Ambient ultrafine particles and asthma onset until age 20: The PIAMA birth cohort

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Supplemental Material

Methods and Materials

Study population and design

The ongoing PIAMA (Prevention and Incidence of Asthma and Mite Allergy) prospective birth cohort started with 3,963 newborns whose mothers were recruited from communities in the North, West, and Central regions of the Netherlands in 1996-1997 (Brunekreef et al. 2002; Wijga et al. 2014). Participants were followed up by repeated questionnaire surveys (parental-completed until age 8, from age 11 onwards also participant completed, and at age 20 only participant completed) including questions about demographic factors and risk factors for asthma and respiratory health. Questionnaires were completed at the child's ages of 3 months and 1 year, then annually until the age of 8 years, and at ages 11, 14, 17, and age 20 years. In the current study, participants with data on incident asthma and data on UFP exposure at the birth and/or current address for at least one of the 12 questionnaire surveys until age 20 were included (n=3,687).

The institutional review boards of the participating institutes approved the study protocol and written informed consent was obtained from the parents or legal guardians of all participants.

Air pollution exposure assessment

Annual average UFP exposures at the participants' birth addresses and current home addresses at the different follow-ups were estimated by our recently developed national UFP model which combines regional background measurements and monitoring from a mobile platform (Table S1) (Kerckhoffs et al. 2021). In brief, regional background UFP data were derived from 20 regional background sites across the Netherlands that were measured each three times for a period of 14 days. Annual average regional background concentrations were estimated using a kriging method (van de Beek et al. 2020). For mobile monitoring, an electric car (REVA, Mahindra Reva Electric Vehicles Pvt.Ltd., Bangalore, India) was used to measure UFP concentrations at 14,392 road segments over a 14-months period from June 2016 to November 2017 (van de Beek et al. 2020). Measurements of UFP started after 9:15 AM and stopped before 4:00 PM to avoid rush hour traffic and increase comparability between road segments (Kerckhoffs et al. 2016). All measurements were performed using a condensation

particle counter (TSI, CPC 3007) installed in the back of the electric car. Routes were sampled between 1 and 3 times (average 2.2 times) and UFP concentrations from repeated samples were averaged per road segment. Road segments were on average 110 m long (SD: 68m) and accumulated on average 43 s of UFP data (IQR: 9-44s) over the study period. Land use predictors such as local traffic intensity variables, population density, were presence of ports within five-kilometer buffers as well as the regional background concentration were selected by supervised stepwise linear regression into the final model to explain the spatial variation in UFP concentrations concentrations. A deconvolution method was applied to segregate the average UFP concentrations into a local and a background signal as this is thought to be more physically realistic. Model performance was evaluated using external validation with 3x24 h measurements at 42 sites in the cities of Amsterdam and Utrecht that have been described elsewhere (van Nunen et al. 2017) and was found to be good (R^2= 0.6).

Long-term exposure to NO_2, $PM_{2.5}$ absorbance ("soot"), and PM mass was assessed using land-use regression models developed within the European Study of Cohorts for Air Pollution Effects (ESCAPE) project as described more extensively in our previous publication (Gehring et al. 2020). In brief, three two-week air pollution monitoring campaigns were performed in the warm, cold, and intermediate seasons in a one-year period in 2008 -2010 (Cyrys et al. 2012; Eeftens et al. 2012b). NO_2, $PM_{2.5}$ absorbance ("soot", calculated from defined as the reflectance of $PM_{2.5}$ filters), $PM_{2.5}$, PM_{10} were measured. PM_{coarse} (PM with aerodynamic diameters between 10 and 2.5 µm) was calculated as the difference between PM_{10} and $PM_{2.5}$. Predictors on nearby traffic, population/household density, and land use derived from geographic information systems were used to explain spatial variation in annual average concentrations (Table S1) (Beelen et al. 2013; Eeftens et al. 2012a). The performance of the LUR models for PM mass, $PM_{2.5}$ absorbance and NO_2 was evaluated using internal leave-one-out cross-validation.

For the current analysis, annual average air pollution exposures at the participants' residential addresses throughout the follow-up were estimated using the (purely spatial) LUR models without adjustment for temporal trends. We defined early life exposure as the annual average exposure at the birth address, and more recent exposure for each of the follow-ups as the

annual average exposure at the home address at the time of that specific follow-up.

