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Prenatal exposure to pyrethroid and organophosphate insecticides and language development at age 20–36 months among children in the Odense Child Cohort

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ABSTRACT

Background: Prenatal exposure to organophosphate and pyrethroid insecticides has been associated with impaired neurodevelopment. Few longitudinal studies have investigated associations with early language development in populations with mainly low dietary exposure.

Objective: To investigate associations between biomarkers of maternal gestational exposure to organophosphate and pyrethroid insecticides and the child's language development at age 20–36 months in the prospective Odense Child Cohort.

Methods: Metabolites of organophosphate and pyrethroid insecticides were measured in maternal urine samples collected at gestational week 28. Language development was assessed among 755 singletons at age 20–36 months using the Vocabulary and Complexity scores of the MacArthur-Bates Communicative Development Inventories, standardized into age and sex specific percentile scores according to a Danish reference study. Multiple logistic regression models were used to estimate the odds of scoring below the 15th percentile scores in relation to maternal urinary insecticide metabolite concentrations after adjustment for confounders.

Results: The generic pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA) and the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCPY) were detectable in more than 90% of the urine samples analyzed. Likewise, 82.2% had detectable concentrations of diethyl phosphates (DE) and 58.4% of dimethyl phosphates (DM), both of which are common metabolites of organophosphate insecticides. None of the metabolites was associated with higher odds of delayed results below the 15th percentile language scores. In contrast, reduced probability for scoring below the 15th percentile Vocabulary score was seen for the highest tertile of 3-PBA in boys and for the upper tertile of TCPY and DE in girls.

Conclusion: In this prospective cohort, with predominantly dietary insecticide exposure, we found no evidence that gestational exposure to organophosphate or pyrethroid insecticides adversely affected early language development in the children. The observed indication of a positive effect of insecticides on language development may be explained by residual and unmeasured confounding from socioeconomic factors and dietary habits. Follow-up of these children should include assessment of more complex cognitive functions in later childhood, as well as associations with their own postnatal insecticide exposure.

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1. Introduction

Pyrethroids and organophosphates are two major classes of insecticides. Due to their acute toxicity, organophosphates were prohibited for indoor use as biocides in both the EU and the U.S. more than a decade ago, but they are still in use in agriculture, although not in Denmark. The organophosphate chlorpyrifos has for decades been one of the most widely used insecticides in agriculture worldwide (Eaton et al., 2008). However, from February 2020 chlorpyrifos and chlorpyrifos-methyl are no longer approved for use in the EU because of concern for developmental neurotoxicity and genotoxicity (EFSA, 2019), although exposure will still occur from produce originating outside the EU. While the use of organophosphates is decreasing, the use of pyrethroids is increasing as substitutes for organophosphates in biocide products and also, to some degree, in agriculture. Human biomonitoring studies suggest widespread exposure to both organophosphates and pyrethroids, also among pregnant women, based on high detection rates of organophosphate and pyrethroid metabolites in urine from pregnant women (Dalsager et al., 2019; Dereumeaux et al., 2018; Sokoloff et al., 2016; Yolton et al., 2013). The insecticides are able to cross the human placenta, thus causing fetal exposures as well, as illustrated by detection of these substances in umbilical cord blood and meconium (Berton et al., 2014; Rauh et al., 2011; Silver et al., 2016).

Most insecticides target the nervous system of insects. Due to similarities in neurochemistry they also have neurotoxic properties in mammals (Abreu-Villaca and Levin, 2017). The developing brain is particularly vulnerable to neurotoxicants, and major windows of vulnerability occur in utero and during early postnatal life (Grandjean and Landrigan, 2014; Rice and Barone, 2000). Several birth cohort studies have reported associations between prenatal exposure to organophosphate and pyrethroid insecticides and impaired childhood neurodevelopment, i.e., cognitive and/or behavioural deficits, although mainly in populations with elevated exposure from occupational or indoor use or from residence in proximity to pesticide-treated agricultural areas (Andersen et al., 2015; Bouchard et al., 2011; Eskenazi et al., 2018; Gunier et al., 2017; Liu et al., 2016; Llop et al., 2013; Rauh et al., 2006; Shelton et al., 2014). A few studies have investigated neurodevelopmental outcomes associated with prenatal organophosphate and pyrethroid exposure in populations considered to be mainly exposed through ingestion of residues in foods (Cartier et al., 2016; Dalsager et al., 2019; Donauer et al., 2016; Jusko et al., 2019; Nkinsa et al., 2020; Tanner et al., 2020; Viel et al., 2015, 2017; Yolton et al., 2013), and the results have been equivocal perhaps in part due to differences in study design. Some of these studies reported on language development as part of IQ assessment in relation to prenatal pyrethroid (Viel et al., 2015) or organophosphate exposure (Cartier et al., 2016; Donauer et al., 2016; Nkinsa et al., 2020). Only one study assessed language skills as a separate outcome and found no relationships between prenatal organophosphate exposure and language development at age 4 years (Donauer et al., 2016). Since early language development can be seen as an important indicator of early neurodevelopment and is correlated to subsequent intelligence and later academic achievement (Bleses et al., 2016; Flensborg-Madsen and Mortensen, 2018; Liao et al., 2015), we aimed to investigate associations between maternal urinary concentrations of organophosphate and pyrethroid metabolites in pregnancy and language development in the children up to age 36 months among mother-child pairs from the Odense Child Cohort (OCC). From this cohort, mainly exposed to organophosphate and pyrethroid insecticides from dietary sources, we have previously reported associations between maternal pyrethroid exposure in pregnancy and more attention deficit hyperactivity disorder (ADHD) symptoms among the children at age 2–4 years (Dalsager et al., 2019).

