

## ORIGINAL ARTICLE

Children's respiratory health and oxidative potential of PM<sub>2.5</sub>: the PIAMA birth cohort studyAileen Yang,<sup>1,2</sup> Nicole A H Janssen,<sup>1</sup> Bert Brunekreef,<sup>2,3</sup> Flemming R Cassee,<sup>1,2</sup> Gerard Hoek,<sup>2</sup> Ulrike Gehring<sup>2</sup>

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**ABSTRACT**

**Introduction** The oxidative potential (OP) of particulate matter (PM) has been proposed as a health-relevant metric, but currently few epidemiological studies investigated associations of OP with health. Our main aim was to assess associations of long-term exposure to OP with respiratory health in children. Our second aim was to evaluate whether OP is more consistently associated with respiratory health than PM mass, PM composition or nitrogen dioxide (NO<sub>2</sub>).

**Methods** For 3701 participants of a prospective birth cohort, annual average concentrations of OP (assessed by spin resonance (OP<sup>ESR</sup>) and dithiothreitol assay (OP<sup>DTT</sup>)), PM with an aerodynamic diameter of less than 2.5 µm (PM<sub>2.5</sub>) mass, NO<sub>2</sub>, and PM<sub>2.5</sub> constituents at the home addresses at birth and at all follow-up addresses were estimated by land-use regression. Repeated questionnaire reports of asthma and hay fever until age 14 years, and measurements of allergic sensitisation, lung function and fractional exhaled nitric oxide at age 12 years were linked with air pollution concentrations.

**Results** Asthma incidence, prevalence of asthma symptoms and rhinitis were positively associated with OP<sup>DTT</sup> (adjusted OR (95% CI) per IQR increase in exposure 1.10 (1.01 to 1.20), 1.08 (1.02 to 1.16), 1.15 (1.05 to 1.26), respectively). These associations persisted after adjustment for most co-pollutants. Forced expiratory volume in 1s and forced vital capacity were negatively associated with OP<sup>DTT</sup>. These associations were sensitive to adjustment for NO<sub>2</sub>. Respiratory health was not significantly associated with PM<sub>2.5</sub> mass and OP<sup>ESR</sup>.

**Conclusions** Respiratory health was more strongly associated with OP<sup>DTT</sup> than with PM<sub>2.5</sub> mass; OP<sup>DTT</sup> associations with lung function, but not symptoms, were sensitive to adjustment for NO<sub>2</sub>.

**INTRODUCTION**

Numerous epidemiological studies have established associations between exposure to ambient air pollution and respiratory health.<sup>1–2</sup> Various exposure metrics have been used to investigate the effect of air pollution on human health, such as particulate matter (PM) with an aerodynamic diameter of less than 2.5 µm (PM<sub>2.5</sub>) and 10 µm (PM<sub>10</sub>); traffic markers (elemental carbon (EC); nitrogen dioxide (NO<sub>2</sub>)); and less frequently, different PM constituents (iron, copper, nickel, zinc, vanadium, sulfur, potassium, silicon).<sup>3–4</sup> However, PM toxicity is likely to be reflected by the sum of multiple toxic components. Since toxicological studies have

**What this paper adds**

- Oxidative potential (OP), which measures the inherent capacity of particulate matter (PM) to oxidise target molecules, has been proposed as a biologically more relevant exposure metric than PM with an aerodynamic diameter of less than 2.5 µm (PM<sub>2.5</sub>).
- This is the first study on the associations between long-term exposure to two measures of OP (assessed by dithiothreitol assay (OP<sup>DTT</sup>) and spin resonance (OP<sup>ESR</sup>)) of PM<sub>2.5</sub> and children's respiratory health.
- Exposure to OP<sup>DTT</sup>, but not OP<sup>ESR</sup> and PM<sub>2.5</sub> mass, was associated with increased risks of asthma and rhinitis until age 14 years, and decreased lung function at age 12 years.
- Associations of OP<sup>DTT</sup> with asthma symptoms and rhinitis were largely independent of exposure to other air pollutants, including NO<sub>2</sub>, PM<sub>2.5</sub> mass and PM<sub>2.5</sub> constituents.
- Our study suggests that OP<sup>DTT</sup> could be a health-relevant metric.

documented the ability of inhaled PM to cause oxidative stress and inflammation in the airways,<sup>5–8</sup> oxidative potential (OP), which measures the inherent capacity of PM to oxidise target molecules, has been proposed as a biologically more relevant exposure metric.<sup>9</sup>

Currently, there is limited epidemiological evidence on the relationship between OP and health; consequently, it is not clear whether OP has more consistent associations with health than other PM characteristics.<sup>10</sup> Recently, a few studies have assessed the acute health effects of OP, especially on respiratory health end points. The findings, so far, did not consistently indicate that OP is a better predictor of acute health effects than other air pollution metrics.<sup>11–16</sup> High correlation between temporal patterns of OP and other pollutants might have contributed to the inconsistency of the two-pollutant findings. To the best of our knowledge, only one publication on the long-term health effects of OP is available. Tonne *et al*<sup>17</sup> compared the associations of carotid intima-media thickness with annual average PM<sub>10</sub> concentrations and PM<sub>10</sub> concentrations weighted by OP, and found that the inclusion of OP did not strengthen the associations.



