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1 **Outdoor air pollution, exhaled 8-isoprostanes and current asthma in adults: the EGEA**
2 **study**

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28

29 **Running title:** outdoor air pollution, exhaled 8-isoprostanes and asthma.

30

31 **Take home message:** Exhaled 8-isoprostanes concentration is associated with both outdoor
32 air pollution and current asthma in adults.

33

34 **Plain language summary:** Associations between outdoor air pollution and asthma in adults
35 are still scarce, and the underlying biological mechanisms are poorly understood. Among
36 adults, we studied the associations between 1) long-term exposure to outdoor air pollution and
37 current asthma 2) exhaled 8-isoprostanes, a biomarker related to oxidative stress and current
38 asthma, and 3) outdoor air pollution and exhaled 8-isoprostanes. We found for the first time
39 associations between long-term exposures to outdoor air pollution estimated at individual
40 level, exhaled 8-iso concentration and current asthma. Traffic intensity and O₃ exposure
41 increased significantly the risk of current asthma. Exhaled 8-iso concentration was positively
42 and significantly associated with current asthma. Among participants without asthma, exhaled
43 8-iso concentration increased significantly with PM_{2.5} exposure and decreased with O₃ and
44 O₃-summer exposures. Results add new insights of a potential role of oxidative stress in the
45 associations between outdoor air pollution and asthma in adults.

46

47

48

49 **ABSTRACT**

50 Associations between outdoor air pollution and asthma in adults are still scarce, and the
51 underlying biological mechanisms are poorly understood.

52 To study the associations between 1) long-term exposure to outdoor air pollution and current
53 asthma 2) exhaled 8-isoprostanes, a biomarker related to oxidative stress and current asthma,
54 and 3) outdoor air pollution and exhaled 8-isoprostanes.

55 Cross-sectional analyses were conducted in 608 adults (39% with current asthma) from the
56 first follow-up of the French case-control and family study on asthma (EGEA). The NO₂,
57 NO_x, PM₁₀, PM_{2.5}, road traffic, and O₃ were assessed by ESCAPE FP7 and IFEN. Models
58 took account city and familial dependence.

59 The risk of current asthma increased with traffic intensity (adjusted (a)OR=1.09 95%CI:
60 [1.00, 1.18] per 5,000 vehicles/day), with O₃ exposure (aOR=2.04 [1.27, 3.29] per 10 µg/m³),
61 and with exhaled 8-iso concentration (aOR=1.50 [1.06, 2.12] per 1 pg/mL). Among
62 participants without asthma, exhaled 8-iso concentration increased with PM_{2.5} exposure
63 (adjusted (a)β=0.23 95 CI [0.005, 0.46] per 5 µg/m³) and decreased with O₃ and O₃-summer
64 exposures (aβ=-0.20 [-0.39, -0.01], aβ=-0.52 [-0.77, -0.26] per 10 µg/m³, respectively).

65 Results add new insights of a potential role of oxidative stress in the associations between
66 outdoor air pollution and asthma in adults.

67

68 **Introduction**

69 According to the World Health Organization, outdoor air pollution caused 3.7 million deaths
70 in the world in 2012, and 9 % of the total mortality in France was recently attributed to
71 particulate matter with a diameter ≤ 2.5 microns (PM_{2.5}) [1]. The most studied pollutants in
72 relation with health effects are nitrogen dioxide (NO₂), ozone (O₃) and particulate matter
73 (PM₁₀ and PM_{2.5}). Evidence of the impact of outdoor air pollution on respiratory health is
74 increasing [2] [3], and the associations between exposure to outdoor air pollution and asthma
75 have been largely studied in children [4]. However, studies on associations between long-term
76 exposure to outdoor air pollution and asthma among adults are still scarce [5].

77 One biological mechanism proposed to partly explain the association between outdoor air
78 pollution and asthma is oxidative stress [6]. Oxidative stress is an imbalance between the
79 increase to reactive oxygen species (ROS) and antioxidant response [7]. Among the biological
80 markers related to oxidative stress, 8-isoprostanes (8-iso) are known as stable and specific
81 products of lipid peroxidation [8]. Interestingly, 8-iso can be measured non-invasively in
82 exhaled breath condensate (EBC), a fluid close to the lungs [7]. An increase of 8-iso
83 concentration along with a decline of antioxidant defense can induce tissue damages and can
84 contribute to pathophysiological changes as those seen in asthma [9, 10]. 8-iso are known to
85 provoke airway hyperresponsiveness, to increase the production of mucus and to promote
86 contraction of smooth muscles [10] [11]. In a recent systematic review, 8-iso concentration
87 was found higher in adults with severe asthma than in those with mild-to-moderate asthma
88 [12]. To date, epidemiological studies on the associations between long-term exposure to
89 outdoor air pollution and asthma including the measurement of 8-iso in adults are lacking.