2. Methods

2.1. Study population

We used data from the prospective Odense Child Cohort (OCC). From January 2010 to December 2012, all pregnant women living in the Municipality of Odense in Denmark were invited to participate in the cohort either at a voluntary meeting about ultrasound, at the first antenatal visit or at the first ultrasound examination at Odense University Hospital at gestational week (GW) 8–16 weeks. A total of 2874 women agreed to participate. Of these women, 52 dropped out before giving birth, 112 had a miscarriages or stillbirth, and 210 withdrew from the cohort after giving birth, leaving 2500 families (2549 children) in the cohort. Participating women tended to be older, more often non-smokers, better educated, had fewer children, and were more often of Danish origin than non-participants (Kyhl et al., 2015). At both inclusion and GW 28 the women filled in questionnaires about health, lifestyle and social factors. All children were examined at birth, and information on birth weight (g), gestational age of the child as well as maternal pre pregnancy BMI (kg/m^2), smoking status, parity and age at delivery was obtained from obstetric records. Maternal country of birth was obtained from a register in the Municipality of Odense. For 228 women, information on education level was missing from the questionnaires. In these cases, information on occupation obtained from obstetric records was used to estimate educational level whenever possible. Information on duration of breastfeeding was obtained from questionnaires answered by the parents when the child was 18 months and supplemented with data from a sub-project on breastfeeding reported via text messages (Bruun et al., 2016). For this study, we excluded twins and children of mothers not born in Denmark (to ensure that all mothers were native Danish speakers).

2.2. Pyrethroid and organophosphate insecticide exposure measurements

Spot urine samples were collected in GW 28 (median 28.7, range 26.4–34.0) after overnight fasting and before 9.30 a.m. The samples were stored at −80 °C until analyses. Out of 1603 women who provided a urine sample, a subset of 1207 samples (including mothers of twins and mothers not born in Denmark) was analyzed for the specific metabolite of chlorpyrifos/chlorpyrifos-methyl, TCPY (3,5,6-trichloro-2-pyridinol), and the generic pyrethroid metabolite, 3-PBA (3-phenoxybenzoic acid), representing exposure to most pyrethroids. In addition, we measured some more specific pyrethroid metabolites: *cis*- and *trans*-DCCA (3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid) representing exposure to the *cis*- and *trans*-isomers of permethrin, cypermethrin, and cyfluthrin; 4-F-3PBA (4-fluoro-3-phenoxybenzoic acid) which is a specific metabolite of cyfluthrin; and *cis*-DBCA (*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid) being a specific metabolite of deltamethrin. The analyses were performed by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) as previously described (Dalsager et al., 2019). Calibration curves, solvent blanks, and quality control samples were included in each batch of samples. In-house made quality control (QC) samples (low and high level) with all compounds were made in diluted urine (1:3). The accuracy of the analysis was also controlled by participation in the German External Quality Assessment Scheme (G-EQUAS), (for 3-PBA, trans-DCCA, and cis-DBCA). Excess sample material from this program was also used as QC samples. The accuracy of the analysis for all the compounds ranged from 92.7 to 103.3%. The between batch variation (CV%) ranged from 3.5 to 17.6%. The limit of detection (LOD) for the compounds were: 0.3 μg/L for TCPY; 0.03 μg/L for 3-PBA, 0.2 μg/L for 4-F-3PBA; 0.4 μg/L for *trans*-DCCA, and 0.5 μg/L for *cis*-DCCA and *cis*-DBCA. Spectrophotometric determination of creatinine concentrations was conducted on a Konelab 20 Clinical Chemistry Analyzer, using a commercial kit (Thermo, Vantaa, Finland).

Out of the 1207 urine samples, a subset of 564 samples were also

analyzed for concentrations of six non-specific dialkyl phosphate (DAP) metabolites of organophosphate insecticides at the Flemish Institute for Technological Research NV (VITO), Belgium, using solid phase extraction (SPE) followed by Ultra Performance Liquid Chromatography-tandem mass spectrometry (UPLC-MS/MS) as previously described (Dalsager et al., 2018). The DAP metabolites comprise three dimethyl (DM) phosphate metabolites (dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP)) and three diethyl (DE) phosphate metabolites (diethyl phosphate (DEP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP)). LODs were 1.49 μg/L for DMP and 0.30 μg/L for DMTP, DMDTP, DEP, DEDTP, and DEDTP. The metabolite concentrations were converted to their molar concentrations and summed to yield total DM, total DE, and total DAP concentrations (nmol/L).

The first 200 urine samples analyzed for insecticides were selected randomly from the 1603 available urine samples, whereas the remaining samples were selected based upon availability of information from baseline questionnaires, birth records and clinical examinations of the child.

2.3. Language development assessment

Language development was assessed using the validated Danish adaption of the MacArthur-Bates Communicative Development Inventories (MB-CDI) (Bleses et al., 2008; Fenson et al., 2000) which is a parent report instrument used for assessing children's early language development (e.g., vocabulary and grammar) at different age steps. Every third month from the age of 20–36 months, the parents completed an electronic version of the MB-CDI: *Words and Sentence* questionnaire regarding their child's current language skills. The questionnaire contained seven subscales, of which two (Vocabulary and Complexity) were included in the present study to represent important domains of language development during the age span of interest (Fenson et al., 2000). The Vocabulary subscale focuses on the child's use of commonly used words for toddlers (lexical development), whereas the Complexity subscale focuses on use of grammar and syntactic complex sentences (morphosyntactic development). In the questionnaire, parents marked all words and sentences their child produced prior to or at the time of reply.

Although parents repeatedly filled in questionnaires during the 16-month period, we included only one questionnaire per category per child because some parents only filled in few questionaries while others filled in many, and at different ages during the 16 month period. For Vocabulary, we used the first questionnaire filled out to create a productive Vocabular summary score (number of words reported to be expressed), whereas for Complexity, we used the first questionnaire after the age of 26 months in girls and 30 months in boys for the purpose of creating a summary score (number of mastered Complexity items). These age limits were applied as more than 15% of children in the reference population had a score of zero before the ages chosen (Bleses et al., 2008). For each child, the summary scores were converted into an age and sex specific percentile score by comparing the obtained score with the sex and age specific score for the Danish reference population (Bleses et al., 2008). A percentile-score ≤ 15 was considered as delayed language development.

2.4. Ethics

The project was performed in accordance with the Helsinki Declaration II, with written informed consent, and approved by the Regional Scientific Ethical Committees for Southern Denmark (Project-ID S-20090130) and the Danish Data Protection Agency (j.no. 2016-231-0188).