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The prospective Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study provided information on the relationship between exposure to air pollution (NO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance, PM<sub>2.5</sub> elemental composition) and the development of asthmatic symptoms and allergic diseases up to the age of 12 years.<sup>18</sup> Another questionnaire survey was completed when the participants were 14 years old. The present study examines the associations of OP of PM<sub>2.5</sub> with asthma incidence and symptoms, hay fever, rhinitis, allergic sensitisation, lung function, and airway inflammation represented by fractional exhaled nitric oxide (FE<sub>NO</sub>)<sup>19</sup> in the PIAMA study.

The first aim of this study was to assess whether OP is associated with the aforementioned health outcomes. The second aim was to assess whether the associations of OP with health were more robust than associations with other air pollutant metrics, especially PM<sub>2.5</sub> mass concentration. These findings will help to elucidate the value of OP as an exposure metric in long-term health effects studies.

## MATERIALS AND METHODS

### Study population

The PIAMA prospective birth cohort study recruited pregnant women during their second trimester in 1996–1997 from various communities in the Netherlands.<sup>20</sup> Non-allergic pregnant women were invited to participate in a 'natural history' study arm; allergic women, identified through a screening questionnaire, were primarily allocated to an intervention arm (involving the use of mite-impermeable mattress and pillow covers) with a random subset allocated to the natural history arm. The study population for the present study consists of all participants of the intervention and natural history studies with data on at least one of the health outcomes studied, and with data on air pollution exposure and potential confounders available to be included in at least one of the adjusted analyses (N=3701). The study protocol was approved by the institutional review boards of participating institutes, and written informed consent was obtained from the parents and legal guardians of all participants.

### Long-term exposure assessment

We estimated annual average outdoor air concentrations of OP, PM<sub>2.5</sub> mass, PM<sub>2.5</sub> absorbance, PM<sub>2.5</sub> elemental composition (iron (Fe), copper (Cu), potassium (K), nickel (Ni), sulfur (S), silicon (Si), vanadium (V) and zinc (Zn)), and NO<sub>2</sub> at the participants home addresses at birth and at the different follow-ups (medical examination at age 12 years and questionnaire follow-ups until age 14 years) by means of land-use regression (LUR) models that have been described previously.<sup>4 21–23</sup> LUR models for OP, PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance and NO<sub>2</sub> are presented in online supplementary table S1. In short, three 2-week measurements of PM<sub>2.5</sub> were performed in the warm, cold and intermediate seasons between February 2009 and February 2010 at 40 sites spread over the Netherlands and Belgium.<sup>24 25</sup> All PM<sub>2.5</sub> filters were analysed for OP and elemental composition.<sup>22 23</sup> NO<sub>2</sub> was measured at 80 sites, including the 40 PM sites.

OP of PM<sub>2.5</sub> was measured with the dithiothreitol (DTT) and spin resonance (ESR) assays.<sup>23</sup> The DTT assay measures the ability of PM<sub>2.5</sub> to transfer electrons from DTT to oxygen and is sensitive towards the organic compounds in the PM mixture.<sup>26</sup> OP<sup>DTT</sup> is reported as the consumption rate of DTT (expressed as nmol DTT/min divided by sampled volume), which is proportional to the concentration of redox reactive compounds (eg, organic compounds) in the sample. The ESR

method is based on the trapping of PM-induced hydroxyl radicals (OH•) mainly generated via Fenton-type reactions in the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin trap.<sup>27 28</sup> OP<sup>ESR</sup> is sensitive towards transition metals in the PM mixture. OP<sup>ESR</sup> is reported as the average of the total amplitudes of the DMPO-OH quartet in arbitrary units (AU) divided by sampled volume.

The temporally adjusted average of the three measurements for each site was used for LUR model development. Predictor variables, such as land use, traffic intensity, roads and population density, used for LUR model development were derived from the geographical information system. The estimated regional background concentration was offered as an additional predictor. For each site, the background concentrations were calculated by inverse distance squared weighted interpolation of concentrations measured at all regional sites.

### Health outcomes

Health outcome information was collected annually from birth until age 8 years, and then at ages 11–12 and 14 years using questionnaires. From the questionnaires, we selected the same health end points as in the previous analyses,<sup>18</sup> namely incident doctor-diagnosed asthma, prevalence of asthma symptoms in the past 12 months, hay fever ever and hay fever symptoms (rhinitis) in the past 12 months.

In addition, at age 12 years, a medical examination was performed on participants with additional consent. IgE levels to common inhalant allergens, including house dust mite (*Dermatophagoides pteronyssinus*), cat, cockroach and birch pollen were measured in serum by a radioallergosorbent test-like method used at the Sanquin Laboratories (Amsterdam, the Netherlands). Sensitisation was defined as a positive reaction (specific IgE level ≥0.70 IU/mL) to one of the allergens tested. Spirometry was done following the guidelines of American Thoracic Society/European Respiratory Society.<sup>29</sup> We measured forced expiratory volume in 1 s (FEV<sub>1</sub>), forced vital capacity (FVC), mid-respiratory flows (FEF<sub>25–75</sub>) and FE<sub>NO</sub> (a biomarker of airway inflammation).

