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# Childhood exposure to non-persistent pesticides and pubertal development in Spanish girls and boys: Evidence from the INMA (Environment and Childhood) cohort☆

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ABSTRACT

This study assessed cross-sectional associations between urinary metabolites of non-persistent pesticides and pubertal development in boys and girls from urban and rural areas in Spain and examined effect modification by body mass index (BMI). Four metabolites of insecticides (TCPy, metabolite of chlorpyrifos; IMPy, metabolite of diazinon; DETP, non-specific metabolite of organophosphates; 3-PBA, metabolite of pyrethroids) and the metabolite of ethylene-bis-dithiocarbamate fungicides (ETU) were quantified in urine collected in 2010–2016 from 7 to 11-year-old children (606 girls, 933 boys) participating in the INMA Project. Pubertal development was ascertained by Tanner stages and/or parent-reported Pubertal Development Scale (PDS). Associations between pesticide metabolites and odds of being in stage 2+ for breast development (girls), genital development (boys), pubic hair growth (girls and boys), and/or overall puberty onset, gonadarche, and adrenarche (PDS for girls and boys) were examined by mixed-effect logistic regression. Effect modification by BMI was explored by interaction terms and stratified analysis. In girls, DETP and ETU concentrations>75th percentile (P75) were associated with higher odds of overall puberty development (OR [95%CI] = 1.86 [1.07–3.24] and 1.71 [1.03–2.83], respectively, for > P75 *vs.* undetected concentrations), while ETU > P75 was also associated with higher odds of breast development (OR [95%CI] = 5.55 [2.83–12.91]), particularly in girls with underweight/normal weight (OR [95%CI] = 10.08 [2.62–38.76]). In boys, detection of TCPy (40%) and 3-PBA (34%) was associated with higher odds of genital development (OR [95%CI] = 1.97 [1.08–3.57] and 2.08 [1.15–3.81], respectively), and the association with 3-PBA was observed in boys with overweight/obesity alone. In addition, ETU > P75 was associated with higher odds of genital development in boys with underweight/normal weight (OR [95%CI] = 2.89 [1.08–7.74]) but higher DETP with lower odds of puberty in boys with overweight/obesity (OR [95%CI] =

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0.94 [0.89–0.99] per log-unit increase in concentration). Results suggest an association of childhood exposure to ETU and certain insecticides with earlier puberty in girls and boys that may be modified by child BMI.

## 1. Introduction

Contemporary pesticides are widely used in food production as well as in non-agricultural urban and domestic settings. The main exposure route for pesticides in the general population is the diet, especially through the consumption of conventionally grown fruits and vegetables (Fortes et al., 2013; Lu et al., 2008). Once in the human body, they are rapidly metabolized and excreted, mainly *via* urine (Barr, 2008; Egeghy et al., 2011). Organophosphate (OP) and pyrethroid insecticides and certain herbicides are the most common pesticides in the European Union (EU) and worldwide. Dithiocarbamate fungicides are another extensively employed class of pesticides. Experimental evidence suggests that several currently used and banned non-persistent pesticides may act as endocrine-disrupting chemicals (EDCs) (Andersen et al., 2002; Kojima et al., 2004; Orton et al., 2011). For instance, *in vitro* studies have shown that OP insecticides such as chlorpyrifos and diazinon interact with the estrogen receptors alpha and beta (ERα, ERβ) and/or the androgen receptor (AR) (Andersen et al., 2002; Chen et al., 2002; Kojima et al., 2004; Manabe et al., 2006; Orton et al., 2011; Shen et al., 2021). Chlorpyrifos also inhibits the expression of key sex steroid synthesizing enzymes, including aromatase (Usmani et al., 2003; Viswanath et al., 2010). Likewise, the pyrethroids deltamethrin, cypermethrin, and λ-cyhalothrin are known to exert estrogenic and anti-androgenic effects *in vitro* (Andersen et al., 2002; Chen et al., 2002; Fujino et al., 2019; Kjeldsen and Ghisari, 2013; Kojima et al., 2004; Orton et al., 2011; Sun et al., 2007). Dithiocarbamate fungicides such as mancozeb have also been shown to antagonize human AR activity *in vitro* (Archer and van Wyk, 2015; Kjeldsen and Ghisari, 2013).

Increasing exposure to EDCs over the past few decades may be one the factors responsible for the consistent secular trend towards earlier puberty onset in girls observed in Western countries (Biro and Kiess, 2016; Toppari and Anders, 2010) and for a more recently described shift towards earlier male puberty onset (Brix et al., 2019; Herman-Giddens and Marcia, 1997). However, few human studies have addressed the relationship between exposure to contemporary pesticides and puberty timing, with conflicting findings (Castiello and Freire, 2021). Specifically, *in utero* exposure to the herbicide atrazine was associated with earlier age of menarche in mother-child pairs from urban and rural areas in the UK (Namulanda et al., 2017), and childhood exposure to OP pesticides was associated with delayed sexual development in urban and rural boys and girls in Belgium (Croes et al., 2015). Pyrethroid exposure in an urban setting was associated with pubertal delay in girls (Ye and Pan, 2017a) and pubertal acceleration in boys (Ye and Pan, 2017b) in a Chinese study, and the occupational exposure of pregnant Danish women to multiple pesticides was linked to earlier breast development in female offspring (Wohlfahrt-Veje et al., 2012a) and lower testicular volume and penile length in the males (Wohlfahrt-Veje et al., 2012b). Other cross-sectional studies found no clear evidence of an association between residence in an agricultural area and pubertal development in South African boys (English et al., 2012) or breast development in Native American girls (Guillette et al., 2006).

Spain is the largest consumer of pesticides in the European Union (EU), using 74,000 tons of pesticides in 2019, including 34,000 tons of fungicides, the largest group (EUROSTAT 2022). With this background, the aim of this study was to investigate the association between concentrations of urinary metabolites of various non-persistent pesticides (OPs, pyrethroids, and dithiocarbamate fungicides) and pubertal development in girls and boys aged 7–11 years.

## 2. Material and methods

### 2.1. Study population

This cross-sectional study was conducted among children participating in the Environment and Childhood (INMA) multicenter birth cohort study, designed to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development in different regions of Spain (Guxens et al., 2012). Five out of seven cohorts in the INMA study collected urine from the children at age 7–11 years. These five INMA cohorts enrolled 3238 women during the first prenatal visit (10–13 weeks of gestation) in Asturias, Gipuzkoa (Basque Country), Sabadell (Catalonia), and Valencia (2003–2008) but at birth in Granada (Andalusia) (2000–2002). All cohorts included boys and girls except for Granada, which recruited boys alone, as the initial aim of this cohort was to investigate the influence of prenatal exposure to endocrine-disrupting chemicals on the risk of male urogenital malformations (Fernandez et al., 2007). The Sabadell cohort in Northeastern Spain recruited mother-child pairs residing in the urban area of Sabadell, a medium-sized city, while the remaining cohorts recruited children from both urban and rural areas. INMA-Asturias and Gipuzkoa study areas, in Northern Spain, are characterized by the presence of urban centers with important industrial activity and small rural towns. The INMA-Granada study area, in Southeastern Spain, comprises the metropolitan area of Granada city and surrounding rural towns and villages. Finally, the Valencia-cohort is located in an area with intense agricultural activity surrounding the city of Valencia in Eastern Spain. Further details on the INMA study areas and recruitment strategies are described elsewhere (Guxens et al., 2012). A total of 1976 out of 3238 children (61%) born to women originally included in these cohorts underwent puberty development assessment at 7–11 years of age between 2010 and 2016 (Granada in 2010–2012, and Asturias, Gipuzkoa, Sabadell and Valencia in 2013–2016). Puberty assessment included physical examination of developmental stage, as described below (section 2.4), and hormonal measurements in Valencia and Granada. In this study, the association of pesticide exposure with pubertal stage was examined in 1539 (606 girls and 933 boys) of the 1976 children from the five cohorts for whom urine samples were available and were analyzed for metabolites of non-persistent pesticides (Fig. 1).

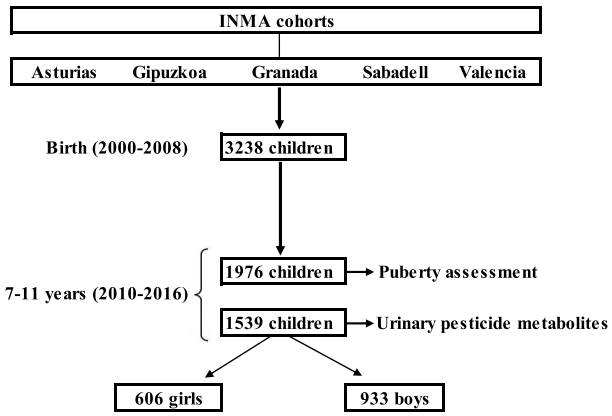


**Fig. 1.** Flow-chart of the study population.

### 2.2. Ethical aspects

The parents or guardians of all participants signed informed consent, and the research protocol was approved by the Ethics Committees of each region: Clinical Research Ethics Committee (CEIC) of the Principado de Asturias (ref. number 29/2003), CEIC Euskadi in Gipuzkoa (ref. number 11/2013), Biomedical Research Ethics Committee in Granada (ref. number 0509-N17), CEIC Parc de Salut MAR in Sabadell (ref. number 2005/2106/l), and CEIC DGSP-CSISP in Valencia (ref. number 20131007).

### 2.3. Measurement of urinary pesticide metabolites

A non-fasting spot urine sample was collected from each participant in Granada (5–8 p.m.) and Valencia (90% in the morning) on the day of the follow-up visit. Samples from Asturias were first morning voids, and samples from Sabadell and Gipuzkoa were pools of first morning and preceding bedtime voids. Urine samples (approx. 30 mL) were collected by study participants at their residences using sterile propylene containers and kept at 4 °C until arrival at the hospital or follow-up site, where they were stored at −80 °C. Pools of first morning and preceding bedtime voids were prepared by qualified research staff in Sabadell and Gipuzkoa following a standardized protocol. Samples were delivered to the ‘‘UNETE Research Unit’’ of the Biomedical Research Center (CIBM), University of Granada (Spain), where pesticide measurements were performed. Urine samples were analyzed for concentrations of 3,5,6-trichloro-2-pyridinol (TCPy), a metabolite of chlorpyrifos and chlorpyrifos-methyl; 2-isopropyl-6-methyl-4-pyrimidinol (IMPy), a metabolite of diazinon; diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP), two dialkyl phosphates (DAPs) that are metabolites of various OP insecticides; 3-phenoxybenzoic acid (3-PBA), a generic metabolite of pyrethroids; and ethylene thiourea (ETU), primary metabolite of ethylene-bis-dithiocarbamate (EBDC) fungicides such as mancozeb and maneb.

The metabolites/pesticides analyzed in this study were selected based on their ubiquity and toxicological/regulatory relevance. TCPy, 3-PBA, and DAP metabolites are widely used as urinary biomarkers of OP and pyrethroid insecticides (Barr, 2008; Yusà et al., 2015, 2022). Importantly, chlorpyrifos and pyrethroids are on the list of priority chemicals established by the European Human Biomonitoring Initiative (HBM4EU) (Ougier et al., 2021). Chlorpyrifos, one of the most widely used pesticides on European crops over the past few years, was banned in 2020 (Regulation (EU) 2020/17). Although diazinon has not been authorized in the EU since 2006, IMPy has been detected in urine samples collected several years after the prohibition of diazinon from Spanish children (Roca et al., 2014) and pregnant women (Bravo et al., 2020). Moreover, diazinon residues were detected in fruit, vegetable, and spices marketed in Spain in 2018 (AESAN 2018). DETP and DEDTP were selected as biomarkers of exposure to several common OP insecticides, including approved and non-approved insecticides such as chlorpyrifos, diazinon, ethion, parathion, phorate, and terbufos. Growing concerns about their potential adverse health effects of pyrethroids has led to the prohibition of some pyrethroids by the EU (PAN International 2021). Urinary ETU is considered a useful biomarker of human exposure to EBDC fungicides (Yusà et al., 2015), which are among the most frequently detected pesticides in food sold in Spain, including organic food (AESAN 2018). Mancozeb lost its approval in the EU in January 2021 (Regulation (EU) 2020/2087), but other EBDC fungicides such as ziram and metiram are still authorized. Notably, ETU is also used in the manufacture of neoprene, polyacrylate rubbers, plastic materials, and pharmaceutical compounds and in paper whitening, dry cleaning, and photography, although the main source of exposure is from agricultural fungicides (Mutic et al., 2017).

Ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS/MS) was used for the analysis of urinary pesticide metabolites. All metabolites were calibrated and extracted according to a previously described methodology (Freire et al., 2021; Suárez et al., 2021). Limits of detection (LD) were 0.039 μg/L for TCPy, 0.117 μg/L for IMPy and 3-PBA, 0.116 μg/L for DETP, 0.142 μg/L for DEDTP, 0.117 μg/L for 3-PBA, and 0.072 μg/L for ETU. In order to correct for urine dilution, urinary creatinine concentrations were measured in a Roche Cobas C-311 system using a commercial kit (CREJ2) based on the Jaffe method.

### 2.4. Puberty development assessment

Puberty development was assessed by clinical Tanner staging (Marshall and Tanner, 1970) and/or the parent-reported Peterson's Pubertal Development Scale (PDS) (Petersen et al., 1988) at a mean age of 8.8 years (5th-95th percentile: 7.7–10.1 years). Puberty development was always assessed by individuals who were blinded to the results of the exposure assessment, eliminating the possibility of bias. Children in Asturias and Valencia (308 girls and 341 boys) were evaluated for Tanner stages of genital development (boys), breast development (girls), and pubic hair development (boys and girls), while boys in Granada ($n = 279$) were evaluated for genital development alone. A pediatrician or trained health care professional assessed genital (G) by visual inspection and palpation, pubic hair development (PH) by visual inspection, and breast development (B) by palpation. The development stage was classified on a scale ranging from 1 (pre-pubertal) to 5 (adult development). Puberty onset was defined as being in stage 2+ for genital development (G2+), pubic hair development (PH2+ or pubarche), or breast development (B2+ or thelarche).

The PDS was used to assess the children in Gipuzkoa and Sabadell (297 girls and 304 boys) and both Tanner staging and the PDS for those in Valencia (184 girls and 191 boys). The PDS is an interview-based continuous measure of pubertal development. Parents (in most cases mothers, 97%) in these 3 cohorts rated the pubertal status of their children using the PDS, which contains items on growth, body hair, and skin changes for all children, with sex-specific items on facial hair and voice change for males and on breast development and menarche for females (Petersen et al., 1988); there are four response options: “not yet started, barely started, definitely started, seems complete”. The questionnaire does not contain any pictures or diagrams. The continuous score was transformed into a five-point ordinal scale (1-pre-puberty, 2-early puberty, 3-midpuberty, 4-late puberty, and 5-post-puberty) in accordance with Carskadon and Acebo (1993) and Shirtcliff et al. (2009). The PDS has proven to be a reliable and valid instrument to assess pubertal development in children (Carskadon and Acebo, 1993; Koopman-Verhoeff and Elisabeth, 2020), and both self-reported and parent-reported PDS have shown strong internal consistency and test-retest reliability (Koopman-Verhoeff and Elisabeth, 2020). The PDS has been validated in previous studies, including the INMA-Valencia cohort, and results were in moderate-to-good agreement with the Tanner stages evaluated by physical examination (Koopman-Verhoeff and Elisabeth, 2020). Although PDS categories cannot be directly translated into the 5-point Tanner scale, it can adequately differentiate between pre-, mid-, and post-pubertal stages (Koopman-Verhoeff and Elisabeth, 2020). The algorithm of Carskadon and Acebo (1993) was used to calculate the pubertal development from three scale items: growth of body hair and the two sex-specific items. The algorithm of Shirtcliff et al. (2009) uses all five PDS indicators of sexual development, differentially gathering gonadal and adrenal signals of physical development. Breast development, menarche, and growth spurt are associated with gonadal hormonal signals in females, while testicular enlargement, growth spurt, voice deepening, and facial hair growth are associated with these signals in males. Pubic/body hair and skin changes are associated with adrenal hormonal signals in both females and males (Shirtcliff et al., 2009). In this way, three distinct pubertal outcomes were derived from PDS results: overall puberty development, adrenal development (adrenarche), and gonadal development (gonadarche). As in the case of Tanner stages, puberty onset was defined as being in stage 2+ (early puberty stage or

higher).

### 2.5. Covariates

Data on covariates were obtained from interviewer-based questionnaires administered to the parents by research staff during pregnancy and at the follow-up visit and from medical records. Maternal variables included age at delivery (years), schooling (up to primary; secondary; university), ethnicity (white; non-white), area of residence during pregnancy (urban; sub-urban; rural), smoking during pregnancy (no; yes), and weight gain during pregnancy (kg). Maternal ethnicity was not considered in analyses because only 2% of mothers were non-white. There were very few mothers that reported any use of pesticides or working in agricultural activities during pregnancy and this information was therefore not considered in this study, and maternal urinary pesticide metabolites (*i.e.*, OP insecticides) were only available for Valencia (Llop et al., 2017). Child-related variables included birth weight (g), gestational length (weeks), and age (years), urinary creatinine (mg/dL), height (cm), and weight status (underweight; normal weight; overweight; obese) at puberty assessment. Weight status was obtained by converting their BMI to a z-score for age and sex based on World Health Organization (WHO) reference curves for children (5–19 years) (WHO 2007) and then classifying the results as underweight (<-1 standard deviation [SD]), normal weight (±1 SD), overweight (>+1 SD, equivalent to BMI≥25 kg/m$^2$ at 19 years), and obese (>+2 SD; equivalent to BMI≥30 kg/m$^2$ at 19 years). Weight status was categorized into underweight/normal weight, overweight, or obese, because only 14 (0.9%) children were underweight.

### 2.6. Statistical analysis

Urinary concentrations of pesticide metabolites were expressed as detection frequencies and 50th, 75th, 90th, and 95th percentiles. Descriptive statistics for pesticide metabolites with and without creatinine correction, participants' characteristics, and pubertal development were reported separately for the girls and boys. Spearman's correlation test was used to examine relationships between pesticide metabolite pairs. Mixed-effect logistic regression models were constructed to examine the association between pesticide exposure and puberty onset (stage 2+). The cohort (Asturias; Gipuzkoa; Granada; Sabadell; Valencia) was treated as a random variable (cluster variable) in models to account for heterogeneity among cohorts. Each urinary pesticide metabolite was separately modeled with each outcome. IMPy and DETP were found above the LD in more than 60% of samples and were modeled as continuous variables. In this case, analytical results below the method LD were imputed with LD/$\sqrt{2}$, and concentrations were natural log transformed before analysis to obtain a normal distribution of residuals. ETU, detected in around 50% of samples, was classified as low (<LD), moderate (LD-75th percentile [P75]), or high (>75th percentile) exposure. IMPy and DETP and were also classified as low, moderate, or high, as for ETU, to assess possible non-monotonic relationships. Detection of TCPy and 3-PBA in <40% of samples only allowed them to be modeled in two broad categories (undetected/detected). DEDTP was detected in <5% of samples and was therefore not considered in analyses. The first model (basic model) included child age and urinary creatinine (log-transformed) as covariates. The second model (model 2) included child age, creatinine, maternal education, and child height, and model 3 (fully-adjusted model) also included child BMI at puberty assessment. The remaining covariates were not included in the models as they did not confound the association between pesticide exposure and puberty onset. Unadjusted urinary pesticide metabolite concentrations and urinary creatinine concentrations were considered as separate independent variables, reported to be a better approach for controlling measurement error bias due to variability in urine concentrations (Barr et al., 2005; O'Brien et al., 2016). Selection of confounders was based on biological considerations and previous studies on pesticide exposure and puberty timing (Wohlfahrt-Veje et al., 2012a, 2012b; Ye and Pan, 2017a; 2017b). Results are presented as odds ratios (ORs) with 95% confidence interval (CI) for being in pubertal stage 2+ per log-unit increase in urinary metabolite concentrations or for detected/moderate/high *versus* undetected/low concentrations.

Given that the BMI is a strong predictor of puberty onset (Loredana Marcovecchio and Chiarelli, 2013; Reinehr and Roth, 2019), the potential effect modification by child BMI was further examined through cross-product terms (metabolite x underweight or normal weight/-overweight or obese) and stratification (underweight or normal weight *vs.* overweight or obese) of logistic models. In addition, the association between pesticide exposure and puberty status as an ordinal variable was examined by multinomial logistic regression in all girls and boys, grouping Tanner/PDS stages into 1, 2, or 3+, because very few girls or boys were in stage 4 or 5, as described below. Sensitivity analysis was further conducted by adjusting regression models for co-exposure, as follows: TCPy and IMPy models were simultaneously adjusted for TCPy, IMPy, 3-PBA, and ETU, as inclusion of DETP in the models may lead to over adjustment; and DETP, 3-PBA, and ETU models were simultaneously adjusted for DETP, 3-PBA, and ETU. The significance level was set at 0.05. The statistical programs R v.4.1.0 package "nlme" (The R Project for Statistical Computing, https://www.r-project.org) and SPSS v.26 (IBM SPSS Statistics for Windows, Armonk, NY) were used for statistical analyses.

## 3. Results

### 3.1. General characteristics of study participants

No significant differences in general characteristics were found between participants included in the present study and those with data on puberty status at age 7–11 years but not on urinary pesticide biomarkers (n = 437), except for a higher percentage of children from Gipuzkoa, Granada, and Valencia and a lower percentage of children from Asturias and Sabadell in the included *versus* non-included children (Supplementary material, Table S1). Children from Gipuzkoa and Valencia represented up to 24.2% and 24.4% of the participants in this study sample, respectively, followed by Asturias (18.4%), Granada (18.2%), and Sabadell (14.8%) (Table 1). The mean age of mothers at delivery was 32 years, 24% had primary schooling, 69% resided in urban areas during pregnancy, 27% reported smoking during pregnancy, 26% were overweight or obese before pregnancy, and they gained an average of 13.8 kg during pregnancy. The mean birth weight of children was 3276 g and the mean gestational length 39.6 weeks. At puberty assessment, the mean height was 132 cm for girls and 134 cm for boys, and 41% of girls and 45% of boys were overweight or obese.

### 3.2. Puberty status of children

Among 308 girls with data on Tanner staging, 38.6% were in stage B2+ (6.5% in stage B3) and 23.8% in stage PH2+ (6.1% in stage PH3 or 4). Among 620 boys with data on genital development, 22.4% were in stage G2+ (1.6% in stage G3), and 6.5% of 341 boys with data on pubic hair growth were in stage PH2 (Table 2). Among 481 girls assessed with the PDS, 45.9% were classified in pubertal stage 2+ (19.7% in stage 3 or 4), 33.3% in adrenal development stage 2+ (10.5% in stage 3, 4 or 5), and 45.1% in gonadal development stage 2+ (9.5% in stage 3, 4, or 5) (Table 3). Among 495 boys assessed with the PDS, 26.3% were in pubertal stage 2+ (1.8% in stage 3), 13.1% in adrenal development stage 2+ (2.2% in stage 3 or 4), and 27.9% in gonadal development stage 2+ (5.6% in stage 3) (Table 3).

### 3.3. Urinary concentrations of pesticide metabolites

The most prevalent pesticide metabolite was IMPy, detected in 63.0% of urine samples from girls (median = 0.277 μg/L) and 63.6% of

**Table 1**
Characteristics of 1539 children in the INMA Project, Spain.

| Characteristics | N (%) or mean ± SD | | |
|---|---|---|---|
| | All | Girls (N = 606) | Boys (N = 933) |
| **Cohort (age range)** | | | |
| Asturias (7.7–9.6 yrs) | 284 (18.4) | 125 (20.6) | 159 (17.0) |
| Gipuzkoa (7.6–8.6 yrs) | 373 (24.2) | 185 (30.5) | 188 (20.1) |
| Granada (8.8–11.3 yrs) | 279 (18.2) | 0 (0) | 279 (29.9) |
| Sabadell (8.0–10.7 yrs) | 228 (14.8) | 112 (18.5) | 116 (12.4) |
| Valencia (8.2–9.8 yrs) | 375 (24.4) | 184 (30.4) | 191 (20.5) |
| **Mother's age at delivery (years)** | 31.8 ± 4.3 | 32.1 ± 4.0 | 31.6 ± 4.4 |
| **Mother's educational attainment during pregnancy** | | | |
| Up to primary | 365 (23.7) | 109 (18.0) | 256 (27.4) |
| Secondary | 626 (40.7) | 254 (41.9) | 372 (39.9) |
| University | 548 (35.6) | 243 (40.1) | 305 (32.7) |
| **Mother's ethnicity** | | | |
| White | 1509 (98.1) | 598 (98.7) | 911 (97.6) |
| Non-white | 30 (1.9) | 8 (1.3) | 22 (2.4) |
| **Mother's area of residence during pregnancy** | | | |
| Urban | 1059 (68.8) | 474 (78.2) | 569 (61.0) |
| Sub-urban | 370 (24.0) | 109 (18.0) | 273 (29.2) |
| Rural | 110 (7.1) | 23 (3.8) | 91 (9.7) |
| **Mother's smoking during pregnancy** | | | |
| Yes | 410 (27.2) | 183 (30.2) | 227 (24.3) |
| No | 1099 (72.8) | 412 (68.0) | 687 (73.6) |
| **Maternal pre-pregnancy BMI** | | | |
| Underweight/normal weight | 1135 (73.7) | 437 (72.1) | 698 (74.8) |
| Overweight/obese | 404 (26.3) | 169 (27.9) | 235 (25.2) |
| **Weight gain during pregnancy (kg)** | 13.8 ± 5.1 | 13.5 ± 5.0 | 14.1 ± 5.2 |
| **Birth weight (g)** | 3276 ± 469 | 3195 ± 458 | 3335 ± 468 |
| **Gestational length (weeks)** | 39.6 ± 1.6 | 39.6 ± 1.6 | 39.5 ± 1.5 |
| **Child urinary creatinine (mg/dL)** | 95.9 ± 68.1 | 86.1 ± 36.8 | 102.3 ± 81.7 |
| **Child age at puberty assessment (years)** | 8.76 ± 0.85 | 8.52 ± 0.72 | 8.92 ± 0.88 |
| **Child's height at puberty assessment (cm)** | 133.4 ± 7.3 | 131.86 ± 7.16 | 134.36 ± 7.20 |
| **Child's weight status at puberty assessment**[a] | | | |
| Underweight or normal weight | 868 (56.4) | 356 (58.7) | 512 (54.9) |
| Overweight | 383 (24.9) | 163 (26.9) | 220 (23.6) |
| Obese | 288 (18.7) | 87 (14.4) | 201 (21.5) |

SD: standard deviation; BMI: Body mass index.
[a] Obtained by converting BMI to z-score for age and sex based on WHO reference curves for children (5–19 years).