90 In the present paper, among adults from the Epidemiological study of the Genetic and
91 Environmental factors of Asthma (EGEA), we first studied the associations between long-
92 term exposure to outdoor air pollution (NO₂, NO_x, PM₁₀, PM_{2.5}, traffic load, traffic intensity,

93 O₃ and O₃-summer) and current asthma. Second, we studied the associations between 8-iso
94 measured in EBC with both long-term exposure to outdoor air pollution and current asthma.

95

96 **Methods**

97 *Study population*

98 EGEA is a cohort study based on an initial group of asthma cases recruited in chest clinics
99 from 5 French cities (1991-1995) along with their first-degree relatives, and a group of
100 controls (<https://egeanet.vjf.inserm.fr/>). Child controls were recruited in surgery hospitals, and
101 adult controls in electoral rolls and from surgery hospitals or check-up centers [13]. The
102 protocol and descriptive characteristics have been described previously [13, 14], and inclusion
103 criteria used to define asthmatic cases and controls were described in the online
104 supplementary material. EGEA collection is certified ISO 9001 [15]. Ethical approval was
105 obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital
106 and Necker-Enfants Malades Hospital, Paris). All participants signed a written informed
107 consent.

108 The present analyses included 608 adults (≥ 16 years old) in the framework of the first follow-
109 up (EGEA2, 2003-2007), with available data on current asthma, outdoor air pollution assessed
110 by the European Study of Cohorts for Air Pollution Effects (ESCAPE) and exhaled 8-iso
111 concentration (figure 1). Among the 608 participants, 5 had no pollution data from the French
112 Institute for the Environment (IFEN) assessment. No significant differences were found
113 between the participants included and those not included (n=963, see online supplementary
114 table E1).

115

116 *Respiratory phenotypes*

117 At EGEA2, the participants with ever asthma answered positively to at least one of the two
118 following questions “*Have you ever had attacks of breathlessness at rest with wheezing?*” or
119 “*Have you ever had asthma attacks?*”, or were recruited as asthmatic cases at EGEA1.
120 Among participants with ever asthma, “*current asthma*” was defined by a report of respiratory
121 symptoms (wheeze, nocturnal chest tightness, attacks of breathlessness following strenuous
122 activity, at rest or at night time), or asthma attacks or use of inhaled and/or oral medicines
123 because of breathing problems in the past twelve months [16] (see the online supplementary
124 material for more details). Only participants with current asthma were included in the
125 analyses because the phenotype “current asthma” which reflects the recent activity of the
126 disease was more relevant than the phenotype “ever asthma” to study the associations
127 between long-term exposure to outdoor air pollution, biological markers and asthma.

128

129 ***Exposure assessment***

130 Thanks to ESCAPE and IFEN assessments, outdoor air pollution exposures (NO₂, NO_x, PM,
131 O₃ and road traffic) were assigned to each participant’s residential address.

132 Annual air pollution levels of NO₂ and particulate matter (PM) were derived from ESCAPE
133 standardized models (www.escapeproject.eu/manuals/). Briefly, the ESCAPE monitoring
134 campaigns took place between 2009 and 2010, including 40 measurement sites for NO₂ and
135 NO_x in Paris, Lyon, Grenoble and Marseille, and 20 PM measurement sites in Paris and
136 Grenoble. Land-use regression (LUR) models were developed and two indicators of road
137 traffic were also calculated. Traffic intensity on nearest road was defined as the number of
138 motor vehicles circulating per day on nearest road to participant’s home, and was expressed in
139 vehicles per day. Total traffic load was defined as the traffic load in all major roads based
140 around a buffer of 100 meters from the participant’s home, and was expressed by traffic
141 intensity multiplied by road length. The back-extrapolation is used to transfer the recent or

142 current LUR models to earlier years. In our study, the estimation of outdoor air pollution by
143 ESCAPE took place after EGEA2, and accordingly we also analyzed the back-extrapolated
144 pollution estimates in order to get a better temporality between outdoor air pollution with
145 current asthma and EBC collection. Back-extrapolated pollution data were available for NO₂,
146 NO_x in all cities, and for PM₁₀ in Paris.

147 In order to supplement the ESCAPE data set, we used O₃ and O₃-summer exposures from the
148 IFEN (see the online supplementary material for more details). The O₃ estimate was the
149 yearly mean of ozone level in 2004 for each participant at the residential address, and derived
150 from a geo-statistical model as previously described [17]. The exposure of O₃-summer was
151 assessed from the monthly means from April to September.

152

153 *Measurement of exhaled 8-isoprostanes*

154 Exhaled Breath Condensate (EBC) was collected at EGEA2 between 2003 and 2006 with an
155 RTubeTM (Respiratory Research Inc., Charlottesville, VA, USA) according to a standardized
156 method, as described previously [18]. Samples were immediately stored at -80°C. Exhaled 8-
157 iso concentration was measured 5.3 to 9.4 years after EBC collection, with a specific enzyme
158 immunoassay (EIA) kit (8-isoprostanes EIA kit Cayman Chemical, Ann Arbor, MI, USA)
159 according to the manufacturer's protocol. Approximately 30% of exhaled 8-iso concentration
160 was below the Limit of Detection (< LD, see the online supplementary material for more
161 details).