### Statistical analysis

#### Longitudinal analysis

Associations of air pollution with asthma incidence were analysed using discrete-time hazard models<sup>30</sup>; associations with asthma symptoms, hay fever and rhinitis were analysed by generalised estimation equations with a logit-link using a seven-dependent correlation matrix.<sup>31</sup> Analyses were done with exposures at the birth address and exposures at the address at the time of the follow-up. We included the same confounders as in the previous analyses: sex, maternal education, parental allergies, breast feeding, maternal smoking during pregnancy, smoking in the child's home, use of gas for cooking, mould/dampness in the child's home, pets at home, daycare attendance during first year of life and neighbourhood percentage of low-income households.<sup>18</sup>

#### Cross-sectional analysis

Owing to high prevalence of allergic sensitisation at age 12 years, we used log-binomial regression to analyse the associations of allergic sensitisation with air pollution levels. For the associations between air pollution and continuous outcome variables (FEV<sub>1</sub>, FVC, FEF<sub>25–75</sub>, FE<sub>NO</sub>), we used linear regression with natural log (ln) transformation of the outcome variable.<sup>18 32</sup> Associations with sensitisation, lung function and FE<sub>NO</sub> were calculated for exposures at the birth address and at the address

## Environment

at age 12 years, when the medical examination took place. Associations with lung function were calculated with adjustment for sex and log-transformed age, height and weight (crude model), and with additional adjustment for respiratory infections in the past 3 weeks and the same set of confounders as in the longitudinal symptom analyses. In addition, we adjusted for short-term average ambient air pollution levels during the 7 days preceding the lung function test by using daily data from the National Air Quality Monitoring Network (<http://www.rivm.nl/milieukwaliteit/lucht/>). Daily data were available for NO<sub>2</sub> and PM<sub>10</sub>, but not for PM<sub>2.5</sub>. Eventually, short-term exposure to NO<sub>2</sub> was included in all models because short-term NO<sub>2</sub> and not short-term PM<sub>10</sub> was a strong confounder of the associations between FE<sub>NO</sub> and long-term air pollution exposure; for FEV<sub>1</sub>, FVC and FEF<sub>25-75</sub>, associations with long-term air pollution exposure were generally the same in models with short-term NO<sub>2</sub> and short-term PM<sub>10</sub>.

Air pollution variables were entered as continuous variables without transformation in all models for effect estimates with pollutants. Effect estimates are presented as relative risk for allergic sensitisation; OR for all other binary outcomes; and percentage change in lung function and FE<sub>NO</sub>, all with 95% CIs, for an IQR increase in exposure at birth address to facilitate comparison. Statistical significance was defined as  $p < 0.05$  and borderline significance as  $p < 0.1$ . We report the results of single-pollutant models, and two-pollutant models for health end points that were significantly associated with OP. Two-pollutant models of OP with other air pollutants were specified to evaluate whether OP is associated with health after adjusting for potentially correlated pollutants.<sup>13</sup>

Analyses were performed with Statistical Analysis System (SAS V9.4) for Windows.

## RESULTS

### Study population characteristics

The distributions of the general characteristics and health outcomes from medical examination for the current study population and the entire cohort are shown in online supplementary table S2. Difference in characteristics between participants and non-participants were generally small. The frequency distribution of questionnaire-based health outcomes shows a decline in asthma incidence and symptom prevalence with age, while hay fever and rhinitis prevalence increased with age.

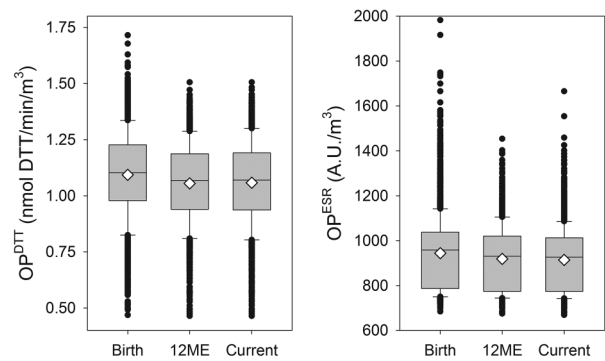
### Distributions and correlations of air pollution exposure

Figure 1 shows the distributions of annual averages of OP<sup>DTT</sup> and OP<sup>ESR</sup> at the participants' addresses at birth, at age 12 years (medical examination) and at age 14 years (questionnaire). Distributions were generally similar at the different time points. The distributions of the annual averages of the other pollutants are shown in online supplementary table S3.

The correlation between the two OP metrics was low ( $R = 0.33$  for birth address, see online supplementary table S4). At the birth address, OP<sup>DTT</sup> correlated highest with NO<sub>2</sub> ( $R = 0.74$ ) and lowest with Zn ( $R = -0.03$ ) and K ( $R = 0.13$ ). OP<sup>ESR</sup> correlated highest with PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance, Cu, Fe, S and Si (all  $R > 0.70$ ). The correlation with PM<sub>2.5</sub> mass concentration was higher for OP<sup>ESR</sup> ( $R = 0.81$ ) than for OP<sup>DTT</sup> ( $R = 0.41$ ). These correlations were similar for exposures at the current address at age 14 years.