**Table 2**
Puberty status of girls and boys based on clinical Tanner stage.

| Tanner stage: AST + GRA + VAL[a] | Girls (N = 308) | | Boys (N = 620) | |
|---|---|---|---|---|
| | mean age | N (%) | mean age | N (%) |
| **Breast development (B)** | | | | |
| B1 | 8.6 | 189 (61.4) | – | – |
| B2 | 9.0 | 99 (32.1) | – | – |
| B3 | 9.0 | 20 (6.5) | – | – |
| **Breast development onset (B2 + )** | 8.7 | 119 (38.6) | – | – |
| AST | 8.4 | 16 (13.0) | – | – |
| VAL | 9.1 | 103 (56.3) | – | – |
| **Genital development (G)** | | | | |
| G1 | – | – | 9.2 | 481 (77.6) |
| G2 | – | – | 9.4 | 129 (20.8) |
| G3 | – | – | 9.1 | 10 (1.6) |
| **Genital development onset (G2 + )** | – | – | 9.4 | 139 (22.4) |
| AST | – | – | 8.3 | 2 (1.3) |
| GRA | – | – | 9.9 | 57 (20.4) |
| VAL | – | – | 9.1 | 80 (44.0) |
| **Pubic hair growth (PH)** | | | | |
| PH1 | 8.7 | 235 (76.3) | 8.7 | 598 (96.4) |
| PH2 | 8.9 | 54 (17.5) | 9.1 | 22 (3.6) |
| PH3 | 9.0 | 17 (5.5) | – | 0 (0) |
| PH4 | 8.8 | 2 (0.6) | – | 0 (0) |
| **Pubic hair growth onset (PH2 + )**[b] | 8.5 | 73 (23.8) | 8.9 | 22 (6.5) |
| AST | 8.3 | 22 (17.6) | 8.5 | 4 (2.5) |
| VAL | 9.1 | 51 (28.0) | 9.1 | 18 (9.9) |

AST: Asturias; GRA: Granada; VAL: Valencia.
[a] VAL: 182 boys and 183 girls with data on Tanner stage.
[b] No data on pubic hair growth for boys in Granada (N = 341 boys with data on pubic hair growth).

those from boys (median = 0.299 μg/L), followed by DETP in 60.6% (median = 0.315 μg/L) and 65.4% (median = 0.401 μg/L), ETU in 53.5% (median = 0.095 μg/L) and 50.0% (median = 0.067 μg/L), TCPy in 34.8% (P75 = 0.085 μg/L) and 40.2% (median = 0.096 μg/L), and 3-PBA in 39.6% (P75 = 0.288 μg/L) and 34.3% (median = 0.184 μg/L), respectively (Table 4). Urinary concentrations of pesticide metabolites were higher in children from Valencia and lower in those from Sabadell and Granada. All metabolites except 3-PBA were detected in at least two-thirds of the children from the Valencia cohort (IMPy: 84%, ETU: 75%, DETP: 60%) (Table S2). In the total sample of girls and boys, all pesticide metabolite pairs except 3-PBA-ETU (rho = 0.09, p = 0.01) showed significant and weak or moderate correlations (rho ranging from 0.14 for 3-PBA-DETP to 0.49 for TCPy-DETP, $p < 0.001$).

### 3.4. Association between pesticide exposure and pubertal development in girls

Results for the association between urinary pesticide metabolites and pubertal development in girls were similar between basic and fully-adjusted models, although ORs were generally higher in the latter (Table 5 and Table S3). In models that included all the girls, ETU and DETP were associated with significantly higher odds of having started puberty. Specifically, higher ETU concentrations were associated with higher odds of being in Tanner stage B2+ (fully-adjusted OR [95% CI] = 4.27 [1.84–9.93] and 5.55 [2.83–12.91] for concentrations below and above the P75, respectively, *vs.* undetected concentrations) and PDS stage 2+ of overall puberty development (OR [95% CI] = 1.71 [1.03–2.83] for concentrations > P75). The odds significantly increased for being in stage B2 *vs.* B1 and for being in PDS stage 3+ *vs.* 1 (Table S5). DETP concentrations > P75 were also associated with higher odds of PDS stage 2+ (OR [95% CI] = 1.86 [1.07–3.24]) and increasing DETP was associated with slightly higher odds of being in stage B2+ and PDS stage 2+ of adrenal development (OR [95% CI] = 1.04 [1.00–1.11] and 1.02 [1.00–1.07], respectively, per log-unit increase in concentration), particularly when comparing stage 3+ *vs.* 1 (Table S5). In addition, the presence of detectable concentrations of TCPy was modestly

**Table 3**
Puberty status of girls and boys based on parent-reported PDS.

| PDS: GIP + SAB + VAL | Girls (N = 481) | | Boys (N = 495) | |
|---|---|---|---|---|
| | Mean age | N (%) | Mean age | N (%) |
| **PDS stage: overall puberty** | | | | |
| 1 (Pre-puberty) | 8.4 | 260 (54.1) | 8.5 | 365 (73.7) |
| 2 (Early puberty) | 8.8 | 126 (26.2) | 8.6 | 121 (24.4) |
| 3 (Midpuberty) | 9.0 | 90 (18.7) | 8.5 | 9 (1.8) |
| 4 (Late puberty) | 8.7 | 5 (1.0) | – | 0 (0) |
| **Puberty onset (PDS stage 2 + )** | 8.9 | 221 (45.9) | 8.7 | 130 (26.3) |
| GIP | 7.7 | 47 (25.4) | 7.7 | 45 (23.9) |
| SAB | 9.3 | 63 (56.3) | 9.2 | 32 (27.6) |
| VAL | 9.1 | 111 (60.3) | 9.1 | 53 (27.7) |
| **PDS stage: adrenal development** | | | | |
| 1 (Pre-puberty) | 8.5 | 321 (66.7) | 8.6 | 430 (86.9) |
| 2 (Early puberty) | 8.8 | 109 (22.7) | 8.6 | 54 (10.9) |
| 3 (Midpuberty) | 8.6 | 44 (9.1) | 8.4 | 9 (1.8) |
| 4 (Late puberty) | 9.2 | 6 (1.2) | 8.9 | 2 (0.4) |
| 5 (Post-puberty) | 9.7 | 1 (0.2) | – | 0 (0) |
| **Adrenarche (PDS adrenal 2 + )** | 8.7 | 160 (33.3) | 8.5 | 65 (13.1) |
| GIP | 7.7 | 42 (22.7) | 7.4 | 21 (11.2) |
| SAB | 9.3 | 50 (44.6) | 9.1 | 14 (12.1) |
| VAL | 9.1 | 68 (37.0) | 9.0 | 30 (15.7) |
| **PDS stage: gonadal development** | | | | |
| 1 (Pre-puberty) | 8.6 | 264 (54.9) | 8.6 | 357 (72.1) |
| 2 (Early puberty) | 8.5 | 171 (35.5) | 8.3 | 110 (22.2) |
| 3 (Midpuberty) | 8.9 | 43 (8.9) | 8.6 | 28 (5.6) |
| 4 (Late puberty) | 9.3 | 1 (0.2) | – | 0 (0) |
| 5 (Post-puberty) | 9.4 | 2 (0.4) | – | 0 (0) |
| **PDS gonadal 2 + (gonadarche)** | 8.7 | 217 (45.1) | 8.7 | 138 (27.9) |
| GIP | 7.7 | 83 (44.9) | 7.7 | 77 (41.0) |
| SAB | 9.4 | 48 (42.9) | 9.3 | 20 (17.2) |
| VAL | 9.1 | 86 (46.7) | 9.0 | 41 (21.5) |

GIP: Gipuzkoa; SAB: Sabadell; VAL: Valencia.
PDS: Pubertal Development Scale.

associated with higher odds of being in stage B2+ (OR [95% CI] = 1.84 [0.97–3.52]).

Table 6 shows that the association between ETU and earlier breast development was stronger in girls with underweight/normal weight. In this way, the odds of being in stage B2+ was two-fold higher in girls with underweight/normal weight *versus* overweight/obesity (*i.e.*, ETU > P75: OR [95%CI] = 10.08 [2.62–38.76] *vs.* 4.56 [1.10–18.92]). Although not significant, there was a marginal association between DETP and earlier breast development in girls with underweight/normal weight (*i.e.*, DETP > P75: OR = 3.01 [0.85–10.68] *vs.* 1.24 [0.30–5.09] in girls with overweight/obesity). In addition, the detection of TCPy was only modestly associated with higher odds of adrenarche in girls with underweight/normal weight alone (OR [95% CI] = 1.50 [0.93–3.78]). However, interaction terms were not statistically significant, *i.e.*, $P_{interaction}$ = 0.75 (ETU), 0.70 (DETP), and 0.13 (TCPy).

Adjustment for co-exposure led to a slightly weaker but significant association between ETU and higher odds of being in stage B2+ (OR [95%CI] = 4.08 [2.10–12.03] and 5.03 [2.39–12.59] for moderate and higher ETU concentrations, respectively), while associations of ETU and DETP with being in PDS stage 2+ did not remain significant (Table S7). Stratification of co-exposure models by weight status revealed a non-significant association between TCPy and higher odds of stage B2+ in girls with underweight/normal weight (OR [95%CI] = 2.55 [0.90–7.24]). The remaining BMI-stratified associations were not substantially different than those from single exposure models (data not shown).

### 3.5. Association between pesticide exposure and pubertal development in boys

Results for boys were again similar between basic and fully-adjusted models, although some associations were strengthened by the inclusion of child BMI (Table 7 and Table S4). In fully-adjusted models that included all the boys, detected *vs.* undetected TCPy and 3-PBA were associated with significantly higher odds of being in Tanner stage G2+ (OR [95% CI] = 1.97 [1.08–3.57] and 2.08 [1.15–3.81], respectively); these odds were higher although not significant when comparing stage G3 *vs.* G1 (Table S6). BMI-stratified models (Table 8) showed a stronger but non-significant association between TCPy and genital development in boys with normal weight ($P_{interaction}$ = 0.52), and a stronger association between 3-PBA and genital development that was significant in boys with overweight/obesity alone ($P_{interaction}$ = 0.03). A higher ETU (>P75) was associated with higher odds of genital development in boys with normal weight alone (OR [95%CI] = 2.89 [1.08–7.74]) ($P_{interaction}$ = 0.07) and DETP with slightly lower odds of being in PDS stage 2+ in boys with overweight/obesity alone (OR [95%CI] = 0.94 [0.89–0.99] per log-unit increase in concentration) ($P_{interaction}$ = 0.03).