162

163 *Statistical analyses*

164 The level of outdoor air pollution was described by city. Due to its skewed distribution, the
165 exhaled 8-iso concentration was log-transformed.

166 Associations between outdoor air pollution and current asthma, and associations between

167 exhaled 8-iso concentration and current asthma were studied with logistic models. In order to
168 study the association between outdoor air pollution and exhaled 8-iso concentration
169 independently of asthma, linear regression models were first performed among participants
170 without asthma and sensitivity analyses were conducted by: a) using back-extrapolated
171 pollution estimates, b) studying the associations by city, and c) using bi-pollutant models
172 (PM_{2.5} and O₃ or O_{3-summer}). Analyses with back-extrapolated pollution estimates were
173 performed to get a better temporality between pollution and EBC collection. We also studied
174 the associations between outdoor air pollution and exhaled 8-iso concentration in all
175 participants, and among participants with current asthma.

176 All estimates were adjusted for age, sex, smoking habits. Further adjustments were conducted
177 for body mass index (BMI), socio-professional category (SPC), and use of cleaning products
178 which is an indicator of exposure to indoor pollution. In order to study only the road traffic
179 effect, estimates of associations between road traffic and current asthma or exhaled 8-iso
180 concentration were also adjusted for background NO₂. As the aim was to study the effect of
181 long-term exposure to outdoor air pollution, we performed sensitivity analyses by excluding
182 participants who lived less than one year at the same address (N=46).

183 In order to take into account the city-effect on outdoor air pollution levels, we performed
184 meta-analyses including city-specific analyses from the Harvard School of Public Health's
185 macro program (<https://www.hsph.harvard.edu/donna-spiegelman/software/metaanal>), and
186 pooled analyses with random effect on city.

187 The above models have taken into account random effects on familial dependence. All the
188 results are expressed for an increase of 1 pg/mL of exhaled 8-iso concentration, an increase of
189 20 µg/m³ of NO_x, 10 µg/m³ of NO₂, PM₁₀, O₃ and O_{3-summer} and 5 µg/m³ of PM_{2.5}. For traffic
190 measures, the results of traffic load are expressed for four million vehicles × m/day in major
191 roads within a 100-m buffer, and the results of traffic intensity are expressed for an increase

192 of 5,000 vehicles/day on major road. Statistical analyses were performed using SAS software,
193 version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA).

194

195 **Results**

196 The mean age of the 608 adults was 43 years, 47% were males, 39% had current asthma, 21%
197 were current smokers, and 37 % were overweight (BMI ≥ 25 kg/m²) (table 1). Participants
198 with current asthma were younger, more often men, current smokers and unemployed, and
199 had more often a secondary education level than those without asthma (table 1). Participants
200 with current asthma had also a lower FEV₁, higher airway hyperresponsiveness, higher
201 allergic sensitization, higher IgE level, and higher exhaled 8-iso concentration than those
202 without asthma.

203 The characteristics of participants were heterogeneous across cities (see the online
204 supplementary table E2 and the online supplementary material for more details).

205 The pollutant and traffic levels were the highest in Paris, except for the PM₁₀ level (online
206 supplementary figures E1 and E2). The PM₁₀ and PM_{2.5} levels were above the values
207 recommended by the WHO. The O₃ and O_{3-summer} levels were the highest in Marseille (online
208 supplementary figure E3). Positive and significant correlations were found between NO₂,
209 NO_x, PM₁₀, and PM_{2.5} (correlation coefficient (r) $0.47 \leq r \leq 0.95$, all P<0.002) whereas O₃ and
210 O_{3-summer} levels were negatively correlated with all pollutants ($-0.50 \leq r \leq -0.15$, all P<0.002)
211 (data not shown). The NO₂ level was significantly higher in managers and technicians, and
212 O_{3-summer} level was significantly higher in manual workers (data not shown).

213 The exhaled 8-iso concentration (25th;75th percentiles) was 3.16 (1.40;7.69) pg/mL in all
214 participants and 3.97 (1.85;9.10) pg/mL among those with current asthma (table 1), and was 7
215 times higher in Paris than in other cities, was higher in women and decreased with age (online
216 supplementary tables E2 and E3). No significant association was found between exhaled 8-iso

217 concentration and smoking expressed as current smoking habits, quantity of tobacco, or
218 number of pack-years (all $P > 0.80$, data not shown). No inter-plate variability was observed
219 (data not shown) and no association was found between storage time and exhaled 8-iso
220 concentration (regression coefficient=0.02, $P=0.39$).

221

222 ***Associations between outdoor air pollution and current asthma***

223 Associations between outdoor air pollution and current asthma were not significantly
224 heterogeneous between cities (Q test, $p\text{-value} > 0.08$). In pooled analyses, the risk of current
225 asthma increased significantly with traffic intensity (adjusted (a)OR=1.09 95%CI [1.00,
226 1.18]) and with O_3 exposure (aOR=2.04 [1.27, 3.29], table 2) whatever the adjustment. The
227 results were similar after excluding participants who lived less than one year at the same
228 address. Back-extrapolated exposure estimates gave similar results (online supplementary
229 table E4).