2.5. Statistical analysis

Insecticide metabolites with detection frequencies below 20% were dichotomized ($\geq$LOD vs $<$ LOD). For all other metabolites, values below the limit of detection (LOD) were substituted by the metabolite specific LOD/$\sqrt{2}$. Urinary concentrations were expressed as volume-based (μg or nmol/L) or creatinine-based (μg or nmol/g creatinine) and reported as medians and 25–75 percentiles. To investigate possible effects of combined exposure to chlorpyrifos and pyrethroids, we also created a variable indicating whether the combined exposure was low (both metabolites below the median), medium (one above the median) or high (both above the median). Correlations between urinary pesticide metabolite concentrations and between Vocabulary and Complexity percentile scores were evaluated by Spearman correlation. The language score percentiles were reported as medians and 25–75 percentiles, and the language scores were further dichotomized (below/above the 15th percentile) to indicate potentially delayed language development (Bleses et al., 2008).

Associations between maternal insecticide metabolite concentrations and the age and sex specific percentile language scores were estimated by logistic regression models. For these analyses, the volume-based metabolite concentrations were divided into tertiles and the continuous concentrations were $\ln_2$ transformed to obtain a normal distribution and ln-transformed creatinine (g/L) was included as a covariate. The odds ratio (OR) for having a language score below the 15th percentile across increasing tertiles of maternal urinary insecticide metabolites concentrations as well as for doubling the concentrations using continuous $\ln_2$-transformed variables were calculated. Potential confounders were identified as variables shown in the literature to be associated with either pesticide exposure or child language development. Of the covariates considered *a priori*, we had information on maternal age, pre-pregnancy body mass index (BMI), educational level, smoking during pregnancy, parity, preterm birth (child born before GW 37), child sex and birth weight and duration of breastfeeding. Breastfeeding information was missing for 11% of the women and a category consisting of these women was added to the breastfeeding variable to avoid exclusion of these data. Differences in characteristics between participants and non-participants as well as differences in exposure and outcome variables in relation to maternal and child characteristics were tested using non-parametric Kruskal Wallis/Mann Whitney tests and chi-square tests. Covariates included in the adjusted analyses were maternal education (as the best available estimate of socio-economic status), breastfeeding and child sex. We did not adjust for child age, as language scores were categorized into age specific percentile scores according to age of the reference population. Although the language scores were also sex standardized, sex was included to account for potential sex-differences in vulnerability towards pesticides, and interaction between exposure and child sex was investigated by including an interaction term in the adjusted analyses. Further, logistic regression analyses were performed for boys and girls separately. In a sensitivity analysis, we extended the adjusted model to include also maternal pre-pregnancy BMI, as this characteristic was associated with both exposure and outcome in our data set. The results are presented as OR with 95% confidence intervals, and p-values < 0.05 were considered statistically significant.

3. Results

Of 2217 singletons of mothers born in Denmark, MB-CDI questionnaires were completed for 1,360, and 1109 had insecticides measured in the urine (Fig. 1). A total of 755 mother-child pairs, with data on both vocabulary outcome and exposure, were included in this study. The participants were more often non-smokers, had fewer preterm deliveries, longer duration of breastfeeding, and the children had higher birth weight compared to non-participating singletons of mothers born in Denmark (N = 1462). There was no difference in maternal age, BMI,

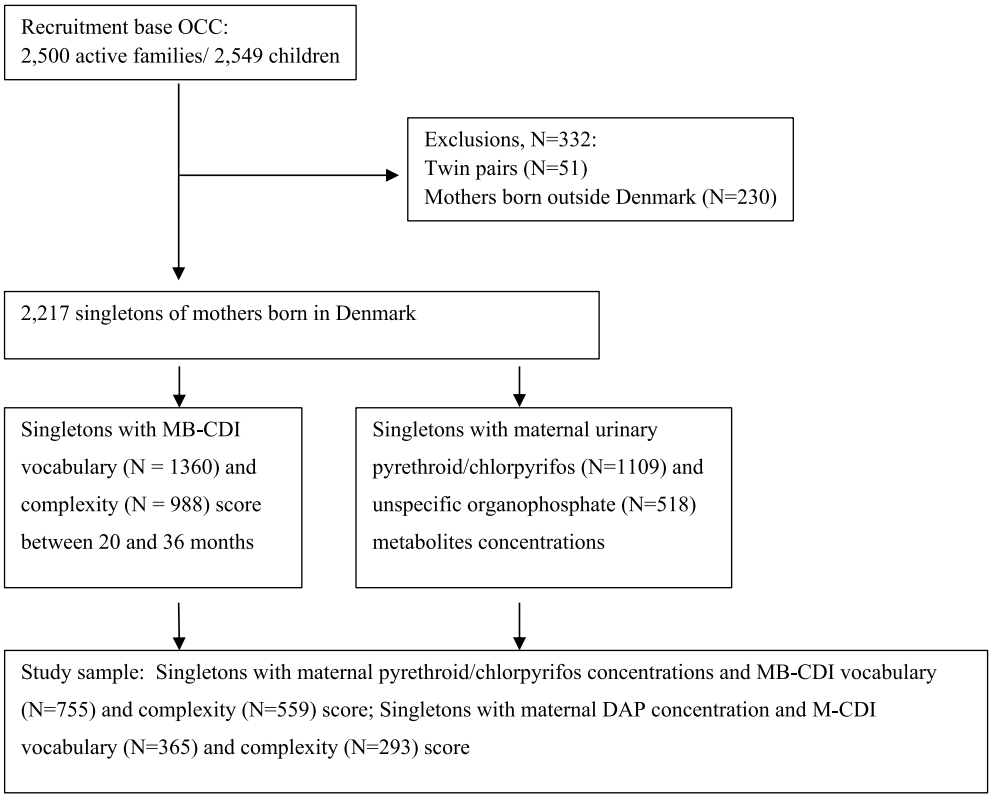

Fig. 1. Flow diagram of study population based on mother-child pairs from the Odense Child Cohort (OCC) with maternal insecticide metabolite concentration and MacArthur-Bates Communicative Development Inventories (MB-CDI) language assessment in the offspring.

level of education or parity (data not shown).