Asthma ascertainment

Information on the participants' respiratory health was collected by repeated questionnaires from birth until age 20 years. We use the same definition of asthma as in our previous analysis, that is a positive answer to at least two of the three following questions: 1) "Has a doctor ever diagnosed asthma in your child (age 20 years, has a doctor ever told you that you have asthma?" 2) "Has your child (age 20 years: Have you) had wheezing or whistling in the chest in the last 12 months?" 3) "Has your child (age 20 years: Have you) been prescribed asthma medication during the last 12 months?". This definition has been developed by a panel of experts within the Mechanisms of the Development of Allergy (MeDALL) consortium (Pinart et al. 2014). Incident asthma was defined as positive the first time a participant fulfilled the criteria for asthma and the participant had non-missing data for all previous follow-ups. Incident asthma was defined negative if a participant did not fulfill the criteria in the respective year and all previous years. Data for participants with missing information on asthma for one or more follow-ups were right censored and incident asthma was defined missing from the first follow-up with missing data onwards.

Covariate assessment

Covariates were selected *a priori* based on literature and to be consistent and facilitate comparisons with previous analyses of the association between air pollution and asthma incidence within the same population (Gehring et al. 2020). Sex, maternal and paternal asthma and/or hay fever (yes/no), Dutch nationality (yes/no), parental education (maximum education attained by the mother or father, low/medium/high), breastfeeding at 12 weeks (yes/no), older siblings (yes/no) and maternal smoking during pregnancy (yes/no) were obtained from questionnaires completed during pregnancy or the child's first year of life; daycare attendance (yes/no) was obtained from the 2-year questionnaire. Smoking in the participants' home (yes/no), mold/damp spots in the living room and/or participants bedroom (yes/no), and gas cooking (yes/no) were obtained from the parental questionnaires from birth

until age 17 years and questionnaires completed by the participants themselves at age 20 years. Information on active smoking of the participants (at least once a week, yes/no) was obtained from the questionnaires completed by the participants from the age of 14 years onwards.

Statistical analysis

Categorical variables were presented as numbers (proportions), continuous variables were presented as mean ± standard deviation. Correlations between different air pollutants and correlations between UFP exposures at different follow-up periods were presented as Spearman correlation coefficients (Figure S1 and S2). Associations of UFP exposure with asthma incidence from birth until age 20 years were analyzed using discrete-time hazard models (Singer et al. 2003) dividing the follow-up from birth until age 20 into 12 discrete periods (that is eight periods of one year and four periods of three years) in between questionnaires and modelling the conditional probability of developing asthma in each discrete time period, given that a participant did not have asthma in any earlier time period in relation to air pollution exposure. Separate analyses were performed with early-life exposure for all time periods and more recent exposure at a specific follow-up for the respective period. To be consistent with our previous analysis (Gehring et al. 2020), models were adjusted for the same set of confounders, including all covariates described above and age.

To explore the shape of the concentration-response curve, we applied natural splines with three degrees of freedom in the adjusted model and tested for linearity by comparing the models with and without splines using the likelihood ratio test and the results showed no deviation from linearity (Figure S3). Two-pollutant models (of UFP with PM mass, $PM_{2.5}$ absorbance, or NO_2) were also performed. Sex-specific analysis was done to test if there is any effect modification (Table S3) . In sensitivity analysis, we restricted the analysis to those participants with nearly complete follow-up (at least 11 out of the 12 questionnaires) to assess potential attrition bias (Table S4). Moreover, we restricted the analysis to data from age 4 onward to assess whether the associations were mainly driven by the high incidence before the age of 4 years and performed stratified analyses by moving (defined as any change in

address since birth) (Table S5). We did not include early life and more recent UFP exposures into one model because that led to multicollinearity problems (variation inflation factors (VIFs) >3). Associations were presented as odds ratios (OR) with 95% confidence intervals (CI) for an interquartile range (IQR) increase in exposure. All analyses were performed with R version 3.6.1 (Team 2019).

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Table S1. Land use regression model and performances for UFP, PM, "soot" ($PM_{2.5}$ absorbance) and NO_2.