230

231 ***Associations between exhaled 8-iso concentration and current asthma***

232 A positive and significant association was found between exhaled 8-iso concentration
233 expressed as a continuous variable and current asthma (aOR=1.50 [1.06, 2.12], figure 2). No
234 significant association was found when exhaled 8-iso concentration was expressed as $<LD$ or
235 $>LD$. Overall, the risk of current asthma increased significantly with exhaled 8-iso
236 concentration expressed as $<LD$, $>LD$ and $\leq\text{median}$, $>LD$ and $>\text{median}$ (trend $p\text{-value}=0.05$).
237 Exhaled 8-iso concentration was unrelated to duration of asthma (years), age of asthma onset
238 expressed continuously (years) or in classes (all $P > 0.40$). Further, no significant association
239 was found between exhaled 8-iso concentration with FEV_1 % predicted continuously or in
240 classes (all $P > 0.60$) or allergic sensitization ($P=0.12$), (data not shown).

241

242 *Associations between outdoor air pollution and exhaled 8-iso concentration among*
243 *participants without asthma*

244 Associations between outdoor air pollution and exhaled 8-iso concentration were not
245 heterogeneous between cities (Q test, p-values>0.20). In pooled analyses, exhaled 8-iso
246 concentration increased significantly with PM_{2.5} exposure (adjusted (a)β=0.23 [0.005, 0.46],
247 and decreased with O₃ and O_{3-summer} exposures (aβ=-0.20 [-0.39,-0.01] and aβ=-0.52 [-0.77,-
248 0.26] whatever the adjustment, table 3). The results were similar after excluding participants
249 who lived less than one year at the same address. Analyses performed with back-extrapolated
250 data also gave similar results (online supplemental table E5). Furthermore, exhaled 8-iso
251 concentration decreased significantly with O₃ and O_{3-summer} exposures in Paris (aβ=-0.22 [-
252 0.42,-0.02] and aβ=-0.53 [-0.90,-0.16], online supplementary tables E6). After excluding
253 participants from Paris (n=111), no significant associations were observed between O₃ and
254 O_{3-summer} and exhaled 8-iso concentration (online supplementary table E7). In models adjusted
255 for both PM_{2.5} and O₃, similar result was found only between O_{3-summer} exposure and exhaled
256 8-iso concentration (aβ=-0.59 [-0.71,-0.47], online supplementary table E8). In models
257 adjusted for both NO₂ and O₃, the negative associations between O₃ and O_{3-summer} and exhaled
258 8-iso concentration remained statistically significant (data not shown). In all participants, only
259 O_{3-summer} exposure was negatively and significantly associated with exhaled 8-iso
260 concentration (aβ=-0.33 [-0.55,-0.11], data not shown). No association was found between
261 outdoor air pollution and exhaled 8-iso concentration in participants with current asthma (data
262 not shown).

263

264 **Discussion**

265 For the first time in adults, we found associations between long-term exposures to outdoor air
266 pollution estimated at individual level, exhaled 8-iso concentration and current asthma.

267 Traffic intensity and O₃ exposure increased significantly the risk of current asthma. Exhaled
268 8-iso concentration was positively and significantly associated with current asthma. Among
269 participants without asthma, exhaled 8-iso concentration increased significantly with PM_{2.5}
270 exposure and decreased with O₃ and O_{3-summer} exposures.

271 Participants with asthma included in the present analyses were mostly recruited in chest
272 clinics as asthma cases, with a careful procedure set up to include true asthmatics using
273 standardized and validated questionnaires. Others were recruited as first-degree relatives of
274 asthmatic cases based on answers to questions on asthma diagnosis. This leads to a group of
275 asthmatics with a wide range of disease expression. In our cross-sectional analyses, cause and
276 consequence cannot be disentangled. It was not possible to study the associations between
277 outdoor air pollution and exhaled 8-iso concentration with the incidence of asthma because
278 only 30 new cases of asthma were reported at EGEA2. But, there is clearly a need for further
279 research to confirm the associations and to clarify its causal underpinnings. Regarding
280 exposure assessment, LUR models are well-adapted to take into account the spatial variation
281 of NO₂, PM_{2.5} [19] and NO_x [20], and ESCAPE resolution is accurate to estimate the
282 exposure to markers of road traffic which have a spatial heterogeneity. In addition, IFEN
283 resolution is larger than ESCAPE but suitable for O₃ and O_{3-summer} which are homogeneous
284 over long distances [21]. We acknowledge that a weakness of our study is the non-compliance
285 of the temporality because outdoor air pollution was estimated by ESCAPE between 2009 and
286 2010 whereas the collection of EBC and phenotype “current asthma” took place at EGEA2
287 between 2003 and 2007. To get a better temporality in our analyses, we used the back-
288 extrapolated pollution estimates which were back-extrapolated at participants’ residential
289 address at EGEA2, and found similar results. Both back-extrapolated and non-back-
290 extrapolated estimates were highly and significantly correlated in our study (correlation
291 coefficients ≥ 0.98 , $P < 0.001$) as previously reported by Beelen *et al.* in the same ESCAPE