### Single-pollutant models

We report the associations of questionnaire-reported symptoms with OP<sup>ESR</sup>, OP<sup>DTT</sup>, NO<sub>2</sub>, PM<sub>2.5</sub> and PM<sub>2.5</sub> absorbance at the



**Figure 1** Distribution of annual averages of oxidative potential assessed by dithiothreitol assay (OP<sup>DTT</sup>) and oxidative potential assessed by spin resonance (OP<sup>ESR</sup>) at the participants' birth address, current address at the time of the medical examination (12ME) and current address at age 14 years. Median (horizontal line in the box), mean (diamond symbol), 25th and 75th centiles (box) are shown, whiskers indicate P10 and P90, and individual outliers are shown as points.

birth address and at the current address from single-pollutant models in table 1. Generally, differences between crude and adjusted associations of annual air pollution level with asthma, hay fever and rhinitis were small; all changes in OR were less than 6%, except for PM<sub>2.5</sub> and hay fever where the change in OR was 11% (data not shown). We found statistically significant positive associations of asthma incidence, prevalence of asthma symptoms and rhinitis with OP<sup>DTT</sup> at the birth address, after adjusting for potential confounders. OP<sup>ESR</sup> was not significantly associated with any health outcome. We also found significant associations of asthma incidence, prevalence of asthma symptoms and rhinitis with NO<sub>2</sub> at the birth address. Effect sizes were similar for OP<sup>DTT</sup> and NO<sub>2</sub>. The adjusted associations with asthma, rhinitis and hay fever were generally weaker and non-significant for air pollution exposure at the current address as compared with air pollution exposure at the birth address. We did not find any significant associations of asthma, hay fever, and rhinitis with PM<sub>2.5</sub> and PM<sub>2.5</sub> absorbance, and few significant associations (eg, K, S and Zn) with PM<sub>2.5</sub> constituents (see online supplementary table S5).

Associations of FE<sub>NO</sub>, FEV<sub>1</sub>, FVC and FEF<sub>25-75</sub> with OP<sup>ESR</sup>, OP<sup>DTT</sup>, NO<sub>2</sub>, PM<sub>2.5</sub> and PM<sub>2.5</sub> absorbance at the birth address and at age 12 years from single-pollutant models are reported in table 2. After adjusting for potential confounders, we found significant negative associations of FEV<sub>1</sub> and FVC with OP<sup>DTT</sup> and NO<sub>2</sub> at age 12 years. Effect estimates and CIs for NO<sub>2</sub> were larger than those for OP<sup>DTT</sup>. Moreover, FEV<sub>1</sub> was associated with PM<sub>2.5</sub>, PM<sub>2.5</sub> absorbance, Cu and Fe (see online supplementary table S6) at age 12 years. Estimated reductions in FEV<sub>1</sub> were generally larger for these pollutants than for OP<sup>DTT</sup>. OP<sup>ESR</sup> was not significantly associated with lung function, allergic sensitisation and FE<sub>NO</sub> at age 12 years. Few significant associations were found between lung function and exposure at the birth address (table 2).

We also analysed the impact of moving behaviour for lung function parameters by performing separate analyses for movers and non-movers, and found no significant differences.

### Two-pollutant models

Only the two-pollutant models for OP<sup>DTT</sup> are presented in detail, since OP<sup>ESR</sup> was not significantly associated with any health outcome.

**Table 1** Adjusted associations† of asthma incidence, asthma symptoms, hay fever and rhinitis with exposure at the birth and current address

Component adjusted	Asthma incidence			Asthma symptoms		Hay fever		Rhinitis	
	EI	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
At birth address									
OP <sup>DTT</sup>	0.2	1.10**	(1.01 to 1.20)	1.08**	(1.02 to 1.16)	1.09	(0.98 to 1.21)	1.15**	(1.05 to 1.26)
OP <sup>ESR</sup>	252	1.03	(0.90 to 1.17)	1.03	(0.93 to 1.13)	1.08	(0.93 to 1.25)	1.08	(0.93 to 1.25)
NO <sub>2</sub>	8.4	1.12**	(1.01 to 1.25)	1.08**	(1.00 to 1.17)	1.10	(0.98 to 1.24)	1.12**	(1.00 to 1.25)
PM <sub>2.5</sub> absorbance	0.29	1.06	(0.96 to 1.16)	1.04	(0.97 to 1.12)	1.02	(0.91 to 1.13)	1.05	(0.96 to 1.16)
PM <sub>2.5</sub>	1.2	1.08	(0.94 to 1.25)	1.03	(0.93 to 1.14)	1.06	(0.90 to 1.24)	1.07	(0.94 to 1.22)
At current address									
OP <sup>DTT</sup>	0.2	1.06	(0.97 to 1.15)	1.03	(0.97 to 1.09)	1.03	(0.93 to 1.13)	1.05	(0.96 to 1.14)
OP <sup>ESR</sup>	252	1.02	(0.88 to 1.17)	1.08	(0.98 to 1.19)	1.03	(0.88 to 1.19)	1.13	(0.98 to 1.32)
NO <sub>2</sub>	8.4	1.08	(0.97 to 1.21)	1.06	(0.98 to 1.14)	1.01	(0.89 to 1.16)	1.12**	(1.00 to 1.26)
PM <sub>2.5</sub> absorbance	0.29	1.03	(0.93 to 1.15)	1.03	(0.95 to 1.11)	0.97	(0.85 to 1.11)	1.09	(0.98 to 1.22)
PM <sub>2.5</sub>	1.2	1.02	(0.87 to 1.18)	1.08	(0.97 to 1.20)	1.01	(0.83 to 1.22)	1.14	(0.97 to 1.34)

\*Associations of NO<sub>2</sub> at current address with rhinitis 1.12\* (1.00, 1.26). \*\*Associations are presented as OR with 95% CIs.