Adjustment for co-exposure led to a stronger association between 3-PBA and higher odds of being in stage G2+ (OR [95%CI] = 2.41 [1.26–4.62]) and a significant association between higher DETP and lower odds of being stage G2+ (OR [95%CI] = 0.93 [0.88–0.99]) and adrenarche (OR [95%CI] = 0.95 [0.91–1.00]) (Table S8). BMI-stratified co-exposure models revealed stronger associations between ETU and

**Table 4**
Distribution of child urinary concentrations of pesticide metabolites.

| Metabolites | % > LD | Percentiles | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Unadjusted (μg/L) | | | | Creatinine adjusted (μg/g) | | | |
| | | 50 | 75 | 90 | 95 | 50 | 75 | 90 | 95 |
| **Girls (N = 606)** | | | | | | | | | |
| TCPy | 34.8 | <LD | 0.085 | 0.301 | 0.511 | <LD | 0.110 | 0.371 | 0.588 |
| IMPy | 63.0 | 0.277 | 1.079 | 2.742 | 3.763 | 0.354 | 1.380 | 3.029 | 4.096 |
| DETP | 60.6 | 0.315 | 2.633 | 9.901 | 20.706 | 0.439 | 3.284 | 12.498 | 24.632 |
| 3-PBA | 39.6 | <LD | 0.288 | 0.935 | 2.047 | <LD | 0.378 | 1.328 | 3.019 |
| ETU | 53.5 | 0.095 | 0.367 | 0.964 | 1.981 | 0.114 | 0.501 | 1.469 | 2.559 |
| **Boys (N = 933)** | | | | | | | | | |
| TCPy | 40.2 | <LD | 0.096 | 0.332 | 0.513 | <LD | 0.118 | 0.359 | 0.639 |
| IMPy | 63.6 | 0.299 | 1.118 | 2.417 | 4.003 | 0.363 | 1.282 | 2.761 | 4.195 |
| DETP | 65.4 | 0.401 | 2.877 | 8.042 | 13.986 | 0.519 | 2.907 | 9.099 | 17.582 |
| 3-PBA | 34.3 | <LD | 0.184 | 0.760 | 1.470 | <LD | 0.251 | 0.819 | 1.699 |
| ETU | 50.0 | 0.067 | 0.286 | 0.798 | 1.212 | 0.081 | 0.349 | 1.054 | 1.778 |

LD: Limit of detection (TCPy: 0.039 μg/L; IMPy: 0.117 μg/L; DETP: 0.116 μg/L; 3-PBA: 0.117 μg/L; ETU: 0.072 μg/L).

**Table 5**
Associations between urinary pesticide metabolites and puberty development in girls.

| Pesticide metabolites | Tanner stage 2 + (N = 308) | | PDS stage 2 + (N = 481) | | |
|---|---|---|---|---|---|
| | Breast development | Pubic hair growth | Overall puberty | Adrenarche | Gonadarche |
| | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) |
| TCPy: > *vs.* < LD | 1.84 (0.97–3.52) | 0.77 (0.42–1.45) | 1.42 (0.83–2.23) | 0.99 (0.71–1.87) | 0.95 (0.62–1.57) |
| IMPy (log) | 0.99 (0.92–1.06) | 0.97 (0.92–1.04) | 1.00 (0.94–1.04) | 0.98 (0.94–1.03) | 1.01 (0.96–1.06) |
| IMPy: > LD-P75 *vs.* < LD | 1.17 (0.56–2.47) | 0.77 (0.39–1.54) | 0.91 (0.55–1.49) | 0.74 (0.46–1.21) | 0.77 (0.48–1.22) |
| IMPy: > P75 *vs.* < LD | 1.70 (0.71–4.06) | 0.69 (0.30–1.55) | 1.36 (0.78–2.40) | 0.94 (0.55–1.63) | 1.25 (0.73–2.12) |
| DETP (log) | 1.05 (1.00–1.11) | 1.04 (0.98–1.09) | 1.04 (1.00–1.11)* | 1.02 (1.00–1.07)* | 1.01 (0.98–1.04) |
| DETP: > LD-P75 *vs.* < LD | 1.99 (0.97–4.12) | 1.30 (0.68–2.51) | 1.31 (0.80–2.14) | 1.21 (0.75–1.95) | 0.97 (0.61–1.52) |
| DETP: > P75 *vs.* < LD | 1.88 (0.79–4.47) | 1.12 (0.50–2.50) | 1.86 (1.07–3.24)* | 1.45 (0.85–2.47) | 1.14 (0.68–1.91) |
| 3-PBA: > *vs.* < LD | 1.16 (0.62–2.15) | 0.76 (0.43–1.36) | 1.19 (0.72–1.76) | 9.94 (0.64–1.55) | 1.00 (0.67–1.55) |
| ETU: > LD-P75 *vs.* < LD | 4.27 (1.84–9.93)** | 1.16 (0.54–2.46) | 1.47 (0.89–2.44) | 1.24 (0.76–2.05) | 1.18 (0.73–1.89) |
| ETU: > P75 *vs.* < LD | 5.55 (2.83–12.91)** | 1.33 (0.64–2.74) | 1.71 (1.03–2.83)* | 1.31 (0.80–2.13) | 1.30 (0.81–2.09) |

LD: Limit of detection.
All models are adjusted for cohort (random effect), urinary creatinine (log-transformed), child age, maternal education, child height, and child weight status (normal weight, overweight, or obese) at 7–11 yrs.
*p < 0.05; **p < 0.001.

**Table 6**
Associations between urinary pesticide metabolites and puberty development in girls according to their weight status.[a].

| Pesticide metabolites | Normal weight | | | | | Overweight/obese | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tanner stage 2 + (N = 176) | | PDS stage 2 + (N = 284) | | | Tanner stage 2 + (N = 130) | | PDS stage 2 + (N = 197) | | |
| | Breast development n B2 + = 37 | Pubic hair growth n PH2 + = 40 | Overall puberty n stage 2 + = 96 | Adrenarche n stage 2 + = 74 | Gonadarche n stage 2 + = 97 | Breast development n B2 + = 120 | Pubic hair growth n PH2 + = 86 | Overall puberty n stage 2 + = 125 | Adrenarche n stage 2 + = 79 | Gonadarche n stage 2 + = 36 |
| | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) |
| TCPy: > *vs.* < LD | 1.72 (0.71–4.24) | 1.33 (0.55–3.20) | 1.55 (0.89–3.73) | 1.50 (0.93–3.78) | 0.99 (0.65–2.29) | 1.83 (0.61–5.45) | 0.51 (0.20–1.27) | 1.37 (0.54–2.38) | 0.72 (0.39–1.53) | 0.92 (0.39–1.57) |
| IMPy (log) | 1.00 (0.91–1.09) | 1.03 (0.93–1.14) | 1.01 (0.94–1.09) | 1.02 (0.95–1.11) | 1.00 (0.95–1.09) | 0.98 (0.87–1.10) | 0.91 (0.83–1.00) | 1.00 (0.90–1.05) | 0.96 (0.90–2.25) | 1.02 (0.94–1.08) |
| IMPy: > LD-P75 *vs.* < LD | 0.96 (0.35–2.61) | 0.94 (0.36–2.48) | 0.89 (0.46–1.73) | 0.91 (0.45; 1.85) | 0.68 (0.36–1.27) | 1.04 (0.29–3.68) | 0.53 (0.19–1.48) | 1.04 (0.49–2.19) | 0.67 (0.34–1.34) | 0.99 (0.45–1.78) |
| IMPy: > P75 *vs.* < LD | 1.71 (0.54–5.41) | 1.31 (0.42–4.09) | 1.54 (0.73–3.23) | 1.39 (0.63–3.07) | 1.06 (0.53–2.15) | 2.16 (0.49–9.48) | 0.37 (0.11–1.26) | 1.31 (0.55–3.12) | 0.77 (0.35–1.69) | 1.35 (0.68–3.52) |
| DETP (log) | 1.08 (1.00–1.17) | 1.04 (0.97–1.12) | 1.03 (0.98–1.08) | 1.04 (0.99–1.10) | 1.00 (0.96–1.05) | 1.04 (0.95–1.14) | 1.04 (0.96–1.12) | 1.05 (0.98–1.09) | 1.02 (0.98–1.08) | 1.02 (0.97–1.07) |
| DETP: > LD-P75 *vs.* < LD | 2.69 (0.94–7.64) | 1.28 (0.49–3.33) | 1.23 (0.64–2.36) | 1.38 (0.68–2.76) | 0.91 (0.50–1.67) | 1.74 (0.52–5.85) | 1.40 (0.56–3.53) | 1.36 (0.63–2.92) | 1.18 (0.59–2.37) | 0.99 (0.49–2.00) |
| DETP: > P75 *vs.* < LD | 3.01 (0.85–10.68) | 1.73 (0.54–5.56) | 1.93 (0.92–4.03) | 1.79 (0.81–3.92) | 1.01 (0.51–2.03) | 1.24 (0.30–5.09) | 0.79 (0.26–2.45) | 1.94 (0.85–4.45) | 1.33 (0.64–2.79) | 1.35 (0.63–2.89) |
| 3-PBA: > *vs.* < LD | 0.75 (0.32–1.75) | 0.67 (0.29–1.55) | 1.05 (0.57–1.84) | 1.08 (0.59–2.07) | 0.85 (0.52–1.60) | 1.26 (0.44–3.62) | 0.93 (0.41–2.13) | 1.49 (0.66–2.67) | 0.95 (0.54–1.91) | 1.18 (0.59–2.14) |
| ETU: > LD-P75 *vs.* < LD | 9.55 (2.27–40.08)** | 1.51 (0.49–4.67) | 1.34 (0.68–2.65) | 1.66 (0.79–3.46) | 1.14 (0.60–2.14) | 3.13 (0.94–10.46) | 0.88 (0.31–2.45) | 1.56 (0.72–3.35) | 0.93 (0.46–1.90) | 1.22 (0.60–2.50) |
| ETU: > P75 *vs.* < LD | 10.08 (2.62–38.76)** | 1.66 (0.60–4.61) | 1.61 (0.84–3.07) | 1.73 (0.87–3.47) | 1.13 (0.61–2.12) | 4.56 (1.10–18.9)* | 1.04 (0.36–3.03) | 1.91 (0.84–4.34) | 1.09 (0.53–2.24) | 1.58 (0.75–3.35) |

All models are adjusted for cohort (random effect), urinary creatinine (log-transformed), child age, cohort, maternal education, and child height.
*p < 0.05; **p < 0.001.
[a] Weight status according to BMI z-score for age and sex.

higher odds of being in stage G2+ in boys with underweight/normal weight (OR [95%CI] = 4.11 [1.36–12.4] for higher ETU concentrations), between 3-PBA and higher odds of being in stage G2+ in boys with overweight/obesity (OR [95%CI] = 4.37 [1.60–11.96]), and between higher DETP and lower odds of adrenarche in boys with overweight/obesity (OR [95%CI] = 0.93 [0.88–0.99]). The remaining results were essentially unchanged (data not shown).

## 4. Discussion

In this large sample of Spanish children, higher urinary concentrations of ETU and DETP, and possibly TCPy, were associated with earlier puberty development in girls, especially with earlier breast development in those with underweight/normal weight exposed to higher ETU. In boys, higher urinary TCPy and ETU concentrations were associated with earlier genital development in boys with underweight/normal weight, higher 3-PBA with earlier genital development in boys with overweight/obesity, and higher DETP with delayed overall puberty development and

**Table 7**
Associations between urinary pesticide metabolites and puberty development in boys.

| Pesticide metabolites | Tanner stage 2 + (N = 620) | | PDS stage 2 + (N = 495) | | |
|---|---|---|---|---|---|
| | Genital development | Pubic hair growth | Overall puberty | Adrenarche | Gonadarche |
| | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) |
| TCPy: > *vs.* < LD | 1.97 (1.08–3.57)* | 0.77 (0.31–1.90) | 0.93 (0.60–1.47) | 0.98 (0.71–2.35) | 1.39 (0.81–1.64) |
| IMPy (log) | 1.07 (0.96–1.19) | 0.97 (0.86–1.09) | 0.98 (0.93–1.03) | 0.99 (0.96–1.08) | 1.01 (0.91–1.05) |
| IMPy: > LD-P75 *vs.* < LD | 2.08 (0.94–4.71) | 1.14 (0.35–3.75) | 1.07 (0.66–1.75) | 1.00 (0.52–1.92) | 0.93 (0.56–1.53) |
| IMPy: > P75 *vs.* < LD | 1.68 (0.70–4.13) | 0.94 (0.25–3.52) | 1.02 (0.59–1.76) | 1.15 (0.57–2.34) | 1.03 (0.60–1.78) |
| DETP (log) | 0.97 (0.92–1.02) | 0.96 (0.89–1.04) | 0.98 (0.94–1.07) | 0.96 (0.90–1.04) | 1.00 (0.95–1.04) |
| DETP: > LD-P75 *vs.* < LD | 0.58 (0.29–1.15) | 0.62 (0.21–1.81) | 0.88 (0.54–1.43) | 0.61 (0.32–1.14) | 1.32 (0.82–2.15) |
| DETP: > P75 *vs.* < LD | 0.79 (0.37–1.69) | 0.96 (0.31–2.97) | 1.02 (0.61–1.72) | 0.76 (0.39–1.49) | 0.88 (0.51–1.52) |
| 3-PBA: > *vs.* < LD | 2.08 (1.15–3.81)* | 1.22 (0.49–3.06) | 0.79 (0.50–1.27) | 1.01 (0.73–1.81) | 1.12 (0.54–1.77) |
| ETU: > LD-P75 *vs.* < LD | 1.58 (0.74–4.34) | 0.74 (0.23–2.43) | 0.79 (0.47–1.31) | 0.67 (0.33–1.38) | 0.81 (0.49–1.35) |
| ETU: > P75 *vs.* < LD | 1.79 (0.89–3.53) | 0.72 (0.25–2.11) | 0.74 (0.44–1.22) | 1.10 (0.59–2.07) | 0.71 (0.42–1.20) |

LD: Limit of detection.
All models are adjusted for cohort (random effect), urinary creatinine (log-transformed), child age, maternal education, child height, and child weight status (normal weight, overweight, or obese) at 7–11 yrs.
*$p < 0.05$.