292 project and for a longer period [22]. Previously in ESCAPE study, associations between NO₂
293 back-extrapolated estimates and asthma incidence were similar to those with non-back-
294 extrapolated estimates [23]. We aimed to study the impact of long-term exposure to outdoor
295 air pollution, and therefore conducted sensitivity analyses by excluding participants who lived
296 less one year at the same residential address that did not change our conclusions. We cannot
297 exclude that some non-differential misclassification of pollution exposure may have occurred
298 because the time-activity patterns of participants were not available in our analyses, but in this
299 case, it would lead to bias towards the null. Furthermore, IFEN resolution can better
300 represented daily participant's exposures to O₃ and O₃-summer, at least for those whom work
301 was close to home. We could not take into account all indoor environmental factors; however,
302 we found similar results after further adjustment for domestic exposure to cleaning products.
303 Furthermore, the adjustment for the socioeconomic position potentially associated with
304 pollutant exposures [24] and asthma gave also similar results. The EGEA study is a case-
305 control and family study. Participants from the same family share genetic background, and
306 also socio-economic and lifestyle factors, that could be associated with the exposure to
307 outdoor air pollution or the asthma risk. We therefore took into account familial dependence
308 through random effects in mixed models. Sub-groups analyses suffer from a lack of power
309 but, as best as possible, we used the most suitable statistical models. Finally, the EIA method
310 has been preferred for dosing exhaled 8-iso concentration rather the GCMS method because
311 the former is better adapted to analyze a larger number of samples as in our study.

312 We found that traffic intensity and O₃ exposure increased the risk of current asthma. Our
313 results add evidence of the impact of long-term exposure to outdoor air pollution on asthma in
314 adults. Our results are partly in accordance with those of a recent study showing that traffic
315 exposure but not NO₂ exposure, assessed by satellite-based LUR model at residential
316 addresses, was positively associated with current asthma in 1367 adults [25]. We also found a

317 positive association between O₃ exposure and current asthma. To our knowledge, the
318 literature assessing outdoor air pollution effect on asthma focused mainly on other asthma
319 phenotypes such as asthma onset, asthma severity, or asthma control. A study conducted in
320 California has reported that long-term exposure to O₃ was associated with development of
321 asthma in adult males [26]. Previously in the EGEA study, long-term exposure to O₃
322 estimated by IFEN was associated with asthma severity [17] and with uncontrolled asthma
323 [27]. Recently, a cohort showed that asthmatic adults exposed to O₃ had a greater risk to
324 develop asthma-chronic obstructive pulmonary disease [28]. Interestingly, various asthma
325 phenotypes were studied such as asthma-onset which reflects the initiation of the disease, and
326 severity and control of asthma which are linked to manifestations of the disease. Asthma
327 reflects both ever asthma and current asthma, and the participants with ever asthma had not
328 necessarily a current asthma. In the EGEA study, the phenotype “current asthma” was defined
329 by the report of respiratory symptoms or asthma attacks or use of inhaled and/or oral
330 medicines because of breathing problems in the past twelve months. To study the associations
331 between long-term exposure to outdoor air pollution, biological markers and asthma, the
332 phenotype “current asthma” was more relevant than the phenotype “ever asthma” because it
333 reflects the recent activity of the disease. Overall, all these findings add evidence of
334 associations between outdoor air pollution and asthma in adults, whatever the studied
335 phenotypes.

336 This study adds new insights into a potential role of oxidative stress in the associations
337 between long-term exposure to outdoor air pollution and asthma in adults. We reported a
338 significant association between exhaled 8-iso concentration and current asthma after
339 adjustment for age, sex, smoking habits and body mass index. We investigated whether other
340 asthma characteristics could explain this association, but we did not find any significant
341 association between exhaled 8-iso concentration with duration of asthma, age of asthma onset,