Most of the women had detectable concentrations of TCPY (92.3%) and 3-PBA (94.3%). The median concentrations were 1.73 μg/g creatinine and 0.24 μg/g creatinine, respectively (Table 1). The specific pyrethroid metabolites were detectable only in 12.2% (*trans*-DCCA), 2.6% (*cis*-DCCA), 3.2% (*cis*-DBCA) while 4-F-3PBA was detected only in a single urine sample (data not shown). For the DAPs, 82.2% of the 365 samples analyzed had detectable concentrations of at least one DE metabolite and 58.4% of at least one DM metabolite. The median molar sum of the six metabolites (DAPs) was 59.6 nmol/g creatinine (Table 1) and 23.7 and 30.9 nmol/g creatinine for DE and DM, respectively (data not shown). Further, 3-PBA was weakly correlated with TCPY (Spearman $r_s = 0.17$). Also, as expected, TCPY and DAP were correlated ($r_s = 0.34$). The TCPY concentration was higher among women with longer education and women with low pre-pregnancy BMI and among women who did not breastfeed (Table 1). None of the selected participant characteristics were statistically significantly associated with 3-PBA or DAP concentrations.

Due to incomplete responses, the Complexity percentile score could be calculated only for 559 of the 755 children with maternal 3-PBA and TCPY concentrations, and for 293 of the 365 with maternal DAPs (Fig. 1). The MB-CDI questionnaires on Vocabulary were completed at a median age of 21 months (range 20–36) for both boys and girls. For the Complexity subscale, the median age was 31 months (range 30–36) for boys and 27 months (range 26–36) for girls. The Vocabulary percentile score median was 50 (55 for boys and 50 for girls), and the median Complexity percentile score was 45 (40 for boys and 45 for girls) (Table 1). Vocabulary and Complexity percentile scores were highly correlated ($r_{s\ Boys} = 0.69$ and $r_{s\ Girls} = 0.67$, $p < 0.0001$). The language percentile scores were higher among children who were breastfed the longest, and the Complexity percentile score was higher among children of women with longer education and women with lower pre-pregnancy BMI (Table 1).

The ORs for scoring below the age and sex standardized 15th percentile on the MB-CDI Vocabulary and Complexity subscales according to maternal urinary insecticide metabolite concentrations are shown in Tables 2 and 3. Generally, none of the insecticide metabolites were associated with higher odds of scoring below the 15th percentile language scores in either the crude or the adjusted analyses. For the highest tertile of 3-PBA, a reduced OR for having a Vocabulary score below the 15th percentile was seen in the adjusted analyses (OR = 0.51 (95% CI: 0.27; 0.94), p = 0.03). This association in a direction opposite to the hypothesis was driven by boys (OR = 0.30 (0.12; 0.72), p = 0.007) while no association was observed for girls. The p-value for sex-exposure interaction was 0.05. For the upper tertile of TCPY and DE, again a reduced OR for scoring below the 15th percentile was found for girls (OR for TCPY = 0.38 (0.15, 0.96), p = 0.04 and OR for DE = 0.21 (0.05, 0.86), p = 0.03) but not for boys or both sexes combined. However, the associations described were not apparent when 3-PBA, TCPY and DE were included as continuous variables. No statistically significant associations were seen between maternal insecticide metabolites and odds of scoring below the 15th percentile on the MB-DI Complexity subscale. Combined maternal 3-PBA and TCPY concentrations were not associated with altered odds of scoring below the 15th percentile language scores, but a tendency of a reduced OR among boys was seen for the two categories with 3-PBA above the median (Table 2). Further adjustment for maternal pre-pregnancy BMI did not affect these results (data not shown).

4. Discussion

In this prospective study of mother-child pairs from the Odense Child Cohort, we did not observe any associations between low-level gestational exposure to pyrethroid and organophosphate insecticides and impaired language development in the children at age 20–36 months. The observed suggestion of a possibly protective effect of pyrethroids

Table 1
Maternal urinary concentrations (Median (M) and 25th −75th percentiles (25–75)) of 3-PBA, TCPY, and DAP in gestational week 28 and M (25–75) of MB-CDI Vocabulary and Complexity score at age 1.5–3 years according to maternal and child characteristics.