Air pollutant	LUR model	R^2 *	Source
UFP	7843+2.289e-02*HHOLD_5000+6.561e-01*HTRAFNEAR+5.417e-04*PORT_5000+1.212e-03*TLOA_50+1.146e-03*TMLOA_50+1.557e-01*TRAFNEAR	0.60	Kerckhoffs et al, 2021
PM_{10}	23.71 + 2.16E-8*TRAFMAJORLOAD_500 + 6.68E-6*POP_5000 + 0.02*MAJORROADLENGTH_50	0.60	Eeftens et al, 2012
$PM_{2.5}$	9.46 + 0.42×REGIONALESTIMATE + 0.01×MAJORROADLENGTH_50 + 2.28×10−9×TRAFMAJORLOAD_1000	0.61	Eeftens et al, 2012
$PM_{2.5}$ abs.	0.07 + 2.95×10−9×TRAFLOAD_500 + 2.93×10−3×MAJORROADLENGTH_50 + 0.85×REGIONALESTIMATE + 7.90×10−9×HLDRES_5000 + 1.72×10−6×HEAVYTRAFLOAD_50	0.89	Eeftens et al, 2012
PM_{coarse}	7.59 + 5.02×10−9×TRAFLOAD_1000 + 1.38×10−7*PORT_5000 + 5.38×10−5×TRAFNEAR	0.38	Eeftens et al, 2012
NO_2	−7.80 + 1.18*REGIONALESTIMATE + 2.30E-5*POP_5000 + 2.46E-6*TRAFLOAD_50 + 1.06E-4*ROADLENGTH_1000 + 9.84E-5*HEAVYTRAFLOAD_25 + 12.19*DISTINVNEARC1 + 4.47E-7*HEAVYTRAFLOAD_25_500	0.81	Beelen et al, 2013

UFP, ultrafine particles. port (PORT_X); natural land (NATURAL_X);the sum of high and low density residential land (HDLDRES_X);the sum of (traffic intensity *length of all road segments) within a buffer (vehicles day^{-1}m) for all roads(TRAFLOAD_X),for all major road segments (TRAFMAJORLOAD_X), for heavy traffic(HEAVYTRAFLOAD_X) and heavy traffic on major roads(HEAVYTRAFMAJORLOAD_X); population data on an European level (N) (POPEEA_X); total length (m) of all roads(ROADLENGTH_X) and all major road segments (MAJORROADLENGTH_X);traffic intensity on the nearest road (TRAFNEAR); X-coordinate (XCOORD); Y-coordinate (YCOORD); the product of inverse distance to the nearest major road and the traffic intensity on this major road (INTMAJORINVDIST); inverse distance (m-1) to the nearest major road in the local network (DISTINVMAJOR1);

* Leave-one-out cross validation R^2 for PM mass, $PM_{2.5}$ absorbance and NO_2, external validation R^2 for UFP.

Table S2. Association estimates for UFP and other air pollutants in single- and two-pollutant models.

		OR (95%CI)	
		Birth address	**Current address**
Single pollutant models			
	UFP	1.08 (1.02, 1.14)	1.06 (1.00, 1.12)
	$PM_{2.5}$	1.15 (1.02, 1.30)	1.19 (1.04, 1.36)
	PM_{10}	1.09 (1.01, 1.18)	1.07 (0.99, 1.15)
	PM_{coarse}	1.12 (1.04, 1.20)	1.11 (1.02, 1.20)
	$PM_{2.5}$ absorbance	1.12 (1.03, 1.22)	1.12 (1.03, 1.23)
	NO_2	1.20 (1.10, 1.32)	1.15 (1.04, 1.27)
Two pollutant model			
UFP+$PM_{2.5}$	UFP	1.06 (0.99, 1.13)	1.06 (0.97, 1.13)
	$PM_{2.5}$	1.06 (0.91, 1.23)	1.14 (0.96, 1.32)
UFP+PM_{10}	UFP	1.07 (0.97, 1.17)	1.05 (0.95, 1.15)
	PM_{10}	1.01 (0.89, 1.15)	1.02 (0.88, 1.16)
UFP+PM_{coarse}	UFP	1.00 (0.90, 1.11)	0.95 (0.85, 1.06)
	PM_{coarse}	1.12 (0.97, 1.29)	1.17 (1.01, 1.35)
UFP+$PM_{2.5}$ absorbance	UFP	1.03 (0.94, 1.14)	0.97 (0.88, 1.07)
	$PM_{2.5}$ absorbance	1.08 (0.93, 1.26)	1.17 (1.01, 1.36)
UFP+NO_2	UFP	0.95 (0.86, 1.05)	0.96 (0.87, 1.06)
	NO_2	1.28 (1.09, 1.51)	1.21 (1.03, 1.44)

This table provides the numeric results for Figure 1 in the main text.