**Table 8**
Associations between urinary pesticide metabolites and puberty development in boys according to their weight status.[a].

| Pesticide metabolites | Normal weight | | | | | Overweight/obese | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tanner stage 2 + (N = 183) | | PDS stage 2 + (N = 282) | | | Tanner stage 2 + (N = 158) | | PDS stage 2 + (N = 213) | | |
| | Genital development n G2 + = 75 | Pubic hair growth n PH2 + = 10 | Overall puberty n stage 2 + = 64 | Adrenarche n stage 2 + = 29 | Gonadarche n stage 2 + = 72 | Genital development n G2 + = 64 | Pubic hair growth n PH2 + = 12 | Overall puberty n stage 2 + = 66 | Adrenarche n stage 2 + = 36 | Gonadarche n stage 2 + = 66 |
| | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) | OR (95%CI) |
| TCPy: > *vs.* < LD | 2.18 (0.91–5.17) | 0.70 (0.18–2.78) | 0.94 (0.49–1.73) | 1.06 (0.43–2.35) | 1.26 (0.66–2.27) | 1.56 (0.68–3.60) | 0.68 (0.20–2.32) | 0.87 (0.47–1.71) | 1.08 (0.41–2.05) | 1.43 (0.92–3.50) |
| IMPy (log) | 1.03 (0.90–1.17) | 0.92 (0.79–1.07) | 0.96 (0.89–1.02) | 0.98 (0.88–1.07) | 1.00 (0.93–1.07) | 1.10 (0.92–1.31) | 1.04 (0.83–1.30) | 1.01 (0.93–1.12) | 0.99 (0.86–1.07) | 1.02 (0.95–1.17) |
| IMPy: > LD-P75 *vs.* < LD | 2.50 (0.79–7.95) | 0.78 (0.14–4.28) | 1.05 (0.54–2.04) | 1.24 (0.48–3.25) | 0.93 (0.47–1.83) | 1.39 (0.44–4.35) | 1.65 (0.29–9.22) | 1.15 (0.55–2.39) | 0.88 (0.36–2.17) | 0.98 (0.47–2.06) |
| IMPy: > P75 *vs.* < LD | 1.27 (0.34–4.73) | 0.78 (0.12–5.06) | 0.87 (0.41–1.87) | 1.42 (0.50–4.00) | 1.12 (0.53–2.35) | 1.56 (0.47–5.14) | 1.03 (0.15–7.11) | 1.18 (0.53–2.63) | 1.00 (0.37–2.65) | 0.94 (0.42–2.10) |
| DETP (log) | 0.96 (0.89–1.03) | 0.98 (0.87–1.10) | 1.02 (0.97–1.07) | 0.97 (0.91–1.04) | 1.00 (0.95–1.05) | 0.98 (0.91–1.05) | 0.94 (0.85–1.04) | 0.94 (0.89–0.99)* | 0.96 (0.90–1.01) | 1.00 (0.93–1.06) |
| DETP: > LD-P75 *vs.* < LD | 0.65 (0.16–1.21) | 0.90 (0.18–5.54) | 0.95 (0.47–1.91) | 0.40 (0.14–1.13) | 1.26 (0.64–2.47) | 0.45 (0.16–1.21) | 0.39 (0.09–1.78) | 0.84 (0.43–1.67) | 0.85 (0.37–1.97) | 1.37 (0.68–2.76) |
| DETP: > P75 *vs.* < LD | 0.42 (0.13–1.37) | 0.97 (0.16–5.94) | 1.72 (0.84–3.53) | 1.10 (0.43–2.83) | 1.07 (0.50–2.29) | 1.28 (0.45–3.65) | 0.94 (0.21–4.26) | 0.57 (0.26–1.26) | 0.62 (0.23–1.65) | 0.71 (0.31–1.61) |
| 3-PBA: > *vs.* < LD | 1.22 (0.52–2.84) | 0.81 (0.21–3.08) | 0.65 (0.32–1.99) | 0.71 (0.27–1.63) | 1.16 (0.61–2.06) | 3.64 (1.51–8.79)* | 1.57 (0.44–5.55) | 0.95 (0.49–1.92) | 1.65 (0.67–3.55) | 0.96 (0.52–2.18) |
| ETU: > LD-P75 *vs.* < LD | 1.48 (0.49–4.53) | 0.29 (0.03–2.79) | 0.84 (0.40–1.75) | 0.39 (0.11–1.39) | 0.94 (0.46–1.95) | 1.59 (0.55–4.56) | 1.29 (0.28–5.89) | 0.75 (0.37–1.55) | 1.11 (0.44–2.80) | 0.69 (0.33–1.42) |
| ETU: > P75 *vs.* < LD | 2.89 (1.08–7.74)* | 0.83 (0.19–3.67) | 0.83 (0.42–1.67) | 1.07 (0.43–2.68) | 0.85 (0.42–1.75) | 1.06 (0.38–2.95) | 0.59 (0.12–2.87) | 0.58 (0.27–1.25) | 1.49 (0.60–3.69) | 0.50 (0.22–1.10) |

All models adjusted for cohort (random effect), urinary creatinine (log-transformed), child age, cohort, maternal education, and child height.
*$p < 0.05$.
[a] Weight status according to BMI z-score for age and sex.

adrenarche in those with overweight/obesity. Most of these novel findings need to be verified in other population-based longitudinal studies; however, the data suggest an association of peri-pubertal exposure to ETU and certain insecticides (OPs, pyrethroids) with earlier puberty in girls and in boys, which may be modified by their BMI.

Exposure to mixtures of different pesticide residues is ubiquitous among the general population, and human biomonitoring studies have shown that children are exposed, mainly *via* their diet, at comparable levels throughout the world (Fernández et al., 2020; Li et al., 2019a, 2019b; Papadopoulou et al., 2019). However, limited biomonitoring studies are available on the pesticide exposure of Spanish children. The detection of TCPy in the present sample of children (35%) is similar to that in pregnant women from the INMA-Valencia cohort (detection frequency [DF], geometric mean [GM] = 39%, 0.49 μg/L) (Llop et al., 2017) and lower than that reported for 5 to 12-year-old participants in the BIOVAL study in the same region (DF, median = 74%, 1.13 μg/L and 86%, 3.40 μg/g, respectively) (Fernández et al., 2020; Roca et al., 2014). However, in the present study, TCPy was detected in the urine of up to 66% of the children from Valencia, an intense area of agricultural production including vegetables, fruits (*e.g.,* citrus), and rice. Detection of

IMPy in two-thirds of the present samples indicates ongoing exposure to diazinon or its residue some years after its prohibition in the EU, most likely from food imported from non-EU countries (AESAN 2018). IMPy concentrations were similar to those reported for children in the BIOVAL study (DF = 57%, median = 5.16 μg/g) (Roca et al., 2014) but higher than those in pregnant women from the INMA-Valencia cohort (DF, GM = 12%, 0.03 μg/L) (Llop et al., 2017). DETP detection was similar to that found in mothers from the INMA-Valencia cohort (DF = 75%, GM = 0.22 μg/L) and more frequent than previously reported in children from Valencia (DF = 21% and 36%, respectively) (Fernández et al., 2020; Roca et al., 2014) and from the Andalusian province of Almería (DF = 18%) (González-Alzaga et al., 2020), whereas 3-PBA detection was less frequent than in children in the BIOVAL study (DF, median = 79%, 1.63 μg/L) (Fernández et al., 2020).

Regarding ETU, urinary concentrations in the present children, particularly those from Valencia, are higher than concentrations in 6- to 18-year-old children participating in the U.S. 2003–2008 National Health and Nutrition Examination Survey (DF, GM = 10.6%, 0.12 μg/L) (NHANES) (Stadler et al., 2022), within the range of urinary concentrations found in 3- to 10 year-old French children living near vineyards frequently treated with dithiocarbamates (median = 0.43 μg/g, range = 0.01–4.45 μg/g) (Raherison et al., 2019), but lower than those in 6- to 9-year-old children from agricultural communities in Costa Rica (GM = 1.2 μg/L) (van Wendel de Joode et al. 2016). Overall, the extent to which children from the general population is exposed to these fungicides or their transformation products remains largely unknown (Stadler et al., 2022).

### 4.1. Fungicides: ETU

One of the most relevant study findings is the association between urinary ETU and earlier breast development in girls, given that girls with higher urinary ETU concentrations had 5-fold higher odds of being in Tanner stage B2+ (up to 10-fold higher odds in girls with underweight/normal weight) in comparison to those with undetected ETU. To our best knowledge, only one previous study in Denmark reported an association between exposure to non-persistent pesticides and earlier breast development, in a cohort of 83 girls whose mothers worked in greenhouses during pregnancy (Wohlfahrt-Veje et al., 2012a). The authors observed earlier breast development in 6- to 11-year-old girls prenatally exposed to multiple pesticides in comparison to non-exposed girls (mean breast development onset = 8.9 *versus* 10.4 years, p = 0.05). In the present study, the association between ETU and breast development was markedly stronger among girls with underweight/normal weight, likely because overweight may hamper the assessment of early breast stages (Wolff et al., 2014). On the other hand, estrogens synthesized by adipose tissue of girls with overweight/obesity (*e.g.*, in mammary fat) may contribute to breast development independently of the hypothalamic-pituitary-gonadal (HPG) axis. In this study, 21% of girls with underweight/normal weight were classified in stage B2+ *versus* 92% of those with overweight/obesity. In parallel, a higher ETU concentration was associated with increased odds of being in Tanner stage G2+ in boys with underweight/normal weight. In the aforementioned Danish study, however, prenatal exposure to pesticides was related to lower testicular volume and penile length rather than increased genital size (*i.e.*, boys in the high exposure group had 24.7% smaller testes [95% CI = −62.2; −10.1] and 9.4% shorter penile length [95%CI = −16.8; −1.1] than the unexposed) (Wohlfahrt-Veje et al., 2012b). In contrast to the consistent evidence of a strong association between higher childhood BMI and earlier female puberty, data on the association between BMI and male puberty timing have been controversial (Busch et al., 2020). Hence, the effect modification of BMI on the association between ETU and genital development suggested by the present results is less clear. However, it may be explained by a greater predisposition to earlier puberty in boys with normal weight (Kleber et al., 2011; Lee et al., 2016), although there was a similar percentage of boys in stage G2+ in the underweight/normal weight (41%) and overweight/obesity (40%) groups.

ETU is an anti-thyroid compound and has been found to interfere with iodide uptake by inhibiting thyroid peroxidase activity (Hurley et al., 1998; Marinovich et al., 1997). In rodent studies, mancozeb was reported to reduce serum thyroxine (T4) levels and increase thyroid-stimulating hormone (TSH) production (Axelstad et al., 2011; Hurley et al., 1998; Kackar et al., 1997). In human studies, occupational exposure to mancozeb and other EBDC fungicides was associated with hypothyroid-like hormone imbalance (increased TSH and decreased T4) and other thyroid disorders in both men (Medda et al., 2017; Panganiban et al., 2004; Piccoli et al., 2016; Steenland et al., 1997) and women (Goldner et al., 2010). This is of interest because of the known cross-talk between thyroid hormones, estradiol, androgens, and gonadotropins (luteinizing hormone [LH] and follicle stimulating hormone [FSH]) (Ren and Zhu, 2022). Indeed, elevated TSH is one of the factors thought to contribute to central precocious puberty in girls (Jung et al., 2019). The specific mechanism underlying this relationship remains unclear, but thyroid hormones may affect the levels of gonadotropin-releasing hormone (GnRH) that are released by the hypothalamus (Ren and Zhu, 2022) and that stimulate the pituitary secretion of LH and FSH, thereby activating the production of gonadal hormones and the progression of secondary sex characteristics.

Rodents exposed to either ETU or mancozeb have evidenced not only thyrotoxic but also reproductive effects, including disrupted estrus cycles, impaired embryo development, and altered reproductive hormone levels (Cecconi et al., 2007; Maranghi et al., 2013; Runkle et al., 2017). The mechanism(s) by which ETU, mancozeb, or other EBDC fungicides may specifically act on the reproductive system are poorly understood; however, the present results suggest that peri-pubertal exposure to ETU or its parent compound(s) may induce the estrogen surge that triggers the initiation of puberty, particularly breast growth onset, either in a direct manner or by affecting mechanisms that directly or indirectly regulate this surge. This is the first report of an association between ETU and earlier puberty development and, given the cross-sectional design of the study, these findings should be interpreted with caution.