		3-PBA μg/g creatinine	TCPY μg/g creatinine	DAP nmol/g creatinine		Vocabulary percentile score		Complexity percentile score	
Maternal characteristics	N	M (25–75)	M (25–75)	N	M (25–75)	N	M (25–75)	N	M (25–75)
All	755	0.24 (0.14–0.45)	1.73 (1.05–3.02)	365	59.6 (37.0–104.8)	755	50 (25–75)	559	45 (15–70)
Maternal age at delivery (years)									
<26	81	0.22 (0.12–0.47)	1.64 (1.06–2.72)	40	69.0 (43.8–117.9)	81	50 (25–75)	61	45 (20–65)
26–34	512	0.24 (0.14–0.45)	1.72 (1.02–3.09)	242	58.4 (34.3–105.6)	512	55 (30–75)	376	45 (20–75)
>34	162	0.23 (0.12–0.45)	1.80 (1.11–3.05)	83	59.8 (44.0–89.8)	162	45 (19–75)	122	35 (15–66)
Pre-pregnancy BMI (kg/m^2)									
<18.5	21	0.16 (0.09–0.37)	2.13 (1.56–3.23)*	12	57.2 (46.2–121.6)	21	60 (40–85)	17	55 (40–75)*
18.5–24.9	450	0.23 (0.14–0.43)	1.78 (1.10–3.31)*	217	64.4 (41.8–107.7)	450	55 (25–75)	319	50 (20–75)*
≥25	284	0.25 (0.14–0.50)	1.63 (0.91–2.73)*	136	55.6 (29.9–89.8)	284	50 (20–75)	223	40 (15–70)*
Smoking									
No	730	0.24 (0.14–0.45)	1.73 (1.05–3.01)	352	59.8 (37.4–104.8)	730	55 (25–75)	539	45 (15–75)
Yes	25	0.29 (0.16–0.48)	1.61 (0.90–3.50)	13	43.1 (20.4–103.7)	25	55 (28–78)	20	35 (15–56)
Education level[a]									
Low	199	0.24 (0.13–0.42)	1.52 (0.93–2.51)*	111	59.8 (37.7–104.4)	199	45 (20–70)	151	35 (20–60)*
Intermediate	389	0.23 (0.14–0.48)	1.78 (1.08–3.26)*	174	59.1 (35.9–105.6)	389	55 (25–75)	280	45 (15–70)*
High	160	0.24 (0.13–0.47)	1.88 (1.17–3.32)*	75	65.8 (38.8–103.7)	160	55 (31–85)	123	55 (25–85)*
missing	7	0.37 (0.21–0.41)	0.78 (0.53–1.34)	5	26.6 (12.9–108.1)	7	85 (55–98)	5	70 (35–98)
Parity									
Nulliparous	427	0.24 (0.13–0.46)	1.73 (1.02–3.03)	197	59.6 (37.6–104.1)	427	55 (25–75)	312	45 (20–70)
Multiparous	328	0.23 (0.14–0.44)	1.69 (1.05–3.02)	168	59.6 (36.0–105.3)	328	53 (25–75)	248	40 (15–75)
p-value		0.46	0.99		0.93		0.45		0.93
Child characteristics									
Sex									
Boy	390	0.24 (0.14–0.46)	1.72 (1.00–3.01)	188	60.6 (38.0–102.9)	390	55 (25–75)	262	40 (15–75)
Girl	365	0.24 (0.14–0.44)	1.73 (1.09–3.05)	177	59.5 (35.1–106.0)	365	50 (25–75)	297	45 (20–70)
Preterm (<37 weeks)									
no	743	0.24 (0.14–0.45)	1.70 (1.02–3.00)	360	59.4 (37.0–105.3)	743	55 (25–75)	549	45 (15–75)
yes	12	0.31 (0.21–0.44)	2.74 (1.89–3.19)	5	62.7 (59.6–75.9)	12	40 (25–84)	10	30 (14–51)
Birth weight (g)									
<3000	84	0.24 (0.12–0.48)	1.68 (0.89–2.74)	39	59.8 (26.6–86.6)	84	45 (16–70)	67	35 (15–65)
3000-4000	521	0.24 (0.14–0.45)	1.77 (1.08–3.23)	253	60.8 (39.7–105.6)	521	55 (25–75)	379	45 (20–75)
≥4000	150	0.24 (0.14–0.43)	1.64 (1.00–2.60)	73	55.6 (34.4–112.7)	150	50 (25–75)	113	40 (15–73)
Total breastfeeding (weeks)									
0	19	0.29 (0.14–0.90)	2.05 (1.25–3.27)*	9	73.63 (35.6–123.2)	19	55 (15–70)*	14	20 (10–36)*
1–25	229	0.24 (0.13–0.42)	1.55 (0.83–2.72)*	100	57.3 (30.9–98.6)	229	45 (20–70)*	161	35 (15–60)*
>25	426	0.24 (0.14–0.48)	1.79 (1.09–3.18)*	199	60.0 (38.3–103.2)	426	60 (30–85)*	330	50 (20–80)*
Missing	81	0.21 (0.13–0.37)	1.91 (1.23–3.03)	57	57.8 (40.6–122.6)	81	40 (20–65)	54	50 (25–65)

*$p < 0.05$ in Kruskal Wallis test.
[a] Low: High school and vocational education or less, Intermediate: High school + 1–4 years, High: High school + 5 years or more.

and chlorpyrifos on language development is considered biologically implausible and may be explained by residual and unmeasured confounding from socioeconomic factors and dietary habits. Fruit, vegetables, and cereals often contain pesticide residues (Lu et al., 2008; Morgan, 2012; Vanacker et al., 2020) but at the same time a high intake of these commodities may be beneficial, thus potentially outweighing detrimental effects of low organophosphate and pyrethroid exposure, as has also been suggested elsewhere (Donauer et al., 2016; Yolton et al., 2013). Unfortunately, we did not have any information on maternal food habits. Further, due to multiple testing, the few statistically significant associations may well be chance findings.

Regarding organophosphates, our findings are in accordance with two other studies with low maternal DAP-concentrations comparable to our study. In an urban/suburban birth cohort (HOME) in Ohio, no associations between maternal DAP concentrations (geometric mean (GM): 73.7 nmol/g creatinine) and child cognition, including language development, at 1–5 years of age were found (Donauer et al., 2016). Further, a study based on a French birth cohort (PELAGIE) reported no consistent pattern of associations between maternal DAP (median:11.3 nmol/L) and cognitive scores, inclusive of verbal comprehension scores, in the offspring at age 6 years (Cartier et al., 2016). As in our study, a few positive associations were reported (Cartier et al., 2016; Donauer et al., 2016), likely due to similar residual confounding. In contrast, a recent study from the Canadian MIREC cohort, with a slightly higher median maternal DAP concentration (86.1 nmol/L), found higher concentrations of DE to be associated with poorer verbal IQ scores at 3–4 years of age among boys, but not girls (Nkinsa et al., 2020). Interestingly, we found a reduced probability for scoring below the 15th percentile Vocabulary score related to high maternal DE and TCPY concentrations among girls only. Assuming this association is due to residual negative confounding, it may suggest that boys' benefit from these confounding factors is hampered by prenatal exposure to chlorpyrifos and other diethyl organophosphates. Higher vulnerability among boys was found in two studies from China, in which maternal DE concentrations were associated with neurodevelopmental delay at 24 months of age in boys only, but mainly in the adaptive (Liu et al., 2016) or social (Wang et al., 2017) domains, while language development was not significantly affected. The exposure levels were considerably higher than in our study, with median DAP concentrations of 295.8 nmol/L and 352.7 nmol/g creatinine, respectively, but similar to the median of 310 nmol/g creatinine reported in the Generation R study from the Netherlands (Jusko et al., 2019). In the Generation R study, lower nonverbal IQ was associated with maternal DM and total DAP concentrations in late pregnancy (above GW 25), though the results were inconsistent across repeated urine sampling periods (<18, 18–25 >25 GW) in pregnancy. Language development was not included in this study, and the associations were not modified by child sex. Maternal DAP concentrations in the Generation R study were markedly higher than reported from other urban/suburban studies in the EU or US and also higher than in the CHAMACOS study (GM: 114.9 nmol/L) from an agricultural community in California (Bouchard et al., 2011). From the CHAMACOS study, adverse associations with neurodevelopment at 2 years of age (Eskenazi

