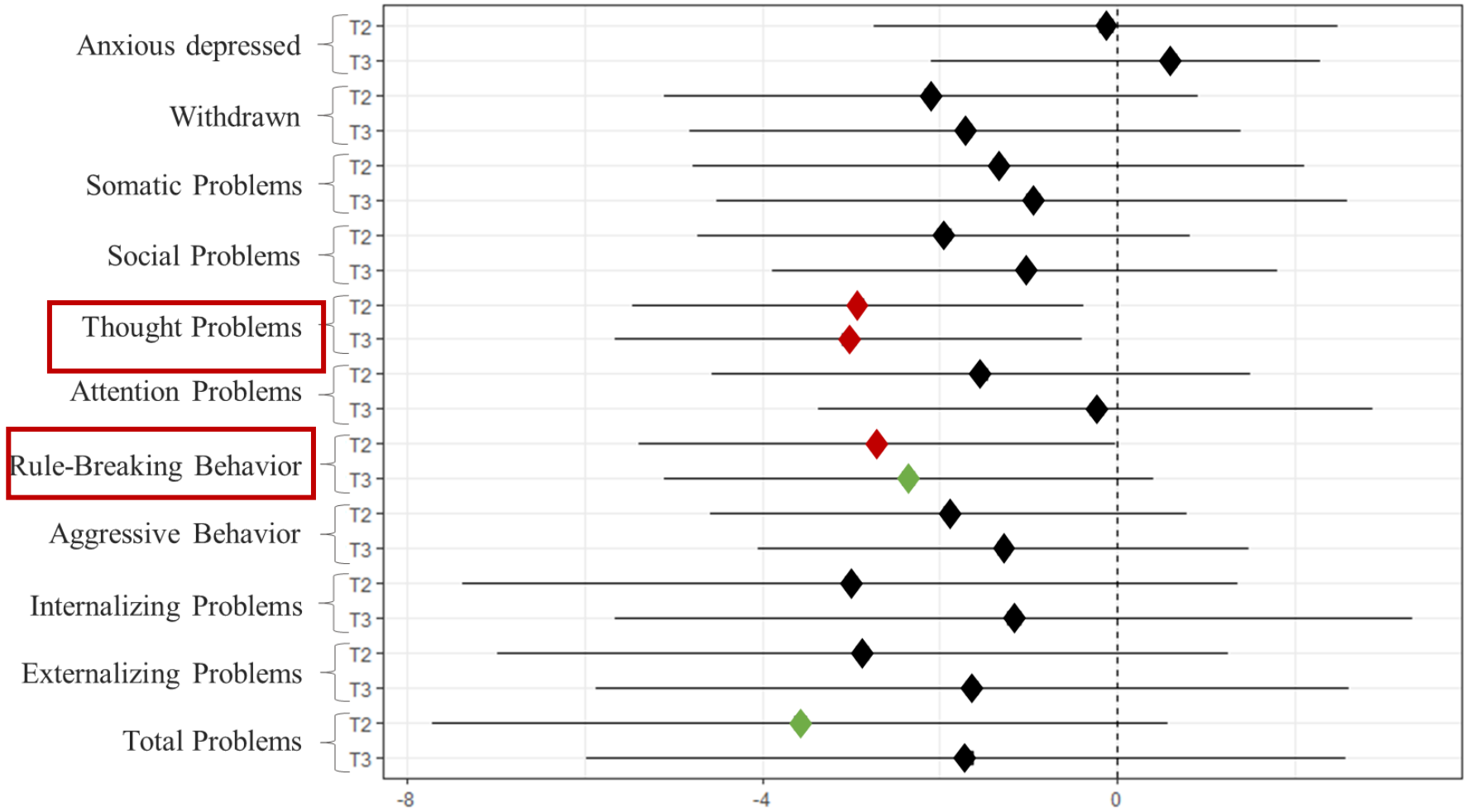
Model adjustment: Age, BMI, alcohol consumption, season of urine collection, urine creatinine, maternal education

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)



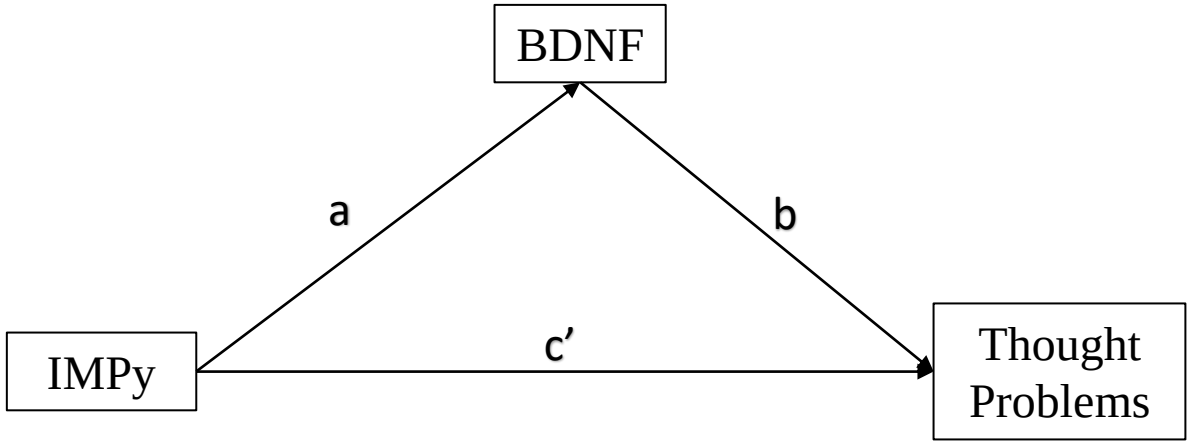
p<0.05; **p<0.10**

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

Children and adolescents are exposed to neurotoxic pesticides that may alter brain development

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.

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