All associations were adjusted for sex, maternal and paternal asthma and/or hay fever, Dutch nationality, parental education, breastfeeding, older siblings, daycare attendance, maternal smoking during pregnancy, parental smoking at home, active smoking (from age 14 years), mold/dampness at home, use of gas for cooking.

Table S3. Sex-specific adjusted* associations of UFP exposure (per interquartile range increase) early in life (i.e. at the birth address) or more recent exposure (i.e. at the current address of the follow-up) with asthma incidence.

Age range	UFP exposure	OR (95%CI)		P for interaction
		Male	Female	
Entire age range (0--20 years)	Birth address	1.06 (0.98, 1.14)	1.10 (1.01, 1.18)	0.765
	Current address	1.03 (0.95, 1.12)	1.06 (0.97, 1.15)	0.871
Up to age 12	Birth address	1.06 (0.97, 1.14)	1.10 (1.01, 1.19)	0.832
	Current address	1.03 (0.95, 1.12)	1.08 (0.99, 1.19)	0.567

Adjusted for maternal and paternal asthma and/or hay fever, Dutch nationality, parental education, breastfeeding, older siblings, daycare attendance, maternal smoking during pregnancy, parental smoking at home, active smoking (from age 14 years), mold/dampness at home, use of gas for cooking.

Table S4. Sensitivity analyses of associations of UFP exposure (per interquartile range increase) early in life (i.e. at the birth address) or more recent exposure (i.e. at the current address of the follow-up) with asthma incidence until age 20 years.

	Total/Cases	OR (95%CI)*	P-value
		Birth address	
Main analyses	3159/545	1.08 (1.02, 1.14)	0.008
Asthma onset from age 4 to age 20	2534/274	1.10 (1.01, 1.19)	0.020
Subjects with at least 11 of 12 follow-ups	1684/371	1.06 (0.99, 1.12)	0.076
		Current address	
Main analyses	3168/547	1.06 (1.00, 1.12)	0.052
Asthma onset from age 4 to age 20	2549/271	1.03 (0.93, 1.14)	0.507
Subjects with at least 11 of 12 follow-ups	1684/371	1.03 (0.96, 1.11)	0.393

Adjusted for sex, maternal and paternal asthma and/or hay fever, Dutch nationality, parental education, breastfeeding, older siblings, daycare attendance, maternal smoking during pregnancy, parental smoking at home, active smoking (from age 14 years), mold/dampness at home, use of gas for cooking.

Table S5. Stratified analysis of associations of UFP exposure (per interquartile range increase) early in life (i.e. at the birth address) or more recent exposure (i.e. at the current address of the follow-up) with asthma incidence until age 20 years by movers or non-movers.

UFP exposure	OR (95%CI)		P for interaction
	Movers	Non-movers	
Birth address	1.06 (1.00, 1.13)	1.13 (0.97, 1.29)	0.353
Current address	1.04 (0.97, 1.11)	1.11 (0.97, 1.26)	0.283

Adjusted for sex, maternal and paternal asthma and/or hay fever, Dutch nationality, parental education, breastfeeding, older siblings, daycare attendance, maternal smoking during pregnancy, parental smoking at home, active smoking (from age 14 years), mold/dampness at home, use of gas for cooking.