Table 2
Crude and adjusted odds ratio (OR) and 95% confidence intervals (95% CI) for scoring below the age and sex standardized 15th percentile on the MB-CDI Vocabulary and Complexity subscales according to maternal urinary concentrations of pyrethroid and chlorpyrifos metabolites (categorical and continuous after ln_2-transformation) among children from the Odense Child Cohort.

	MB-CDI Vocabulary score below 15th percentile				MB-CDI Complexity score below 15th percentile			
	All (N = 755)	All (N = 748)	Boys (N = 389)	Girls (N = 359)	All (N = 559)	All (N = 554)	Boys (N = 261)	Girls (N = 293)
	Crude	Adjusted[a]	Adjusted[a]	Adjusted[a]	Crude	Adjusted[a]	Adjusted[a]	Adjusted[a]
3-PBA								
1the tertile (<0.13 μg/L)	Reference				Reference			
2nd tertile (0.13–0.36 μg/L)	1.21 (0.74; 1.98)	0.99 (0.59; 1.66)	0.71 (0.34; 1.48)	1.38 (0.64; 2.95)	1.17 (0.70; 1.97)	1.06 (0.60; 1.83)	0.53 (0.23; 1.18)	2.04 (0.93; 4.49)
3rd tertile (>0.36 μg/L)	0.71 (0.41; 1.23)	0.51 (0.27; 0.94)	0.30 (0.12; 0.72)	0.88 (0.36; 2.17)	0.78 (0.45; 1.34)	0.66 (0.35; 1.23)	0.49 (0.20; 1.19)	0.95 (0.40; 2.38)
p-trend	0.24	0.03	0.007	0.80	0.37	0.19	0.12	0.89
Continuous[b]	0.94 (0.83; 1.05)	0.87 (0.76; 1.00)	0.81 (0.67; 0.98)	0.93 (0.75; 1.15)	0.92 (0.82; 1.05)	0.89 (0.77; 1.02)	0.91 (0.74; 1.10)	0.87 (0.70; 1.07)
Trans-DCCA								
<LOD (0.4 μg/L)	Reference				Reference			
≥LOD	1.12 (0.64; 2.19)	1.09 (0.58; 2.04)	1.22 (0.53; 2.81)	0.96 (0.35; 2.66)	0.80 (0.39; 1.63)	0.74 (0.35; 1.53)	0.83 (0.31; 2.23)	0.63 (0.21; 1.93)
TCPy								
1the tertile (<1.13 μg/L)	Reference				Reference			
2nd tertile (1.13–2.50 μg/L)	1.20 (0.72; 2.00)	1.06 (0.61; 1.82)	1.37 (0.61; 3.10)	0.88 (0.42; 1.85)	0.98 (0.58; 1.64)	0.87 (0.50; 1.53)	1.06 (0.48; 2.35)	0.78 (0.36; 1.72)
3rd tertile (>2.50 μg/L)	1.00 (0.60; 1.70)	0.79 (0.44; 1.43)	1.49 (0.66; 3.40)	0.38 (0.15; 0.96)	0.72 (0.42; 1.24)	0.61 (0.33; 1.12)	0.65 (0.27; 1.58)	0.59 (0.24; 1.38)
p-trend	0.99	0.41	0.35	0.04	0.23	0.10	0.33	0.22
Continuous[b]	1.00 (0.87; 1.15)	0.93 (0.79; 1.10)	1.05 (0.84; 1.31)	0.83 (0.65; 1.05)	0.90 (0.78; 1.04)	0.84 (0.71; 1.00)	0.87 (0.68; 1.12)	0.82 (0.63; 1.06)
Combined 3-PBA and TCPy								
Both≤p50	Reference				Reference			
3-PBA > p50 & TCPy≤p50	1.15 (0.63; 2.09)	0.94 (0.50; 1.79)	0.42 (0.14; 1.21)	1.69 (0.72; 3.96)	1.06 (0.56; 1.98)	0.97 (0.50; 1.90)	0.88 (0.32; 2.40)	1.12 (0.44; 2.83)
3-PBA≤p50 & TCPy > p50	1.11 (0.60; 2.04)	0.91 (0.48; 1.73)	1.23 (0.51; 2.99)	0.67 (0.25; 1.79)	1.14 (0.62; 2.10)	1.03 (0.54; 1.98)	0.94 (0.34; 2.61)	1.06 (0.43; 2.58)
Both > p50	1.15 (0.63; 2.09)	0.94 (0.50; 1.79)	0.42 (0.14; 1.21)	1.69 (0.72; 3.96)	1.06 (0.56; 1.98)	0.97 (0.50; 1.90)	0.88 (0.32; 2.40)	1.12 (0.44; 2.83)

P50: median (0.21 μg/L for 3-PBA and 1.69 μg/L for TCPy).
[a] Adjusted for creatinine (ln-transformed), maternal education, weeks of breastfeeding, and child sex (for all children).
[b] For doubling the concentration.

et al., 2007) and with full-scale IQ, including poorer scores for processing speed and verbal comprehension, at 7 years of age were reported (Bouchard et al., 2011).