342 lung function, or allergic sensitization. Our results added new evidence to the previous
343 associations reported in the literature with asthma severity and asthma control [12]. We found
344 for the first time that exhaled 8-iso concentration was positively associated with PM_{2.5}
345 exposure in participants without asthma. Unfortunately, we did not have back-extrapolated
346 data for PM_{2.5}. In France, PM_{2.5} level has overall decreased between 2002 and 2012 and the
347 association between PM_{2.5} exposure and exhaled 8-iso concentration may be underestimated
348 [29]. The literature mainly focused on short-term exposure to outdoor air pollution and on
349 other biological compartments. In fact, short-term exposure to PM_{2.5} was previously found to
350 be positively associated with EBC 8-iso concentration among healthy adolescents [30], and
351 with 8-iso concentration in urine among adults [31]. We also found counterintuitive negative
352 associations between O₃ and O_{3-summer} exposures and exhaled 8-iso concentration. Our results
353 are not in accordance with those of a previous study showing that O₃ exposure assigned to
354 residential location was associated with higher plasma 8-iso concentration in 120 healthy
355 students [32]. The inconsistency with our findings may be partially attributed to differences in
356 the study design, in the biological compartment, and in the spatial resolution which was less
357 accurate than in our study. We showed that O₃ exposure and exhaled 8-iso concentration
358 increased the risk of current asthma, and we did not expect negative associations between O₃
359 and O_{3-summer} exposures and exhaled 8-iso concentration. We investigated more thoroughly
360 why these associations were negative. The analyses conducted by city showed that Paris
361 heavily weighted the negative associations between O₃ and O_{3-summer} exposures and exhaled 8-
362 iso concentration. Previously, a study has also reported a negative association between short-
363 term exposure to O₃ and exhaled 8-iso in New Yorker's adolescents [33]. Another explanation
364 is that O₃ is a secondary pollutant mainly produces through complex chemical reactions from
365 NO₂. In our study, O₃ and NO₂ levels were highly and negatively correlated, and NO₂
366 exposure was also positively associated with exhaled 8-iso concentration but not significantly.

367 One hypothesis is likely that the positive association between NO₂ and exhaled 8-iso partly
368 explained the negative associations observed for O₃ and O₃-summer. Overall, the specific effects
369 of pollutants are hard to disentangle even in bi-pollutant models given the strong correlations
370 between pollutants. It is interesting to note that participants from Paris had particular
371 characteristics as an exhaled 8-iso concentration 7 times higher, were exposed to higher levels
372 of road traffic, NO₂ and PM, and to lower levels of O₃ and O₃-summer than in other cities,
373 suggesting that Parisians may have particular characteristics that could partly explain the
374 negative associations between ozone and exhaled 8-iso concentration. O₃ and O₃-summer were
375 negatively correlated with PM_{2.5}, but the negative association between O₃-summer exposure and
376 exhaled 8-iso concentration remained significant in a bi-pollutant model. Previously in EGEA
377 adults, O₃ assessed by IFEN was also found to be negatively associated with the EBC total
378 nitrites/nitrates level, a biological marker of nitrosative stress [34]. In the literature, complex
379 interplays between nitrosative and oxidative stress pathways have been reported [35],
380 including a reciprocal regulation. An alternative explanation is that our results were likely due
381 to other factors that are not included in the present analyses. In all participants, only a
382 negative and significant association between O₃-summer exposure and exhaled 8-iso
383 concentration was found, and no association was found in participants with current asthma. In
384 a directed acyclic graph (DAG), the covariate “current asthma” may be a collider, which
385 makes the interpretation of our results even more difficult in a context of cross-sectional
386 analyses [36].

387 Several biological mechanisms by which outdoor air pollution may be associated with asthma
388 have been suggested in previous reviews [37, 38]. Outdoor air pollution exposure may
389 increase oxidative stress in airways through the production of reactive oxygen species and
390 local inflammation [9]. The PM can be supports for allergens and their small size gives them
391 a large surface related to oxidative potential [39]. To disentangle the complexity of the

392 associations between asthma and oxidative stress, and 8-iso in particular, there is a need for
393 longitudinal epidemiological studies. Overall, our results add new insights of a potential role
394 of oxidative stress in the association between long-term exposure to outdoor air pollution and
395 asthma in adults. To better understand the underlying biological pathways between outdoor
396 air pollution and asthma, future epidemiological studies should use individual portable
397 sensors in order to improve the pollution assessment, study the different windows of exposure
398 and try to collect multiple exposures in order to identify exposure profiles through clustering
399 methods.

400 **Conclusions**

401 We found that traffic intensity, O₃ exposure and exhaled 8-iso concentration increased the risk
402 of current asthma, and that among participants without asthma exhaled 8-iso concentration
403 increased with PM_{2.5} exposure, and decreased with O₃ and O₃-summer exposures. Exhaled 8-iso
404 seems to be an interesting oxidative stress-related biomarker adapted to epidemiological
405 studies. Overall, our study adds new insights in the associations between long-term exposure
406 to outdoor air pollution and asthma in adults, and suggests that oxidative stress may partly
407 explained such associations. Longitudinal studies with larger samples are now needed to
408 confirm such results.

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Table 1. Characteristics of adult participants according to asthma status.