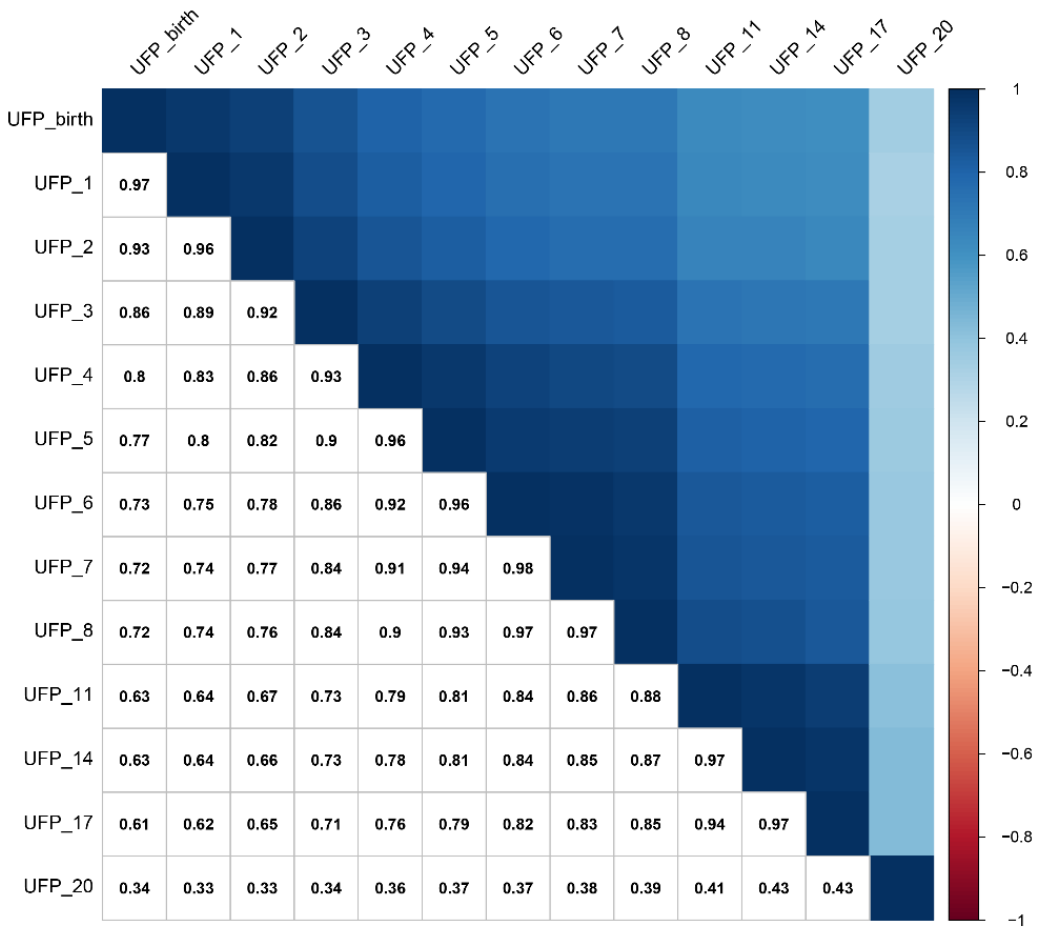

Figure S1. Correlation matrix (Pearson R) of UFP concentrations at the birth address and home addresses at the different follow-ups.