\*\*p<0.05, \*p<0.1.

†Adjusted for sex, maternal education, parental allergies, breast feeding, maternal smoking during pregnancy, smoking in the child's home, use of gas for cooking, mould/dampness in the child's home, pets at home, daycare attendance during first year of life and neighbourhood percentage of low-income households.

EI, exposure increment; NO<sub>2</sub>, nitrogen dioxide; OP<sup>ESR</sup>, oxidative potential assessed by spin resonance; OP<sup>DTT</sup>, oxidative potential assessed by dithiothreitol assay; PM<sub>2.5</sub>, particulate matter with an aerodynamic diameter of less than 2.5 μm.

In all two-pollutant models, associations of rhinitis with OP<sup>DTT</sup> remained statistically significant (figure 2A). For asthma symptoms, the associations only lost significance after adjusting for NO<sub>2</sub>. For asthma incidence, the associations lost significance after adjusting for several co-pollutants, including NO<sub>2</sub> and PM<sub>2.5</sub>. The effect estimates for OP<sup>DTT</sup>, however, were generally of similar magnitude in single-pollutant and two-pollutant OP<sup>DTT</sup> models (figure 2A).

For both FEV<sub>1</sub> and FVC (figure 2B), the effect estimates and CIs were largely unaffected by adjustment for most

co-pollutants. After adjustment for NO<sub>2</sub>, the estimated effects of OP<sup>DTT</sup> on FEV<sub>1</sub> and FVC were reduced by approximately 50%, and the CIs increased (figure 2B). The variance inflation factor was less than 3, indicating that the correlation between NO<sub>2</sub> and OP<sup>DTT</sup> did not result in questionable models.

Owing to the high correlations of OP<sup>DTT</sup> with NO<sub>2</sub>, we also evaluated whether the single-pollutant associations of NO<sub>2</sub> remained after adjusting for OP<sup>DTT</sup> (see online supplementary table S7). For asthma and rhinitis, the effect estimates of NO<sub>2</sub> decreased substantially and lost significance when adjusted for

**Table 2** Adjusted associations† between lung function, FE<sub>NO</sub>, allergic sensitisation and exposure at the birth address and at the current address at the time of the medical examination at age 12 years

	FEV <sub>1</sub>		FVC		FEF <sub>25-75</sub>		FE <sub>NO</sub>		Allergic sensitisation‡	
	EI	%diff (95% CI)	%diff (95% CI)	%diff (95% CI)	%diff (95% CI)	%diff (95% CI)	%diff (95% CI)	RR (95% CI)	RR (95% CI)	
At birth address										
OP <sup>DTT</sup>	0.2	-0.50 (-1.23 to 0.24)	-0.27 (-0.96 to 0.43)	-1.11 (-3.4 to 1.23)	-0.51 (-4.71 to 3.87)	1.04 (0.96 to 1.12)				
OP <sup>ESR</sup>	252	-0.04 (-1.07 to 1.01)	0.16 (-0.82 to 1.15)	-2.07 (-5.28 to 1.24)	-1.18 (-6.95 to 4.95)	1.08 (0.98 to 1.19)				
NO <sub>2</sub>	8.4	-0.38 (-1.34 to 0.58)	-0.19 (-1.09 to 0.72)	-2.54 (-5.46 to 0.48)	0.09 (-5.4 to 5.91)	1.09 (0.99 to 1.19)				
PM <sub>2.5</sub> absorbance	0.29	-0.32 (-1.15 to 0.52)	0.12 (-0.67 to 0.91)	-2.74* (-5.19 to -0.22)	-0.72 (-5.42 to 4.21)	1.08 (0.99 to 1.16)				
PM <sub>2.5</sub>	1.2	-0.17 (-1.26 to 0.94)	0.26 (-0.78 to 1.31)	-2.63 (-5.91 to 0.76)	-0.74 (-6.97 to 5.91)	1.10 (0.98 to 1.23)				
At age 12 years										
OP <sup>DTT</sup>	0.2	-0.94** (-1.66 to -0.22)	-0.62* (-1.30 to 0.06)	-1.49 (-3.75 to 0.82)	0.61 (-3.63 to 5.05)	1.04 (0.97 to 1.12)				
OP <sup>ESR</sup>	252	-0.79 (-1.93 to 0.36)	-0.59 (-1.67 to 0.50)	-0.10 (-3.66 to 3.58)	0.51 (-6.03 to 7.52)	1.08 (0.98 to 1.19)				
NO <sub>2</sub>	8.4	-1.46** (-2.60 to -0.51)	-1.09** (-2.07 to -0.09)	-2.61 (-5.77 to 0.65)	-0.71 (-6.83 to 5.80)	1.07 (0.97 to 1.18)				
PM <sub>2.5</sub> absorbance	0.29	-1.20** (-2.18 to -0.22)	-0.62 (-1.55 to 0.32)	-2.82* (-5.76 to 0.22)	-1.90 (-7.53 to 4.07)	1.05 (0.96 to 1.15)				
PM <sub>2.5</sub>	1.2	-1.11* (-2.34 to 0.13)	-0.53 (-1.70 to 0.65)	-2.84 (-6.65 to 1.13)	1.66 (-5.67 to 9.56)	1.10 (0.96 to 1.25)				