### 4.2. OP insecticides

The suggestive association between TCPy and higher odds of being in Tanner stage G2+ in boys is consistent with the estrogenic action exerted by chlorpyrifos *in vitro* (Andersen et al., 2002; Yu et al., 2015) but is not supported by experimental evidence on the anti-androgenic effects of chlorpyrifos and TCPy (Gao et al., 2021; Hazarika, 2022; Viswanath et al., 2010). However, after adjusting for co-exposure, TCPy was not associated with male genital development but was modestly associated with higher odds of being in Tanner stage B2+ in girls with underweight/normal weight, again suggesting an estrogenic effect. Other proposed mechanisms on how chlorpyrifos could accelerate puberty development are activation of the HPG axis through hypothalamic inflammation (Valsamakis et al., 2021) and immunotoxic effects (Bouman et al., 2005), as it has been shown to influence the activity of pro-inflammatory molecules (Camacho-Pérez et al., 2022) and interfere with the immune function (Lee and Choi, 2020).

To the best of our knowledge, there are no published human data on TCPy/chlorpyrifos exposure and puberty development, and epidemiological studies in males have reported associations of urinary TCPy with reduced estradiol in adults (Meeker et al., 2008) and with reduced estradiol and FSH in adolescents (Suárez et al., 2021) rather than with increased levels. Animal studies have also found a reduction in estradiol, testosterone, LH, and FSH, and an induction of hypothyroidism after exposure to chlorpyrifos/chlorpyrifos-methyl in males and females (Abd-Elhakim and Yasmina, 2021; Jeong et al., 2006; Li et al., 2019a, 2019b; Peiris and Dhanushka, 2017; Ventura et al., 2016), in disagreement with the present findings.

DETP is a metabolite of several OP insecticides, including

chlorpyrifos and diazinon. In animal models, early-life exposure to some of these insecticides produced toxic effects on the HPG axis (Jayachandra and UrbanD'Souza, 2014; Maitra and Mitra, 2008). In partial agreement with the findings for TCPy, a higher DETP concentration was associated with earlier overall puberty development (higher odds of PDS stage 2+) in girls, but with delayed genital (lower odds of Tanner stage G2+) and adrenarche (lower odds of PDS adrenal stage 2+) in boys, although the former effect was not evident in co-exposure models. In partial agreement with the associations observed for DETP in the present boys, a study in Belgium reported that the sum of dimethyl phosphate metabolites (∑DMPs) and diethyl phosphate metabolites (∑DEPs) were associated with lower odds of having completed genital development (OR [95%CI] = 0.46 [0.22–0.96] and 0.53 [0.29–0.96], respectively) or having reached adult phase of estradiol and testosterone levels in 14–15-year-old adolescent males (Croes et al., 2015), suggesting an anti-androgenic action of OP exposure. However, ∑DEPs was associated with lower odds of complete breast development (OR [95%CI] = 0.78 [0.61–1.00]) in Belgium adolescent females (Croes et al., 2015). Unlike observed for ETU, the association between DETP and delayed overall puberty development (lower odds of PDS stage 2+) was stronger in the boys with overweight/obesity. Overall, these results suggest that postnatal exposure to OP insecticides or metabolites may impact on pubertal development centrally by activating the HPG axis and thereby triggering puberty initiation or exerting an anti-androgenic action; however, the possible mechanism(s) underlying the associations observed with TCPy and DETP, and the role of BMI in these associations, remain to be elucidated.

### 4.3. Pyrethroids

3-PBA was only detected in one-third of the urine samples; nevertheless, boys with detected 3-PBA had increased odds of being in Tanner stage G2+, and this association was stronger in the boys with overweight/obesity. In the same line, a Chinese study found that urinary 3-PBA in boys aged 9–16 years (n = 463), although at several-fold higher concentrations than in the present boys (P75: 2.98 *vs.* 0.288 ng/mL), was associated with earlier genital development (*i.e.*, the odds of being in stage G3 and G4 increased by 275 and 280%, respectively, per one-unit increase in log-transformed urinary 3-PBA) and higher FSH and LH levels (Ye and Pan, 2017b). However, Tanner stages were self-assessed in their study, and 3-PBA was associated with delayed breast development and pubic hair growth and with lower odds of having reached menarche in Chinese girls aged 9–17 years (Ye and Pan, 2017a). Observations in boys are supported by the finding of accelerated puberty onset in male mice after postnatal exposure to pyrethroids (Ye and Li, 2017). In addition, two epidemiological studies found an association between urinary 3-PBA and elevated LH and FSH in men occupationally exposed (Han et al., 2008) and non-occupationally exposed (Meeker et al., 2009) to pyrethroids, consistent with an acceleration of puberty onset. With this background, although no direct evidence is available, it can be hypothesized that exposure to pyrethroids or their metabolites may lead to early puberty in boys through gonadotropin stimulation, possibly *via* oxidative stress and inflammation (Zhang et al., 2017). It remains unclear whether this association is limited to boys with overweight/obesity.

### 4.4. Limitations and strengths

One of the major limitations of this study is its cross-sectional design, preventing the inference of causality. Moreover, the determination of non-persistent pesticide metabolites in spot urine samples or pools of two urine samples is limited to reflecting long-term exposure, because urinary metabolites have a short biological half-life. In fact, once in the human body, pesticides such as OPs and pyrethroids are typically metabolized and excreted in urine within 4–48 h after exposure, depending on the compound (Egeghy et al., 2011), and several studies have indicated moderate temporal reliability for urinary metabolites. Thus, the intra-class correlation coefficient (ICC) for urinary DETP in 7-year-old European children was 0.37 for between-day variability and 0.35 for between-season variability (Casas et al., 2018). In pregnant Spanish women, the ICC for IMPy was in the range 0.40–0.50 (Bravo et al., 2020). A study of Costa Rican children aged 6–9 years exposed to agricultural pesticides found fair reliability for urinary ETU (ICC = 0.67) and TCPy (ICC = 0.52) but moderate reliability (ICC = 0.32) for 3-PBA (van Wendel de Joode e al. 2016). However, in populations that are mainly exposed through their diet, it can be assumed that their exposure to pesticides is relatively continuous (Côté et al., 2014). In this regard, non-specific urinary metabolites such as 3-PBA or ETU may be valid biomarkers of chronic or sub-chronic exposure to dietary mixtures of pyrethroids or EBDC fungicide residues, while the specific metabolites (TCPy, IMPy) are dependent on recent exposure to the parent compound and may therefore show higher intra-individual variation. Another limitation is the variation in the timing of spot urine sample collection among cohorts in Granada (afternoon), Asturias (morning), and Valencia (morning), and it is not known whether concentrations differ between afternoon and morning urine samples, and this issue should be addressed in future studies by measuring repeated urine samples. The boys in the Granada cohort were only assessed for genital development using Tanner; hence, the results for genital development should be interpreted with greater caution. In addition, we cannot rule out that concentrations may have changed as a result of storage time, particularly for samples from Granada (collected in 2010–2012). However, any change in concentrations over time would produce an underestimation rather than overestimation of associations with pubertal development, given that misclassification is expected to be non-differential.

It is also possible that urinary 3-PBA concentrations were substantively underestimated, given that pyrethroid metabolites such as 3-PBA are largely present as phase II conjugates (glucuronide and/or sulfate) in urine (up to 85%) (Baker et al., 2004), and this deconjugation step was omitted, although misclassification is again likely to be non-differential. The relatively low detection frequency of 3-PBA, TCPy, and ETU prevented assessment of the potential effect of the pesticide mixture, and a confounding effect of prenatal or postnatal exposure to other pesticides or EDCs cannot be ruled out. Moreover, diethyl phosphate (DEP) and DMP metabolites could not be measured due to the non-availability of reference standards, and measurement of total DAP concentration would have yielded information on exposure to a wider range of OP pesticides. Additionally, the use of two different instruments for the assessment of puberty development may represent a study limitation. However, Tanner and PDS outcomes were analyzed separately because these different measures of pubertal development are not entirely comparable as they yield different outcomes. The PDS assesses secondary sexual characteristics that are not captured by Tanner stages, including growth spurt, voice deepening, facial and body hair growth, and skin changes, providing an overall score for puberty development and additional outcomes related to the maturation of the HPG and hypothalamic-pituitary-adrenal (HPA) axes (gonadarche and adrenarche). Hence, Tanner and the PDS may be considered complementary instruments to assess pubertal development. Finally, the small number of boys who had reached pubarche or adrenarche limited the possibility of detecting associations with male adrenal development.

Study strengths include the contribution of data from a large sample of children on the association of ETU, TCPy, and DETP exposure with earlier puberty, adding to the scant information available on the relationship between pesticides and puberty timing. Furthermore, the sample derived from five different geographical locations and contained a sufficient proportion of children with overweight/obesity to permit evaluation of effect modification by BMI. It cannot be ruled out that some of the significant or suggestive results in the present study were due to chance, given the performance of multiple comparisons (5 exposure biomarkers x outcomes = 25 comparisons in either girls or boys; 50 comparisons in BMI-stratified analysis). Nevertheless, these

findings represent a potential cause of concern, due to the widespread exposure of children in the general population to pesticides and the possibility that altered pubertal timing may increase the risks of behavioral disorders during adolescence and of obesity, cardiovascular disease, and endocrine-related cancers in later life (Golub et al., 2008; Lakshman et al., 2008, 2009).

## 5. Conclusions

This study provides evidence that peri-pubertal exposure to ETU and certain insecticides might be associated with pubertal outcomes, especially earlier breast development in girls and earlier genital development in boys. These results suggest that interference with the HPG axis by certain contemporary pesticides during childhood may potentially impact pubertal timing. To our best knowledge, this is the first study to report that exposure to ETU and TCPy, respectively, is associated with puberty timing in girls and boys, and it is the first to investigate effect modification by BMI on this association. Population-based longitudinal studies of large samples of children are needed to fully elucidate the role of exposure to pesticides in the population trend toward earlier puberty.

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## Credit author statement

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors do not have permission to share data.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120571.

## References

Abd-Elhakim, Yasmina, M., et al., 2021. Iprodione and/or chlorpyrifos exposure induced testicular toxicity in adult rats by suppression of steroidogenic genes and SIRT1/TERT/PGC-1α pathway. Environ. Sci. Pollut. Res. Int. 28 (40), 56491–56506 (April 21, 2022). https://pubmed.ncbi.nlm.nih.gov/34060014/.

Andersen, Helle, et al., 2002. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. Toxicol. Appl. Pharmacol. 179 (1), 1–12.

Archer, E., van Wyk, J.H., 2015. The potential anti-androgenic effect of agricultural pesticides used in the western cape: in vitro investigation of mixture effects. WaterSA 41 (1), 129–137. https://doi.org/10.4314/wsa.v41i1.16. Availableonwebsite. http://www.wrc.org.za (June 9, 2021).

Axelstad, Marta, et al., 2011. Exposure to the widely used fungicide mancozeb causes thyroid hormone disruption in rat dams but No behavioral effects in the offspring. Toxicol. Sci. : Off. J. Soc. Toxicol. 120 (2), 439–446. https://pubmed.ncbi.nlm.nih.gov/21266532/. (Accessed 21 April 2022).

Baker, Samuel E., Olsson, Anders O., Barr, Dana B., 2004. Isotope dilution high-performance liquid chromatography-tandem mass spectrometry method for quantifying urinary metabolites of synthetic pyrethroid insecticides. Arch. Environ. Contam. Toxicol. 46 (3), 281–288. https://pubmed.ncbi.nlm.nih.gov/15195798/. (Accessed 25 April 2022).

Barr, Dana B., et al., 2005. Urinary creatinine concentrations in the U.S. Population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113 (2), 192–200. https://pubmed.ncbi.nlm.nih.gov/15687057/. (Accessed 21 April 2022).

Barr, Dana B., 2008. Biomonitoring of exposure to pesticides. J. Chem. Health Saf. 15 (6), 20–29. https://pubs.acs.org/doi/abs/10.1016/j.jchas.2008.07.001. (Accessed 21 April 2022).

Biro, Frank M., Kiess, Wieland, 2016. Contemporary trends in onset and completion of puberty, gain in height and adiposity. Endocr. Dev. 29, 122–133. https://pubmed.ncbi.nlm.nih.gov/26680575/. (Accessed 21 April 2022).

Bouman, A., et al., 2005. Sex hormones and the immune response in humans. *Human Reproduction*. Update 11, 411–423. https://doi.org/10.1093/humupd/dmi008.