For chlorpyrifos, dose-response associations have been reported between the concentration in umbilical cord blood and delayed psychomotor development in 3-year-old children (Rauh et al., 2006) and cognitive development at age 7 years (Rauh et al., 2011) in a New York inner-city birth cohort established before the ban of chlorpyrifos for residential use. However, only two previous studies have included TCPY in maternal urine as biomarker for chlorpyrifos exposure (Eskenazi et al., 2007; Guo et al., 2019). Both studies reported higher TCPY concentrations than in our study, with median TCPY concentrations of 3.54 and 5.39 μg/L, respectively. Neither of these studies found adverse associations with cognitive development in the children, although associations with maternal DAP concentrations were seen. Thus, adverse neurodevelopment seems more strongly correlated to prenatal exposure to organophosphates overall than to chlorpyrifos specifically, which is reasonable assuming additive effects of organophosphates on neurodevelopment (EFSA et al., 2019).

Regarding pyrethroids, our findings are in accordance with some other studies with low maternal pyrethroid exposure. In the French birth cohort, PELAGIE, verbal comprehension and working memory scores at age 6 years were not associated with maternal pyrethroid metabolites (Viel et al., 2015), but some associations with behavioural problems were reported (Viel et al., 2017) as also seen in the OCC (Dalsager et al., 2019) and in a study from New York (Furlong et al., 2017). However, a recent study from Sweden found associations between maternal 3-PBA and reduced IQ at age 7 years (Tanner et al., 2020), and a study from Mexico City reported association between maternal 3-PBA and delayed mental development at 2 years of age (Watkins et al., 2016). A direct comparison of the urinary pyrethroid metabolite concentrations is hampered by differences in analytical methods and LODs but reported maternal 3-PBA concentrations were below 0.25 μg/L in all the studies. Cognitive deficits have also been associated with higher maternal exposure levels from residential proximity to agricultural pyrethroid use (Gunier et al., 2017; Xue et al., 2013) or high indoor use for malaria control (Eskenazi et al., 2018). Maternal median 3-PBA concentrations reported from these studies were between 0.70 μg/L (Eskenazi et al., 2018) and 2.24 μg/L (Xue et al., 2013).

The neurotoxic properties of organophosphates and pyrethroids are well-known, and several animal studies have demonstrated long-lasting alterations in brain function, including deficits in learning and memory, related to prenatal or early postnatal exposure to organophosphates and pyrethroids (Abreu-Villaca and Levin, 2017; Aldridge et al., 2005; Laugeray et al., 2017). Nonetheless, the present study failed to identify adverse associations between maternal exposure to these insecticides and early language development. Differences in instruments used to assess cognitive function as well as child age at examination might explain some of the inconsistency across the studies. Furthermore, variability and differences in maternal exposure levels, heterogeneity in exposure routes, exposure patterns and individual susceptibility are other likely explanations for the disparate findings. Dermal and inhalation exposures might lead to higher fetal exposure to the parent compounds because maternal hepatic first-pass metabolism is avoided. Interestingly, in the PELAGIE cohort, no association between maternal metabolite concentrations and impaired cognitive development was seen, despite the children's own urinary concentration of DE being associated with lower working memory scores (Cartier et al., 2016) and

Table 3
Crude and adjusted odds ratio (OR) and 95% confidence intervals (95% CI) for scoring below the age and sex standardized 15th percentile on the MB-CDI Vocabulary and Complexity subscales according to maternal urinary concentrations of dialkyl phosphate metabolites of organophosphate insecticides (categorical and continuous after ln_2-transformation) among children from the Odense Child Cohort.

	MB-CDI Vocabulary score below 15th percentile				MB-CDI Complexity score below 15th percentile			
	All (N = 365)	All (N = 360)	Boys (N = 187)	Girls (N = 173)	All (N = 293)	All (N = 290)	Boys (N = 140)	Girls (N = 150)
	Crude	Adjusted[a]	Adjusted[a]	Adjusted[a]	Crude	Adjusted[a]	Adjusted[a]	Adjusted[a]
Diethyl phosphates (DE)								
1the tertile (<13.6 nmol/L)	Reference				Reference			
2nd tertile (13.6–35.9 nmol/L)	1.17 (0.61; 2.23)	1.04 (0.51; 2.11)	1.39 (0.47; 4.09)	0.68 (0.24; 1.90)	0.94 (0.47; 1.88)	0.78 (0.36; 1.67)	1.39 (0.45; 4.26)	0.37 (0.12; 1.15)
3rd tertile (>35.9 nmol/L)	0.62 (0.30; 1.28)	0.52 (0.23; 1.20)	0.82 (0.24; 2.78)	0.21 (0.05; 0.86)	0.71 (0.34; 1.46)	0.57 (0.25; 1.33)	0.93 (0.26; 3.33)	0.33 (0.10; 1.10)
p-trend	0.21	0.12	0.56	0.03	0.35	0.19	0.93	0.07
Continuous[b]	0.89 (0.74; 1.07)	0.83 (0.67; 1.03)	0.87 (0.64; 1.18)	0.76 (0.55; 1.05)	0.99 (0.82; 1.20)	0.96 (0.76; 1.20)	1.14 (0.82; 1.59)	0.80 (0.57; 1.12)
Dimethyl phosphates (DM)								
1the tertile (<14.3 nmol/L)	Reference				Reference			
2nd tertile (14.3–41.1 nmol/L)	0.50 (0.25; 1.01)	0.46 (0.22; 0.96)	0.39 (0.14; 1.07)	0.73 (0.23; 2.30)	1.25 (0.59; 2.65)	1.21 (0.55; 2.68)	1.38 (0.46; 4.14)	1.26 (0.37; 4.27)
3rd tertile (>41.1 nmol/L)	0.75 (0.39; 1.44)	0.68 (0.34; 1.37)	0.52 (0.20; 1.35)	0.94 (0.31; 2.89)	1.53 (0.75; 3.15)	1.44 (0.67; 3.09)	1.21 (0.41; 3.62)	2.11 (0.67; 6.71)
p-trend	0.37	0.28	0.20	0.91	0.24	0.31	0.78	0.19
Continuous[b]	1.00 (0.84; 1.22)	1.00 (0.82; 1.22)	0.96 (0.74; 1.25)	1.02 (0.75; 1.40)	1.10 (0.91; 1.33)	1.09 (0.88; 1.34)	1.10 (0.83; 1.48)	1.08 (0.79; 1.47)
Dialkyl phosphates (DAP)								
1the tertile (<34.5 nmol/L)	Reference				Reference			
2nd tertile (34.5–89.0 nmol/L)	0.61 (0.30; 1.23)	0.57 (0.27; 1.21)	0.78 (0.27; 2.29)	0.36 (0.11; 1.13)	1.56 (0.74; 3.29)	1.57 (0.71; 3.48)	2.11 (0.65; 6.89)	1.24 (0.41; 3.78)
3rd tertile (>89.0 nmol/L)	0.90 (0.47; 1.72)	0.81 (0.39; 1.70)	0.98 (0.34; 2.86)	0.55 (0.18; 1.75)	1.45 (0.69; 3.04)	1.45 (0.63; 3.37)	1.67 (0.48; 5.76)	1.43 (0.42; 4.89)
p-trend	0.73	0.63	0.95	0.24	0.36	0.43	0.53	0.57
Continuous[b]	0.96 (0.78; 1,19)	0.93 (0.73; 1.19)	0.97 (0.70; 1.36)	0.84 (0.58; 1.23)	1.09 (0.88; 1.36)	1.07 (0.84; 1.40)	1.25 (0.86; 1.83)	0.96 (0.66; 1.39)