Characteristics	In all participants n=608	Participants without asthma n=368	Participants with current asthma n=240	p-value
Age (years), mean ± SD	42.5 ± 17.2	45.5 ± 26.3	37.9 ± 17.5	< 0.0001 ⁺
Sex, Men, n (%)	288 (47.4)	160 (43.5)	128 (53.3)	0.02 ⁺
Smoking habits, n (%)	312 (51.3)	189 (51.4)	123 (51.3)	
Never smokers	168 (27.6)	113 (30.7)	55 (22.9)	0.02 ⁺
Ex-smokers	128 (21.1)	66 (17.9)	62 (25.8)	
Current smokers				
BMI (kg/m ²), n (%)	70 (11.5)	38 (10.3)	32 (13.3)	
<20	316 (52.0)	193 (52.4)	123 (51.3)	0.62
[20-25[165 (27.1)	104 (28.3)	61 (25.4)	
[25-30[57 (9.4)	33 (9.0)	24 (10.0)	
>=30				
Socio-professional category, n (%)	n=605	n=366	n=239	
Unemployed	72 (11.9)	29 (7.9)	43 (18.0)	
Manager	212 (35.0)	138 (37.7)	74 (31.0)	0.001 ⁺
Technician	248 (41.0)	149 (40.7)	99 (41.4)	
Manual worker	73 (12.1)	50 (13.7)	23 (9.6)	
Number of years at the same residential address, mean ± SD (min;max)	n=607 12.4 ± 10.7 (0;48)	n=366 13.0 ± 10.9 (0;48)	n=239 11.4 ± 10.4 (0;46)	0.07 [†]
Asthma, n (%)				
Current	240 (39.5)	/	/	
Duration of asthma (years), mean ± SD (min;max)	/	/	n=229 15.9 ± 11.6 (0;59.6)	
Age of asthma onset (years)			n=228	
≤4	/	/	80 (35.1)	
[4-16]			79 (34.7)	
>16			69 (30.3)	
FEV1 % predicted, mean ± SD	103 ± 18.9	108 ± 17.3	95.2 ± 18.5	< 0.0001 ⁺
Allergic sensitization [‡] , n (%)	n=602 327 (54.3)	(n=363) 137 (37.7)	n=239 190 (79.5)	< 0.0001 ⁺
Exhaled 8-iso concentration, pg/mL, GM (q1;q3)	3.16 (1.41;7.69)	2.69 (1.14;6.79)	3.97 (1.85;9.10)	0.004 ⁺
Exhaled 8-iso concentration >LD, n (%)	431 (70.9)	253 (68.8)	178 (74.2)	0.15

BMI, body mass index; 8-iso, 8-isoprostanes; GM, geometric mean; q1 and q3, the 25th and the 75th percentiles of the GM; LD, limit of detection of the 8-iso concentration. FEV₁, forced expiratory volume; [†]: defined by at least one weal ≥ 3 mm to 12 tested allergens; [‡] Results in bold represent significant results (p-values ≤ 0.05).

Table 2. Associations between outdoor air pollution and current asthma.

		NO ₂	NO _x	PM ₁₀	PM _{2.5}	Total traffic load on major roads in a 100-m buffer of the home [#]	Traffic intensity at the road nearest to a participant's home [#]	O ₃	O ₃ -summer
Model 1	n	608	608	437	437	605	605	603	603
	OR crude (95% CI)	0.99 (0.87,1.15)	1.05 (0.92,1.19)	1.10 (0.65,1.85)	0.89 (0.54,1.45)	1.16 (0.96,1.41)	1.09 (1.00,1.19)[†]	2.01 (1.26,3.23)[†]	1.29 (0.68,2.46)
Model 2	n	608	608	437	437	608	608	603	603
	OR adjusted (95% CI)	0.99 (0.86,1.14)	1.04 (0.91,1.18)	1.05 (0.62,1.79)	0.82 (0.49,1.36)	1.15 (0.95,1.38)	1.09 (1.00,1.19)[†]	1.93 (1.21,3.09)[†]	1.10 (0.67,1.81)
Model 3	n	605	605	436	436	605	605	600	600
	OR adjusted (95% CI)	1.00 (0.87,1.16)	1.05 (0.92,1.19)	1.05 (0.61,1.81)	0.84 (0.50,1.39)	1.15 (0.95,1.39)	1.10 (1.00,1.20)[†]	1.89 (1.19,3.02)[†]	1.10 (0.67,1.81)
Model 4	n	603	603	435	435	603	603	598	598
	OR adjusted (95% CI)	0.98 (0.85,1.14)	1.03 (0.90,1.17)	1.03 (0.59,1.80)	0.82 (0.49,1.39)	1.14 (0.94,1.37)	1.09 (1.00,1.18)[†]	2.04 (1.27,3.29)[†]	1.24 (0.71,2.18)
Model 5	n	557	557	400	400	557	557	553	553
	OR adjusted (95% CI)	0.99 (0.86,1.16)	1.04 (0.91,1.19)	1.03 (0.57,1.85)	0.89 (0.52,1.53)	1.18 (0.97,1.45)	1.10 (1.00,1.19)[†]	1.67 (1.06,2.63)[†]	1.06 (0.77,1.45)

Data are presented as OR and 95% confidence intervals (95% CI) with the participants without asthma as reference. The logistic models were conducted with random effects on familial dependence (level 2) and city (level 3). Results are expressed per 20 µg/m³ increase of NO_x exposure, per 10 µg/m³ increase of NO₂, PM₁₀, O₃ and O₃-summer exposures, per 5 µg/m³ increase of PM_{2.5} exposure, per 4.000.000 vehicles x m /day increase of total traffic load and per 5.000 vehicles/day increase of traffic intensity.