Bravo, Natalia, et al., 2020. Mother/child organophosphate and pyrethroid distributions. Environ. Int. 134. https://pubmed.ncbi.nlm.nih.gov/31706197/. (Accessed 21 April 2022).

Brix, Nis, et al., 2019. Timing of puberty in boys and girls: a population-based study. Paediatr. Perinat. Epidemiol. 33 (1), 70–78. https://pubmed.ncbi.nlm.nih.gov/30307620/. (Accessed 21 April 2022).

Busch, Alexander S., Højgaard, Brigitte, Hagen, Casper P., Teilmann, Grete, 2020. Obesity is associated with earlier pubertal onset in boys. J. Clin. Endocrinol. Metab. 105 (4), E1667–E1672 (April 21, 2022). https://pubmed.ncbi.nlm.nih.gov/31761939/.

Camacho-Pérez, M.R., et al., 2022. Organophosphorus pesticides as modulating substances of inflammation through the cholinergic pathway. Int. J. Mol. Sci. 23 (9), 4523. https://doi.org/10.3390/ijms23094523.
Casas, M., et al., 2018. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ. Int. 121 (Pt 1), 561. https://doi.org/10.1016/j.envint.2018.09.046, 73.
Carskadon, Mary A., Acebo, Christine, 1993. A self-administered rating scale for pubertal development. J. Adolesc. Health 14 (3), 190–195. July 4, 2021.
Castiello, Francesca, Freire, Carmen, 2021. Exposure to non-persistent pesticides and puberty timing: a systematic review of the epidemiological evidence. Eur. J. Endocrinol. 184 (6), 733–749 (June 11, 2021). https://pubmed.ncbi.nlm.nih.gov/33769962/.
Cecconi, Sandra, Paro, Rita, Rossi, Gianna, Guido, Macchiarelli, 2007. The effects of the endocrine disruptors dithiocarbamates on the mammalian ovary with particular regard to mancozeb. Curr. Pharmaceut. Des. 13 (29), 2989–3004. https://pubmed.ncbi.nlm.nih.gov/17979742/. (Accessed 21 April 2022).
Chen, Haiyan, et al., 2002. Estrogenicity of organophosphorus and pyrethroid pesticides. J. Toxicol. Environ. Health, Part A 65 (19), 1419–1435. https://pubmed.ncbi.nlm.nih.gov/12396874/. (Accessed 21 April 2022).
Commission Implementing Regulation (EU) 2020/17 of 10 January 2020 Concerning the Non-Renewal of the Approval of the Active Substance Chlorpyrifos-Methyl, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market, and Amending the Annex to Commission Implementing Regulation (EU) No 540/2011 (Text with EEA Relevance) - Publications Office of the EU. https://op.europa.eu/en/publication-detail/-/publication/1bfbf3c3-35de-11ea-ba6e-01aa75ed71a1/language-en (April 21, 2022).
Commission Implementing Regulation (EU) 2020/2087 of 14 December 2020 Concerning the Non-Renewal of the Approval of the Active Substance Mancozeb, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market, and Amending the Annex to Commission Implementing Regulation (EU) No 540/2011. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32020R2087 (April 21, 2022).
Côté, Jonathan, et al., 2014. A novel toxicokinetic modeling of cypermethrin and permethrin and their metabolites in humans for dose reconstruction from biomarker data. PLoS One 9 (2). https://pubmed.ncbi.nlm.nih.gov/24586336/. (Accessed 21 April 2022).
Croes, K., et al., 2015. Endocrine actions of pesticides measured in the flemish environment and health studies (FLEHS I and II). Environ. Sci. Pollut. Control Ser. 22 (19), 14589–14599.
Egeghy, Peter P., et al., 2011. Review of pesticide urinary biomarker measurements from selected US EPA children's observational exposure studies. Int. J. Environ. Res. Publ. Health 2011 8, 1727–1754, 8(5): 1727–54. https://www.mdpi.com/1660-4601/8/5/1727/htm. (Accessed 18 August 2021).
English, René Glynnis, et al., 2012. Farm residence and reproductive health among boys in rural South Africa. Environ. Int. 47, 73–79. https://doi.org/10.1016/j.envint.2012.06.006.
Fernández, Sandra, Pardo, Olga, Corpas-Burgos, Francisca, Yusà, Vicent, 2020. Exposure and cumulative risk assessment to non-persistent pesticides in Spanish children using biomonitoring. Sci. Total Environ. 746. https://pubmed.ncbi.nlm.nih.gov/32750575/. (Accessed 21 April 2022).
Fernandez, M.F., et al., 2007. Human exposure to endocrine-disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias: a nested case-control study. Environ. Health Perspect. 115, 8–14. https://doi.org/10.1289/ehp.9351. Suppl 1(Suppl 1).
Fortes, C., et al., 2013. The relation between dietary habits and urinary levels of 3-phenoxybenzoic acid, a pyrethroid metabolite. Food Chem. Toxicol. : Int. J. Publ. Brit. Indus. Biol. Res. Assoc. 52, 91–96. https://pubmed.ncbi.nlm.nih.gov/23146693/. (Accessed 21 April 2022).
Freire, Carmen, et al., 2021. Urinary metabolites of non-persistent pesticides and serum hormones in Spanish adolescent males. Environ. Res. 197 (December 2020).
Fujino, Chieri, et al., 2019. Activation of PXR, CAR and PPARα by pyrethroid pesticides and the effect of metabolism by rat liver microsomes. Heliyon 5 (9), e02466. https://doi.org/10.1016/j.heliyon.2019.e02466.
Gao, Haina, Li, Jinwang, Zhao, Guoping, Li, Yixuan, 2021. 3,5,6-Trichloro-2-Pyridinol intensifies the effect of chlorpyrifos on the paracrine function of sertoli cells by preventing binding of testosterone and the androgen receptor. Toxicology 460. https://pubmed.ncbi.nlm.nih.gov/34352351/. (Accessed 21 April 2022).
Goldner, Whitney S., et al., 2010. Pesticide use and thyroid disease among women in the agricultural health study. Am. J. Epidemiol. 171 (4), 455–464 (April 21, 2022). https://pubmed.ncbi.nlm.nih.gov/20061368/.
Golub, Mari S., et al., 2008. Public health implications of altered puberty timing. Pediatrics 121 (Suppl. 3). Suppl 3. https://pubmed.ncbi.nlm.nih.gov/18245514/. (Accessed 21 April 2022).
González-Alzaga, B., et al., 2020. Urinary levels of organophosphate pesticides and predictors of exposure in pre-school and school children living in agricultural and urban communities from South Spain. Environ. Res. 186. https://pubmed.ncbi.nlm.nih.gov/32335427/. (Accessed 21 April 2022).
Guillette, Elizabeth A., et al., 2006. Altered breast development in young girls from an agricultural environment. Environ. Health Perspect. 114 (3), 471–475.
Guxens, Mònica, et al., 2012. Cohort profile: the INMA-INfancia y medio ambiente-(environment and childhood) Project. Int. J. Epidemiol. 41 (4), 930–940. https://academic.oup.com/ije/article/41/4/930/683988. (Accessed 12 June 2021).
Han, Yan, et al., 2008. The relationship of 3-PBA pyrethroids metabolite and male reproductive hormones among non-occupational exposure males. Chemosphere 72 (5), 785–790. https://pubmed.ncbi.nlm.nih.gov/18479728/. (Accessed 21 April 2022).
Hazarika, Jnyandeep, et al., 2022. Disruption of androgen receptor signaling by chlorpyrifos (CPF) and its environmental degradation products: a structural insight. J. Biomol. Struct. Dynam. 40 (13), 6027–6038. https://doi.org/10.1080/07391102.2021.1875885. https://pubmed.ncbi.nlm.nih.gov/33480323/. (Accessed 21 April 2022).
Herman-Giddens, Marcia, E., et al., 1997. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the pediatric research in office settings network. Pediatrics 99 (4), 505–512.
Hurley, Pamela M., Hill, Richard N., Whiting, Rick J., 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ. Health Perspect. 106 (8), 437–445. https://pubmed.ncbi.nlm.nih.gov/9681970/. (Accessed 21 April 2022).
Jayachandra, Srinivasa, Urban, Ja D'Souza, 2014. Prenatal and postnatal exposure to diazinon and its effect on spermatogram and pituitary gonadal hormones in male offspring of rats at puberty and adulthood. J. Environ. Sci. Health - Part B Pesticides, Food Contam. Agric. Wastes 49 (4), 271–278.
Jeong, Sang Hee, et al., 2006. Effect of chlorpyrifos-methyl on steroid and thyroid hormones in rat F0- and F1-generations. Toxicology 220 (2–3), 189–202. https://pubmed.ncbi.nlm.nih.gov/16472551/. (Accessed 21 April 2022).
Jung, Geehae, et al., 2019. Thyroid function in girls with central precocious puberty. Ann. Pediatr. Endocrinol. Metab. 24 (2), 124–128 (April 21, 2022). https://pubmed.ncbi.nlm.nih.gov/31261477/.
Kackar, Reena, Srivastava, Mithilesh K., Raizada, Rajendra B., 1997. 17 JOURNAL of APPLIED TOXICOLOGY *Studies On Rat Thyroid After Oral Administration Of Mancozeb: Morphological And Biochemical Evaluations*.
Kjeldsen, Lisbeth Stigaard, Ghisari, Mandana, Eva Cecilie Bonefeld-Jørgensen, 2013. Currently used pesticides and their mixtures affect the function of sex hormone receptors and aromatase enzyme activity. Toxicol. Appl. Pharmacol. 272 (2), 453–464. https://pubmed.ncbi.nlm.nih.gov/23871939/. (Accessed 21 April 2022).
Kleber, Michaela, Schwarz, Alexandra, Reinehr, Thomas, 2011. Obesity in children and adolescents: relationship to growth, pubarche, menarche, and voice break. J. Pediatr. Endocrinol. Metab. : JPEM (J. Pediatr. Endocrinol. Metab.) 24 (3–4), 125–130. https://pubmed.ncbi.nlm.nih.gov/21648278/. (Accessed 21 April 2022).
Kojima, Hiroyuki, et al., 2004. Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. Environ. Health Perspect. 112 (5), 524–531.
Koopman-Verhoeff, Elisabeth, Maria, et al., 2020. Classifying pubertal development using child and parent report: comparing the pubertal development scales to tanner staging. J. Adolesc. Health : Off. Publ. Soc. Adolesc. Med. 66 (5), 597–602. https://pubmed.ncbi.nlm.nih.gov/31974011/(March 24, 2022).
Lakshman, R., et al., 2008. Association between age at menarche and risk of diabetes in adults: results from the EPIC-norfolk cohort study. Diabetologia 51 (5), 781–786.
Lakshman, Rajalakshmi, et al., 2009. Early age at menarche associated with cardiovascular disease and mortality. J. Clin. Endocrinol. Metab. 94 (12), 4953–4960.
Lee, Joyce M., et al., 2016. Timing of puberty in overweight versus obese boys. Pediatrics 137 (2). https://pubmed.ncbi.nlm.nih.gov/26817933/. (Accessed 21 April 2022).
Lee, G.H., Choi, K.C., 2020. Adverse effects of pesticides on the functions of immune system. Sep Comp. Biochem. Physiol. C Toxicol. Pharmacol. 235, 108789. https://doi.org/10.1016/j.cbpc.2020.108789.
Li, Jinwang, et al., 2019a. Chlorpyrifos induces metabolic disruption by altering levels of reproductive hormones. J. Agric. Food Chem. 67 (38), 10553–10562 (April 21, 2022). https://pubmed.ncbi.nlm.nih.gov/31490076/.
Li, Yan, et al., 2019b. Pesticide metabolite concentrations in queensland pre-schoolers - exposure trends related to age and sex using urinary biomarkers. Environ. Res. 176. https://pubmed.ncbi.nlm.nih.gov/31226627/. (Accessed 21 April 2022).
Llop, Sabrina, et al., 2017. Distributions and determinants of urinary biomarkers of organophosphate pesticide exposure in a prospective Spanish birth cohort study. Environ. Health : Global Access Sci. Source 16 (1). https://pubmed.ncbi.nlm.nih.gov/28514952/. (Accessed 21 April 2022).
Loredana Marcovecchio, M., Chiarelli, Francesco, 2013. Obesity and growth during childhood and puberty. World Rev. Nutr. Diet. 106, 135–141. https://pubmed.ncbi.nlm.nih.gov/23428692/. (Accessed 21 April 2022).
Lu, Chensheng, Barr, Dana B., Pearson, Melanie A., Waller, Lance A., 2008. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environ. Health Perspect. 116 (4), 537–542. https://pubmed.ncbi.nlm.nih.gov/18414640/. (Accessed 21 April 2022).
Maitra, Saumen Kumar, Mitra, Anindita, 2008. Testicular functions and serum titers of LH and testosterone in methyl parathion-fed roseringed parakeets. Ecotoxicol. Environ. Saf. 71 (1), 236–244 (April 21, 2022). https://pubmed.ncbi.nlm.nih.gov/18029014/.
Manabe, Mari, et al., 2006. Evaluation of the estrogenic activities of some pesticides and their combinations using MtT/Se cell proliferation assay. Int. J. Hyg Environ. Health 209 (5), 413–421. https://pubmed.ncbi.nlm.nih.gov/16781191/. (Accessed 21 April 2022).
Maranghi, Francesca, et al., 2013. Reproductive toxicity and thyroid effects in sprague dawley rats exposed to low doses of ethylenethiourea. Food Chem. Toxicol. : Int. J. Publ. Brit. Indus. Biol. Res. Assoc. 59, 261–271. https://pubmed.ncbi.nlm.nih.gov/23774258/. (Accessed 21 April 2022).
Marinovich, M., et al., 1997. Thyroid peroxidase as toxicity target for dithiocarbamates. Arch. Toxicol. 71 (8), 508–512. https://pubmed.ncbi.nlm.nih.gov/9248629/. (Accessed 21 April 2022).