[a] Adjusted for creatinine (ln-transformed), maternal education, weeks of breastfeeding, and child sex (for all children).
[b] For doubling the concentration.

their 3-PBA concentration being negatively associated with verbal comprehension scores (Viel et al., 2015). Similar associations between child urinary concentrations of metabolites from pyrethroids and/or organophosphates and neurodevelopment have been reported in several other recent studies (Guo et al., 2019; Oulhote and Bouchard, 2013; Wagner-Schuman et al., 2015; Wang et al., 2016). These findings could indicate that the child's own dietary exposure to the parent compounds may be more hazardous for neurodevelopment than maternal exposure in pregnancy. Unfortunately, we did not have information on postnatal exposure at this young age, but it will be interesting to include the children's own metabolite concentrations when more complex cognitive functions can be assessed later in childhood.

In our study, language development was reported by parents, which can be seen as both a strength and a limitation, since it is independent of the current well-being of the child and test setting, but at the same time dependent on the subjective perception of the parents. Thus, some measurement error is likely, but since the women were unaware of their pesticide exposure status when responding to the questionnaire, this is expected to be non-differential and may contribute to our null findings. The major strengths of the present study are the longitudinal design, the high number of mother-child pairs enrolled, and the access to a large reference population of more than 3500 Danish children for language development (Bleses et al., 2008). The MB-CDI instrument applied at preschool age has shown good predictive validity in regard to language development (Bleses et al., 2008; Fenson et al., 2000). However, the young age of the children when assessing their language skills could limit the ability to detect subtle effects that might manifest later in childhood because of cascading developmental processes (Rice and Barone, 2000) and better opportunity for examination of more complex language skills and other cognitive functions. Among other limitations was that only 43% of the eligible pregnant women (N = 2874) were recruited and included in the OCC, and these women were better educated and more likely to be non-smokers, older and nulliparous than non-participants (Kyhl 2015). We cannot rule out the possibility that this enrollment bias might attenuate potential neurotoxic impacts of the insecticides due to more stimulating environments and healthier lifestyles. Furthermore, it was only possible to analyze urine samples and obtain information about child language development from around a third of the eligible women in the OCC. Participating families may differ from non-participating families leading to selection bias. They were probably more likely to have a healthier lifestyle (e.g., higher intake of organic food and thereby lower pesticide exposure) and exposure contrast may therefore be smaller reducing the possibility to find an association. In addition, families of children with poor language development may have been more or less likely to complete the MB-CDI questionnaire. However, participants were unaware of their urinary pesticide concentration when they responded to the questionnaire and it is therefore unlikely to have affected our findings. An additional concern is the fact that the insecticide metabolite concentrations were determined in a single spot urine sample. Pyrethroids and organophosphates are rapidly metabolized and excreted from the body within few days. When combining this with within-subject variability, misclassification is likely and may bias again the findings toward the null (Spaan et al., 2015). Under the assumption of stable dietary habits, the exposure variation in our study might be lower than in studies with additional exposure from agricultural or indoor use of insecticides. Furthermore, the urinary metabolites may not entirely reflect exposure of the parent compounds but also intake of metabolites preformed in food items. Thus, food samples have been found to contain TCPY and DAP residues (Morgan et al., 2011; Zhang et al., 2008), while the content of pyrethroid

degradates was reported to be very low (Morgan et al., 2018). Finally, we cannot exclude the possibility of residual and unmeasured confounding by socioeconomic, dietary, lifestyle or behavioural factors. Accordingly, the women with high education had highest urinary concentrations of TCPY, and the same tendency was seen for DAPs. Most likely organophosphate and pyrethroid exposure was primarily through intake of fruit, vegetables, and cereals (Lu et al., 2008; Morgan, 2012; Vanacker et al., 2020), which also contain essential vitamins and antioxidants with beneficial effects that may diminish potential harmful effects of insecticides as has been reported for other environmental exposures (Choi et al., 2014; Kim et al., 2011). Finally, we did not have information on parental IQ, although we did take into account maternal education. Given these considerations, the study findings suggest that no detectable pesticide-associated deficits in language development occurred below age 3 years, but extended follow-up will be needed to obtain more conclusive data.

5. Conclusion

In this relatively large prospective study, more than 90% of the pregnant women had measurable, though low, concentrations of organophosphate and pyrethroid metabolites in urine. We found no adverse associations between maternal insecticide exposure and early language development in their children at age 20–36 months among these rather privileged families with mainly dietary exposure to insecticides. The unexpected suggestion of a positive effect of pyrethroids and chlorpyrifos on language development could likely be explained by residual and unmeasured confounding from socioeconomic factors and dietary habits that may mask early adverse effects of insecticides. Follow-up of these children should include assessment of more complex language skills and cognitive functions in later childhood as well as associations with their own postnatal insecticide exposure.

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Declaration of competing interest

None.

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