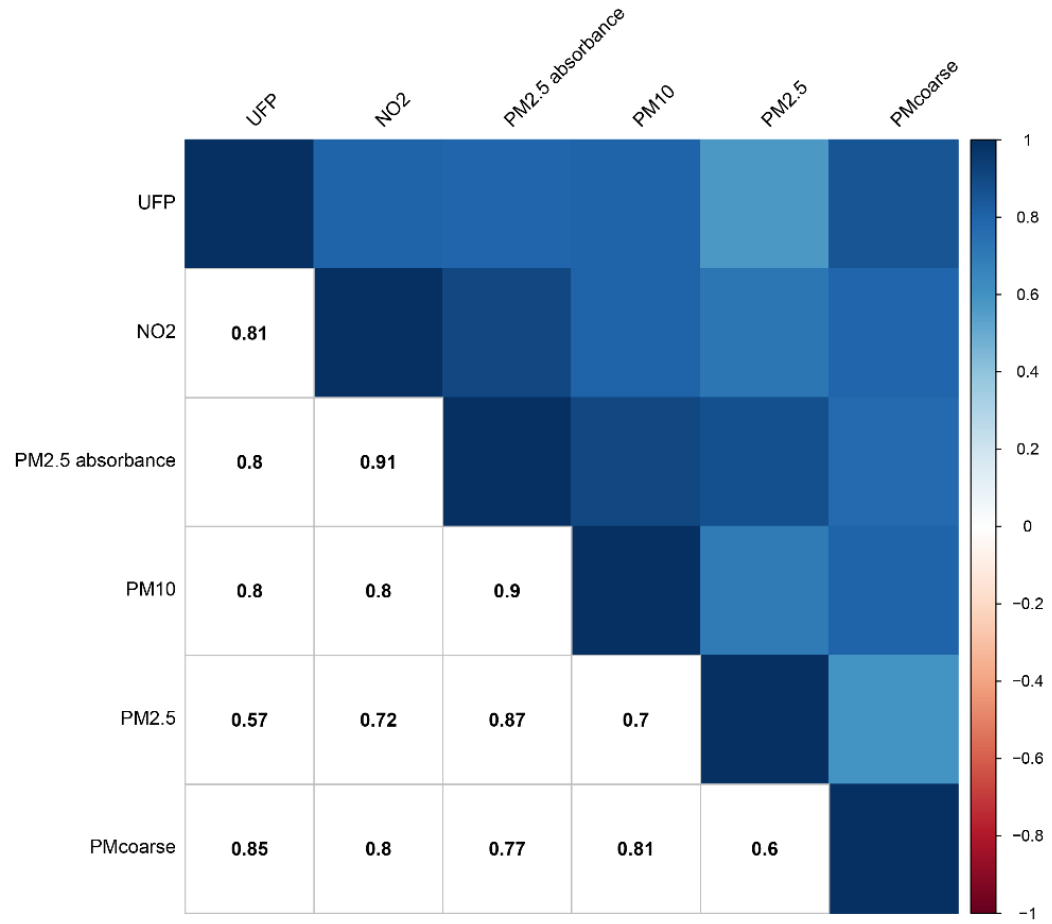

Figure S2. Correlation matrix (Pearson R) of concentrations of ultrafine particles, NO_2, soot and particulate matter mass at the birth address.