Marshall, W.A., Tanner, J.M., 1970. Variations in the pattern of pubertal changes in boys. Arch. Dis. Child. 45 (239), 13–23 (June 12, 2021). https://pubmed.ncbi.nlm.nih.gov/5440182/.

Medda, Emanuela, et al., 2017. Iodine nutritional status and thyroid effects of exposure to ethylenebisdithiocarbamates. Environ. Res. 154, 152–159. https://pubmed.ncbi.nlm.nih.gov/28073049/. (Accessed 21 April 2022).

Meeker, John D., Barr, Dana B., Hauser, Russ, 2009. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod. Toxicol. 27 (2), 155–160. https://pubmed.ncbi.nlm.nih.gov/19429394/. (Accessed 21 April 2022).

Meeker, John D., Ravi, Sarena R., Barr, Dana B., Hauser, Russ, 2008. Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. Reprod. Toxicol. 25 (2), 184–191. https://pubmed.ncbi.nlm.nih.gov/18249523/. (Accessed 21 April 2022).

Mutic, Abby D., Baker, Brenda J., McCauley, Linda A., 2017. Deleterious effects from occupational exposure to ethylene thiourea in pregnant women. Workplace Health & Saf. 65 (12), 595–602. https://pubmed.ncbi.nlm.nih.gov/28511580/. (Accessed 21 April 2022).

Namulanda, Gonza, et al., 2017. In utero exposure to atrazine analytes and early menarche in the avon longitudinal study of parents and children cohort. Environ. Res. 156 (March), 420–425. https://doi.org/10.1016/j.envres.2017.04.004.

O'Brien, Katie M., Upson, Kristen, Cook, Nancy R., Weinberg, Clarice R., 2016. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. Environ. Health Perspect. 124 (2), 220–227. https://pubmed.ncbi.nlm.nih.gov/26219104/. (Accessed 25 April 2022).

Orton, Frances, Rosivatz, Erika, Martin, Scholze, Kortenkamp, Andreas, 2011. Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens. Environ. Health Perspect. 119 (6), 794–800.

Ougier, Eva, et al., 2021. Chemical prioritisation strategy in the European human biomonitoring initiative (HBM4EU) - development and results. Int. J. Hyg Environ. Health 236. https://pubmed.ncbi.nlm.nih.gov/34089975/. (Accessed 21 April 2022).

PAN International Consolidated List of Banned Pesticides | PAN International. https://pan-international.org/pan-international-consolidated-list-of-banned-pesticides/(April 21, 2022).

Panganiban, Lynn R., et al., 2004. Correlation between blood ethylenethiourea and thyroid gland disorders among banana plantation workers in the Philippines. Environ. Health Perspect. 112 (1), 42–45 (April 21, 2022). https://pubmed.ncbi.nlm.nih.gov/14698929/.

Papadopoulou, E., et al., 2019. Diet as a source of exposure to environmental contaminants for pregnant women and children from six European countries. Environ. Health Perspect. 127 (10). https://pubmed.ncbi.nlm.nih.gov/31617753/. (Accessed 20 August 2021).

Peiris, Dinithi Champika, Dhanushka, Thamali, 2017. Low doses of chlorpyrifos interfere with spermatogenesis of rats through reduction of sex hormones. Environ. Sci. Pollut. Res. Int. 24 (26), 20859–20867. https://pubmed.ncbi.nlm.nih.gov/28721614/. (Accessed 21 April 2022).

"PESTICIDE RESIDUE CONTROL RESULTS NATIONAL SUMMARY REPORT Year: 2018 Country: SPAIN.". https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/control_oficial/PESTICIDE_RESIDUE_CONTROL_RESULTS_2018.pdf (April 21, 2022).

Petersen, Anne C., Crockett, Lisa, Richards, Maryse, Boxer, Andrew, 1988. A self-report measure of pubertal status: reliability, validity, and initial norms. J. Youth Adolesc. 17 (2), 117–133 (June 12, 2021). https://pubmed.ncbi.nlm.nih.gov/24277579/.

Piccoli, Camila, et al., 2016. Pesticide exposure and thyroid function in an agricultural population in Brazil. Environ. Res. 151, 389–398. https://pubmed.ncbi.nlm.nih.gov/27540871/. (Accessed 21 April 2022).

Raherison, C., et al., 2019. Pesticides exposure by air in vineyard rural area and respiratory health in children: a pilot study. Environ. Res. 169, 189–195. https://pubmed.ncbi.nlm.nih.gov/30466012/. (Accessed 21 April 2022).

Reinehr, Thomas, Roth, Christian Ludwig, 2019. Is there a causal relationship between obesity and puberty? Lancet. Child Adolesc. Health 3 (1), 44–54. https://pubmed.ncbi.nlm.nih.gov/30446301/. (Accessed 21 April 2022).

Ren, Bingtao, Zhu, Yan, 2022. A new perspective on thyroid hormones: crosstalk with reproductive hormones in females. Int. J. Mol. Sci. 23 (5). https://pubmed.ncbi.nlm.nih.gov/35269847/. (Accessed 21 April 2022).

Roca, Marta, et al., 2014. Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. Environ. Res. 131, 77–85. https://doi.org/10.1016/j.envres.2014.02.009.

Runkle, Jennifer, Flocks, Joan, Economos, Jeannie, Dunlop, Anne L., 2017. A systematic review of mancozeb as a reproductive and developmental hazard. Environ. Int. 99, 29–42.

Shen, Chao, et al., 2021. Screening of potential oestrogen receptor α agonists in pesticides via in silico. In: Vitro and in Vivo Methods. Environmental pollution (Barking, Essex : 1987) 270. https://pubmed.ncbi.nlm.nih.gov/33352482/. (Accessed 21 April 2022).

Shirtcliff, E.A., Dahl, R.E., Pollak, S.D., 2009. Pubertal development: correspondence between hormonal and physical development. Child Dev. 80 (2), 327–337. October 21, 2021. https://pubmed.ncbi.nlm.nih.gov/19466995/.

Stadler, Katrina, et al., 2022. Systematic Review of Human Biomonitoring Studies of Ethylenethiourea, a Urinary Biomarker for Exposure to Dithiocarbamate Fungicides." *Environmental Pollution (Barking, Essex : 1987)* 292(Pt B). https://pubmed.ncbi.nlm.nih.gov/34751155/. (Accessed 21 April 2022).

"Statistics | Eurostat.". https://ec.europa.eu/eurostat/databrowser/view/aei_fm_salpest09/default/table?lang=en (April 21, 2022).

Steenland, Kyle, et al., 1997. Thyroid hormones and cytogenetic outcomes in backpack sprayers using ethylenebis(dithiocarbamate) (EBDC) fungicides in Mexico. Environ. Health Perspect. 105 (10), 1126–1130. https://pubmed.ncbi.nlm.nih.gov/9349837/. (Accessed 21 April 2022).

Suárez, Beatriz, et al., 2021. Organophosphate pesticide exposure, hormone levels, and interaction with PON1 polymorphisms in male adolescents. Sci. Total Environ. 769, 144563 https://doi.org/10.1016/j.scitotenv.2020.144563.

Sun, Hong, et al., 2007. Antiandrogenic activity of pyrethroid pesticides and their metabolite in reporter gene assay. Chemosphere 66 (3), 474–479. https://pubmed.ncbi.nlm.nih.gov/16857237/. (Accessed 21 April 2022).

Toppari, Jorma, Anders, Juul, 2010. Trends in puberty timing in humans and environmental modifiers. Mol. Cell. Endocrinol. 324 (1–2), 39–44. https://pubmed.ncbi.nlm.nih.gov/20298746/. (Accessed 21 April 2022).

Usmani, Khawja A., Rose, Randy L., Hodgson, Ernest, 2003. Inhibition and activation of the human liver microsomal and human cytochrome P450 3A4 metabolism of testosterone by deployment-related chemicals. Drug Metabol. Dispos.: Biol. Fate Chem. 31 (4), 384–391. https://pubmed.ncbi.nlm.nih.gov/12642463/. (Accessed 21 April 2022).

Valsamakis, G., et al., 2021. Diet-induced hypothalamic inflammation, phoenixin, and subsequent precocious puberty. Nutrients 13 (10), 3460. https://doi.org/10.3390/nu13103460.

Ventura, Clara, et al., 2016. Pesticide chlorpyrifos acts as an endocrine disruptor in adult rats causing changes in mammary gland and hormonal balance. J. Steroid Biochem. Mol. Biol. 156, 1–9. https://pubmed.ncbi.nlm.nih.gov/26518068/. (Accessed 21 April 2022).

Viswanath, Gunda, et al., 2010. Anti-androgenic endocrine disrupting activities of chlorpyrifos and piperophos. J. Steroid Biochem. Mol. Biol. 120 (1), 22–29. https://pubmed.ncbi.nlm.nih.gov/20211256/. (Accessed 21 April 2022).

van Wendel de Joode, Berna, et al., 2016. Pesticide exposure and neurodevelopment in children aged 6-9 Years from talamanca, Costa Rica. Cortex; J. Devot. Study Nerv. Syst. Behav. 85, 137–150. https://pubmed.ncbi.nlm.nih.gov/27773359/. (Accessed 21 April 2022).

Wohlfahrt-Veje, C., Andersen, H.R., Schmidt, I.M., et al., 2012a. Early breast development in girls after prenatal exposure to non-persistent pesticides. Int. J. Androl. 35 (3), 273–282.

Wohlfahrt-Veje, C., Andersen, H.R., Jensen, T.K., et al., 2012b. Smaller genitals at school age in boys whose mothers were exposed to non-persistent pesticides in early pregnancy. Int. J. Androl. 35 (3), 265–272.

Wolff, M.S., et al., 2014. Phthalate exposure and pubertal development in a longitudinal study of US girls. Hum. Reprod. 29 (7), 1558–1566. https://pubmed.ncbi.nlm.nih.gov/24781428/. (Accessed 21 April 2022).

Ye, Xiaoqing, Pan, Wuye, et al., 2017a. Association of pyrethroids exposure with onset of puberty in Chinese girls. Environ. Pollut. 227, 606–612. https://doi.org/10.1016/j.envpol.2017.04.035.

Ye, Xiaoqing, Pan, Wuye, et al., 2017b. Relationships of pyrethroid exposure with gonadotropin levels and pubertal development in Chinese boys. Environ. Sci. Technol. 51 (11), 6379–6386.

Ye, Xiaoqing, Li, Feixue, et al., 2017. Pyrethroid insecticide cypermethrin accelerates pubertal onset in male mice via disrupting hypothalamic-pituitary-gonadal Axis. Environ. Sci. Technol. 51 (17), 10212–10221.

Yu, Kaimin, et al., 2015. Chlorpyrifos is estrogenic and alters embryonic hatching, cell proliferation and apoptosis in zebrafish. Chem. Biol. Interact. 239, 26–33. https://pubmed.ncbi.nlm.nih.gov/26079056/. (Accessed 21 April 2022).

Yusà, Vicent, et al., 2015. Occurrence of biomarkers of pesticide exposure in non-invasive human specimens. Chemosphere 139, 91–108. https://pubmed.ncbi.nlm.nih.gov/26070147/. (Accessed 21 April 2022).

Yusà, Vicent, et al., 2022. Exposure to Non-persistent Pesticides in the Spanish Population Using Biomonitoring: A Review, vol. 205. Environmental Research. https://pubmed.ncbi.nlm.nih.gov/34838757/. (Accessed 19 April 2022).

Zhang, Q., Zhang, Y., Du, J., Zhao, M., 2017. Environmentally relevant levels of λ-cyhalothrin, fenvalerate, and permethrin cause developmental toxicity and disrupt endocrine system in zebrafish (Danio rerio) embryo. Chemosphere 185, 1173–1180. https://doi.org/10.1016/j.chemosphere.2017.07.091.