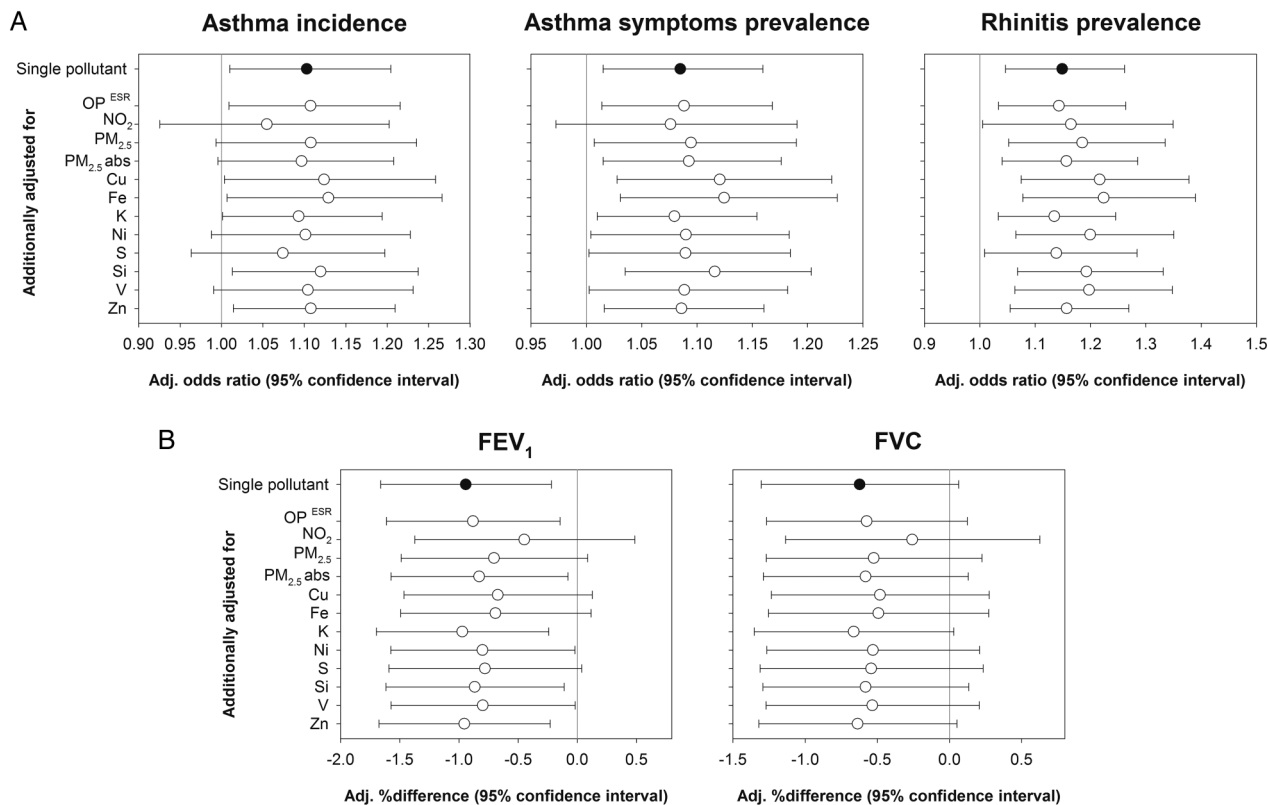
Associations are presented as percentage difference (%diff) and RR with 95% CIs.

\*\*p<0.05, \*p<0.1.

†Confounding factors differ from those by Gehring *et al*<sup>18</sup> as we adjusted for acute NO<sub>2</sub> effects in addition to sex, ln age, ln height, ln weight, respiratory infections during the past 12 weeks, maternal education, parental allergies, breast feeding, maternal smoking during pregnancy, smoking in the child's home, use of gas for cooking, mold/dampness in the child's home, pets at home, daycare attendance during first year of life and neighbourhood percentage of low-income households.

‡Adjusted for sex, maternal education, parental allergies, breast feeding, maternal smoking during pregnancy, smoking in the child's home, use of gas for cooking, mould/dampness in the child's home, pets at home, daycare attendance during first year of life and neighbourhood percentage of low-income households.

EI, exposure increment; FEF<sub>25-75</sub>, mid-respiratory flows; FE<sub>NO</sub>, fractional exhaled nitric oxide; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity; NO<sub>2</sub>, nitrogen dioxide; OP<sup>ESR</sup>, oxidative potential assessed by spin resonance; OP<sup>DTT</sup>, oxidative potential assessed by dithiothreitol assay; PM<sub>2.5</sub>, particulate matter with an aerodynamic diameter of less than 2.5 μm; RR, relative risk.