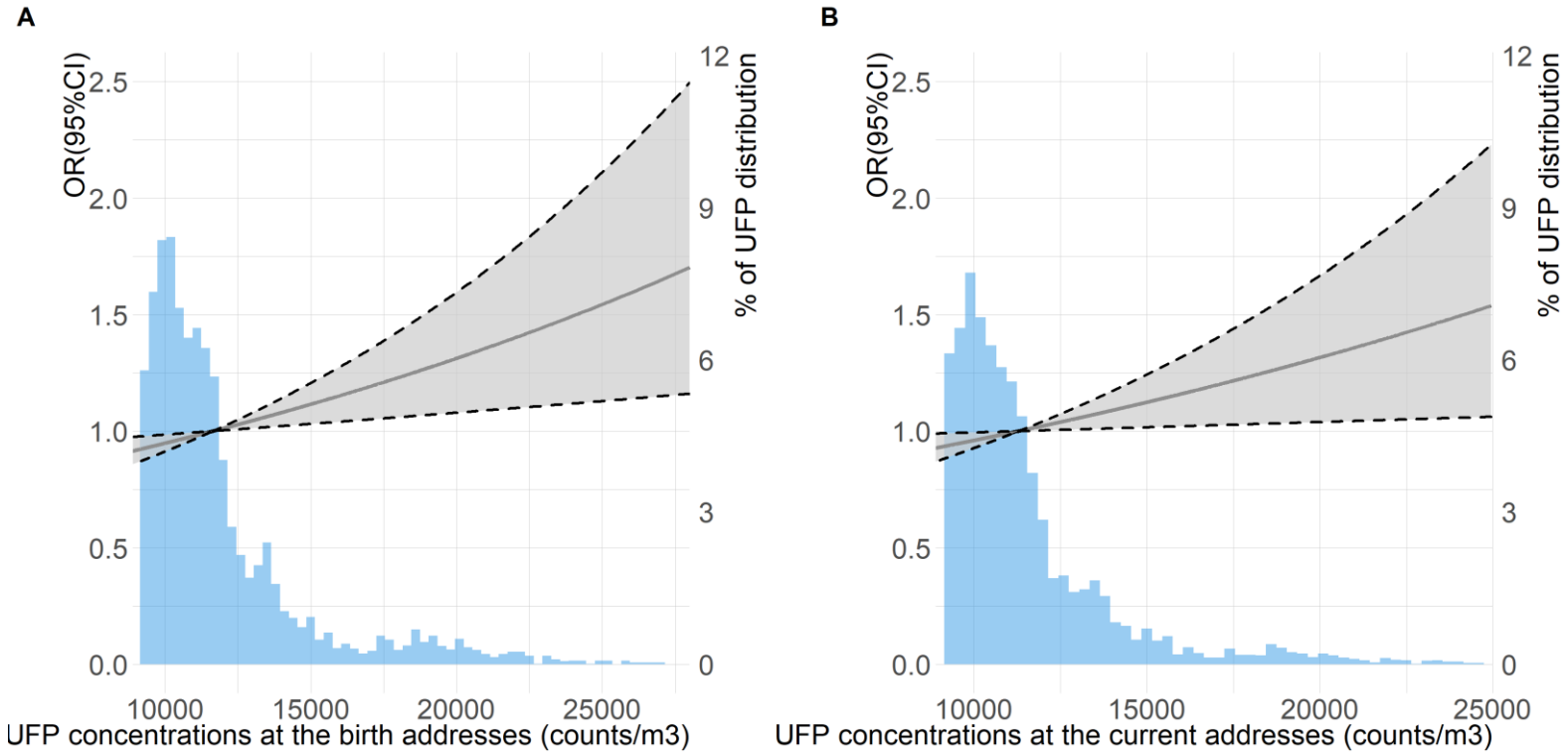

Figure S3. Exposure-response curves using natural splines with 3 degrees of freedom for the association of UFP exposure early in life (i.e. at the birth address) or more recently exposure (i.e. at the current address of the follow-up) with asthma incidence until age 20 years. Adjusted for sex, maternal and paternal asthma and/or hay fever, Dutch nationality, parental education, breastfeeding, older siblings, daycare attendance, maternal smoking during pregnancy, parental smoking at home, active smoking (from age 14 years), mold/dampness at home, use of gas for cooking.

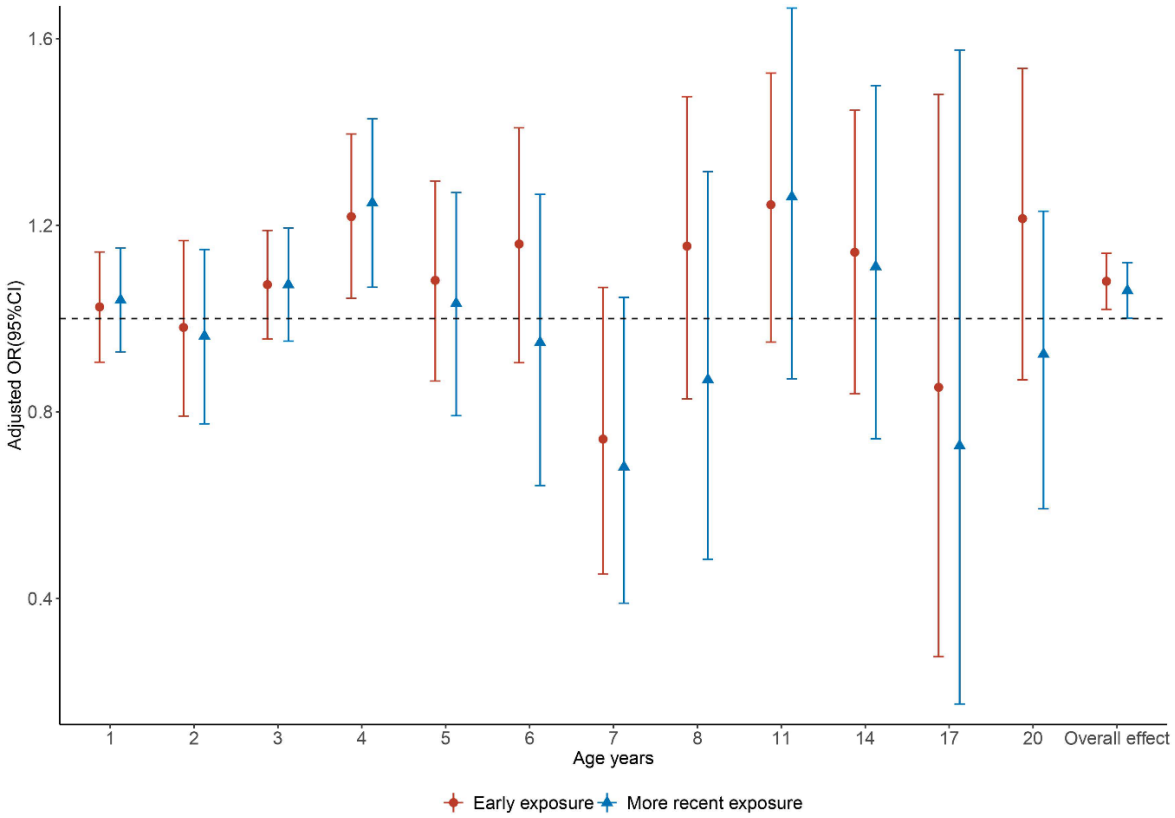

Figure S4. Adjusted* age-specific associations of UFP exposure (per interquartile range increase) early in life (i.e. at the birth address) or more recent exposure (i.e. at the current address of the follow-up) with asthma incidence until age 20 years. Adjusted for sex, maternal and paternal asthma and/or hay fever, Dutch nationality, parental education, breastfeeding, older siblings, daycare attendance, maternal smoking during pregnancy, parental smoking at home, active smoking (from age 14 years), mold/dampness at home, use of gas for cooking.

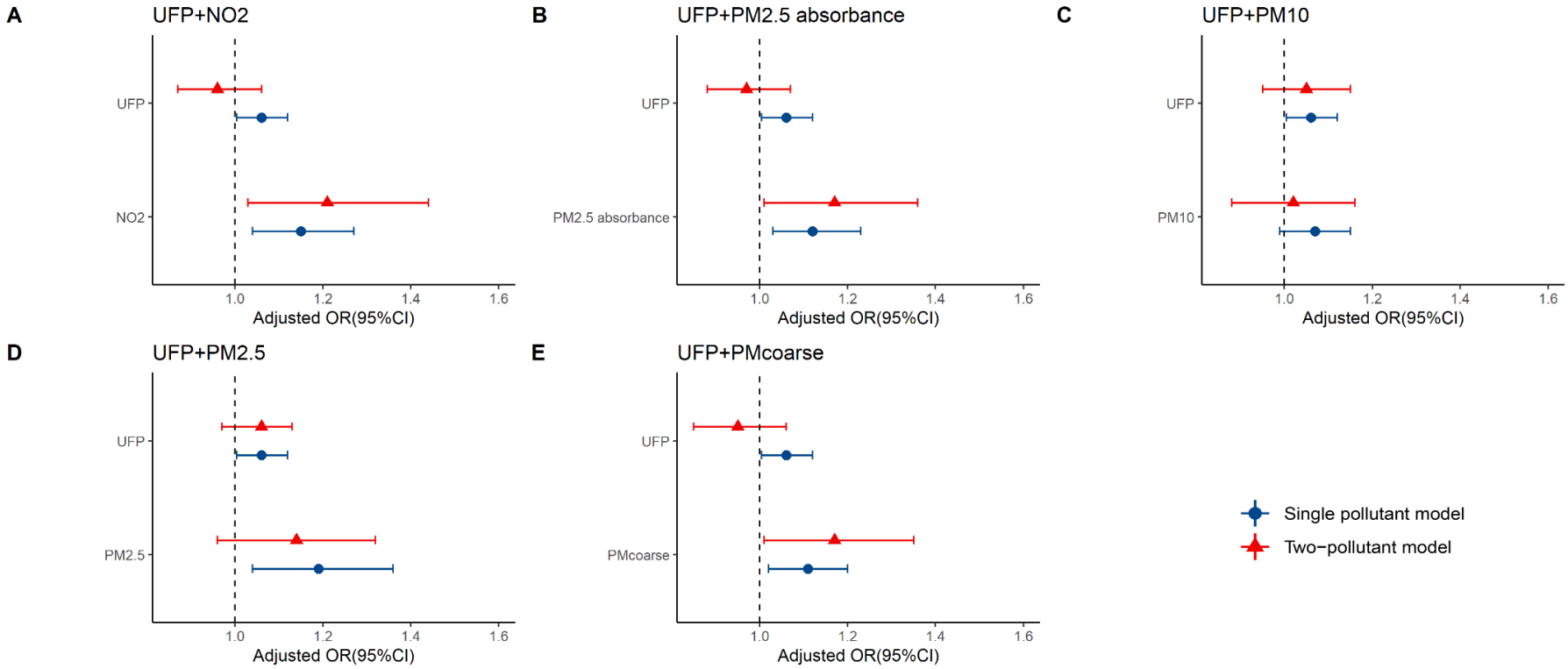

Figure S5. Adjusted associations of ultrafine particles exposure (per interquartile range increase) in more recent exposure (i.e. current address at the time of the follow-up) with asthma incidence until age 20 years in single pollutant and two-pollutant models. Panel A: UFP and nitrogen dioxide; panel B: UFP and particulate matter with a diameter <2.5 µm ($PM_{2.5}$) absorbance; panel C: UFP and particulate matter with a diameter <10 µm; panel D: UFP and $PM_{2.5}$; panel E: UFP and particulate matter with a diameter 2.5-10 µm. Adjusted for sex, age, maternal and paternal asthma and/or hay fever, Dutch nationality, parental education, breastfeeding, older siblings, daycare attendance, maternal smoking during pregnancy, parental smoking at home, active smoking (from age 14 years), mould/dampness at home, use of gas for cooking. Variance inflation factors ranging from 3.21 to 3.82. All other variance inflation factors were below 3.