**Figure 2** (A) Associations of asthma incidence, prevalence of asthma symptoms and rhinitis with OP<sup>DTT</sup> at the birth address from single-pollutant and two-pollutant models; (B) adjusted associations of lung function (FEV<sub>1</sub>, FVC,) with OP<sup>DTT</sup> at the time of the medical examination at age 12 years from single-pollutant and two-pollutant models. Cu, copper; Fe, iron; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity; K, potassium; Ni, nickel; NO<sub>2</sub>, nitrogen dioxide; OP<sup>ESR</sup>, oxidative potential assessed by spin resonance; OP<sup>DTT</sup>, oxidative potential assessed by dithiothreitol assay; PM<sub>2.5</sub>, particulate matter with an aerodynamic diameter of less than 2.5 μm; S, sulfur; Si, silicon; V, vanadium; Zn, zinc.

OP<sup>DTT</sup>. For lung function, the effect estimates for NO<sub>2</sub> were reduced by approximately 20% after adjustment for OP<sup>DTT</sup>. Most respiratory health end points were also more consistently associated with OP<sup>DTT</sup> than with PM<sub>2.5</sub> mass concentrations (see online supplementary table S8).

## DISCUSSION

We investigated the associations between long-term exposure to two measures of OP (OP<sup>DTT</sup> and OP<sup>ESR</sup>) of PM<sub>2.5</sub> and respiratory health in the Dutch PIAMA birth cohort. In single-pollutant models, we found significant associations of asthma incidence, prevalence of asthma symptoms, rhinitis, FEV<sub>1</sub> and FVC with OP<sup>DTT</sup>. In two-pollutant models, these associations with OP<sup>DTT</sup> were insensitive to adjustment for most co-pollutants, including PM<sub>2.5</sub> mass concentration. However, associations of OP<sup>DTT</sup> with lung function were sensitive to adjustment for NO<sub>2</sub>. We did not find significant associations of PM<sub>2.5</sub> mass concentration and OP<sup>ESR</sup> with any of the health end points.

Few studies have assessed the acute respiratory health effects of OP, but the findings from these studies were inconclusive.<sup>11–14 16</sup> In a study where healthy young adults were exposed for 5 h to air pollution at different locations in the Netherlands, three different measures of OP (OP<sup>DTT</sup>, OP<sup>ESR</sup> and the depletion of ascorbate acid) were significantly associated with markers of airway and nasal inflammation (FE<sub>NO</sub> and NAL IL-6) in single-pollutant models.<sup>14</sup> However, in two-pollutant models, these associations only remained significant when measurements at a site with extremely high levels of OP were

excluded.<sup>14</sup> In the same study, lung function was not consistently associated with OP.<sup>13</sup> In a panel study consisting of school children with persistent asthma, Delfino *et al*<sup>11</sup> found significant associations of OP<sup>DTT</sup> and the rat alveolar macrophage assay with FE<sub>NO</sub> in single-pollutant models, but these associations were reduced and lost significance after adjusting for co-pollutants, including NO<sub>2</sub> and EC. The macrophage assay is a measure of the production of reactive oxygen species (ROS) within cells that can be induced by many other factors, including the endogenous formation of H<sub>2</sub>O<sub>2</sub>, and cannot easily be compared with the OP measure that were applied in the present study. In another panel study of elderly people by Delfino *et al*,<sup>12</sup> macrophage ROS production was significantly associated with FE<sub>NO</sub> and blood marker IL-6 in single-pollutant models, but these associations lost significance in two-pollutant models after adjusting for other co-pollutants (polycyclic aromatic hydrocarbons, organic acids). Generally, these studies did not demonstrate that the intrinsic OP of PM is a better predictor of acute health effects than other exposure metrics.

To the best of our knowledge, no data have been published on the effects of long-term exposure to OP of PM<sub>2.5</sub> on the respiratory health of children. We found a more consistent association of most respiratory health end points with OP<sup>DTT</sup> than with PM<sub>2.5</sub> mass concentration and OP<sup>ESR</sup>, suggesting that OP<sup>DTT</sup> could be a more health relevant measure than PM<sub>2.5</sub> mass concentration. Especially for rhinitis, the effect estimates from the single-pollutant and two-pollutant OP<sup>DTT</sup> models remained significant and largely unchanged after adjusting for different co-pollutants, including PM<sub>2.5</sub> and NO<sub>2</sub>. The low

spatial correlation between  $OP^{DTT}$  and  $PM_{2.5}$  mass concentration is consistent with previous results, and provides support for the possibility of distinguishing the independent effects of  $OP^{DTT}$  and  $PM_{2.5}$  mass concentration.<sup>23</sup> The lack of associations of  $PM_{2.5}$  concentration with respiratory health end points is in line with previous results in PIAMA of the same study population at age 12 years.<sup>18</sup>

Few studies have investigated associations with exposure at different time points. The more consistent associations of asthma with early life exposure are in line with earlier findings.<sup>33 34</sup> With regard to lung function, findings of other studies are inconsistent.<sup>3 35 36</sup> The stronger association of lung function with current exposure compared with early life exposure in the present study is supported by studies showing that air pollution effects on lung function in children may be reversible.<sup>37 38</sup>

We previously validated the LUR models used in the current study, and found a significant correlation ( $R=0.65$ ,  $p<0.01$ ) of LUR modelled  $OP^{DTT}$  with measured home-outdoor  $OP^{DTT}$ , and a moderate correlation ( $R=0.50$ ) with measured personal  $OP^{DTT}$ .<sup>39</sup> We found lower correlations of LUR modelled outdoor  $OP^{ESR}$  with personal measurements ( $R=0.24$ ), mostly due to the influence of indoor sources.<sup>39</sup> The higher correlations of LUR modelled OP at the home address with personal exposures for  $OP^{DTT}$  than for  $OP^{ESR}$  may explain why we found consistent associations with  $OP^{DTT}$ , but not with  $OP^{ESR}$ . Moreover, our findings of  $OP^{ESR}$  are consistent with the results from a previous study in PIAMA where weak associations were found for the transition metals which  $OP^{ESR}$  primarily responds to.<sup>18</sup> Also, the differential findings for OP characterised by DTT and ESR could be due to the fact that the assays respond to different components in the PM mixture. Also, there is little external evidence for organic compounds which affect  $OP^{DTT}$  primarily being associated with stronger respiratory health effects than the transition metals which affect  $OP^{ESR}$ .<sup>11 40</sup>

We also observed consistent associations of  $NO_2$  with the majority of health end points studied.  $NO_2$  was highly ( $R=0.70$ – $0.74$ ) correlated with  $OP^{DTT}$ , complicating the disentangling of the separate effects of  $OP^{DTT}$  and  $NO_2$ . Variation inflation factors suggested, however, that the correlation between  $OP^{DTT}$  and  $NO_2$  did not result in multicollinearity problems. Our findings with regard to  $OP^{DTT}$  and  $NO_2$  are dependent on the relevant health outcomes. For asthma incidence, prevalence of asthma symptoms and rhinitis, the effect estimates of  $OP^{DTT}$  largely remained unaffected by adjustment for co-pollutants, including  $NO_2$ . In contrast, the effect estimates of  $NO_2$  decreased and the CIs increased substantially after adjusting for  $OP^{DTT}$ . However, for lung function, the effect estimates of  $OP^{DTT}$  decreased by more than half after adjusting for  $NO_2$ . To date, no consensus has been reached about the associations with  $NO_2$  observed in epidemiological studies reflecting causal effects of  $NO_2$  itself, or  $NO_2$  mostly or partly acting as a surrogate of the mixture of traffic-related air pollutants due to the high correlations between  $NO_2$  and other traffic markers.<sup>41</sup> If  $NO_2$  is mainly an indicator for a mixture, the results of the two-pollutant models with OP are more difficult to interpret as both the metrics may be related to the same causal characteristics of the mixture. More studies with different ratios between OP and  $NO_2$  are needed to determine which metric predicts respiratory health better across different settings.

### Strengths and limitations

Important strengths of this study include the prospective study design and detailed individual residential air pollution exposure assessment. This enabled us to compare the health effects

performance of OP with more frequently used exposure metrics. We further assessed two OP assays responding to different components of the PM mixture.<sup>42 43</sup> The availability of a validation study for the LUR models used in this study allowed us to interpret the epidemiological findings better.

Limitations of our study include the application of acellular OP assays, resulting in the inability to assess the interaction of PM with airway cells which can elicit oxidative stress through alternative pathways. We also did not evaluate the genetic information on single-nucleotide polymorphisms related to oxidative stress, especially with respect to asthma and inflammation.<sup>44</sup> Another limitation is modelling OP using LUR models since OP is considered to be an indicator of PM-induced oxidative stress and we had no specific predictor variables for the biological activity. Predictor variables were similar for OP and other pollutants, but the relative importance of these predictors differed in the OP model versus models for other pollutants, often resulting in only moderate correlations between model-predicted pollutant concentrations. Both OP models included as predictor, the regional background OP derived from interpolation of measurements, a variable that was specific for OP models. The LUR models were based on measurements taken in 2009–2010, which is just before the 14-year questionnaire follow-up (2010–2011) and the medical examination at age 12 years (2008–2009), thus reflecting exposures at the most recent follow-ups well. By applying the models to the children's historical addresses, we assume that the spatial patterns remained stable from the baseline period of the cohort (ie, 1996–1997). Spatial stability was documented by studies where measured and modelled spatial  $NO_2$  contrasts were stable for periods of 7–12 years.<sup>45 46</sup> We assume these findings can be applied to OP as well, supported by the moderate to high correlations of measured OP with  $NO_2$ .

### CONCLUSION

Asthma incidence, prevalence of asthma symptoms and rhinitis were more consistently associated with  $OP^{DTT}$  than with  $PM_{2.5}$  mass concentration. These associations were robust to adjustment for co-pollutants. The associations of lung function with  $OP^{DTT}$  were more sensitive to adjustment for co-pollutants, particularly  $NO_2$ . Respiratory health was not significantly associated with  $OP^{ESR}$ .

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## Children's respiratory health and oxidative potential of PM<sub>2.5</sub>: the PIAMA birth cohort study

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