Model 1: unadjusted; Model 2: adjusted for age, sex, smoking habits and body mass index. Model 3: adjusted for age, sex, smoking habits, body mass index and socio-professional category. Model 4: adjusted for age, sex, smoking habits, body mass index, socio-professional category and cleaning products. Model 5: after excluding participants who lived less than one year at the same address (N=46) and adjusted for age, sex, smoking habits, body mass index, socio-professional category and cleaning products.

[#]Estimates were also adjusted for background NO₂.

[†]Results in bold represent significant results (p-values≤0.05).

Table 3. Associations between outdoor air pollution and exhaled 8-iso concentration among participants without asthma.

		NO ₂	NO _x	PM ₁₀	PM _{2.5}	Total traffic load on major roads in a 100-m buffer of the home [#]	Traffic intensity at the road nearest to a participant's home [#]	O ₃	O ₃ -summer
Model 1	n	253	253	185	185	253	253	250	250
	β crude (95% CI)	0.05 (-0.008,0.11)	0.03 (-0.02,0.08)	0.13 (-0.10,0.36)	0.27 (0.05,0.49)[†]	0.07 (-0.04,0.18)	0.03 (-0.02,0.08)	-0.22 (-0.41,-0.03)[†]	-0.55 (-0.81,-0.28)[†]
	p-value	0.08	0.22	0.30	0.02	0.18	0.14	0.02	0.001
Model 2	n	253	253	185	185	253	253	250	250
	β adjusted (95% CI)	0.04 (-0.02,0.10)	0.02 (-0.03,0.07)	0.12 (-0.11,0.36)	0.25 (0.03,0.46)[†]	0.04 (-0.07,0.15)	0.02 (-0.03,0.07)	-0.20 (-0.38,-0.02)[†]	-0.52 (-0.78,-0.26)[†]
	p-value	0.24	0.59	0.33	0.03	0.50	0.37	0.03	0.002
Model 3	n	253	253	185	185	253	253	250	250
	β adjusted (95% CI)	0.03 (-0.03,0.09)	0.01 (-0.05,0.07)	0.11 (-0.12,0.34)	0.23 (0.005,0.46)[†]	0.03 (-0.09,0.15)	0.02 (-0.02,0.06)	-0.20 (-0.39,-0.01)[†]	-0.52 (-0.77,-0.26)[†]
	p-value	0.29	0.63	0.33	0.04	0.56	0.35	0.04	0.002
Model 4	n	233	233	172	172	233	233	230	230
	β adjusted (95% CI)	0.03 (-0.03,0.10)	0.008 (-0.05,0.07)	0.11 (-0.14,0.36)	0.23 (-0.002,0.46)[†]	0.04 (-0.08,0.16)	0.02 (-0.03,0.07)	-0.23 (-0.43,-0.03)[†]	-0.56 (-0.83,-0.29)[†]
	p-value	0.34	0.79	0.39	0.054	0.53	0.34	0.03	0.001

Data are presented as crude β and 95% confidence intervals (95% CI). The linear regression models were conducted with random effects on familial dependence (level 2) and city (level 3). 8-iso concentration was log10 transformed. Results are expressed per 20 μg/m³ increase of NO_x exposure, per 10 μg/m³ increase of NO₂, PM₁₀, O₃ and O₃-summer exposures, per 5 μg/m³ increase of PM_{2.5} exposure, per 4.000.000 vehicles x m/day increase of total traffic load and per 5.000 vehicles/day increase of traffic intensity.

Model 1: unadjusted; Model 2: adjusted for age, sex and smoking habits; Model 3: adjusted for age, sex, smoking habits and cleaning products. Model 4: after excluding participants who lived less than one year at the same address (N=46) and adjusted for age, sex, smoking habits, body mass index, socio-professional category and cleaning products.

[†]Estimates were also adjusted for background NO₂.

[#]Results in bold represent significant results (p-values≤0.05).

Figure legends

Figure 1. Flow chart of the studied population.

ESCAPE, European Study of Cohorts for Air Pollution Effects; 8-iso, 8-isoprostanes; LD, limit of detection of the 8-iso concentration; EBC, Exhaled Breath Condensate.

Figure 2. Associations between exhaled 8-iso concentration and current asthma.

8iso, 8-isoprostanes; LD, limit of detection of the 8-iso concentration.

The logistic models were conducted with random effects on familial dependence.

Estimates were adjusted for age, sex, smoking habits and body mass index. 8-iso concentration was log₁₀ transformed. Figures are OR (95% confidence intervals) with participants without asthma as reference.

#Result is expressed of an increase for one unit of the concentration in 8-iso.

¶The 8-iso concentration below the limit of detection was used as reference category.

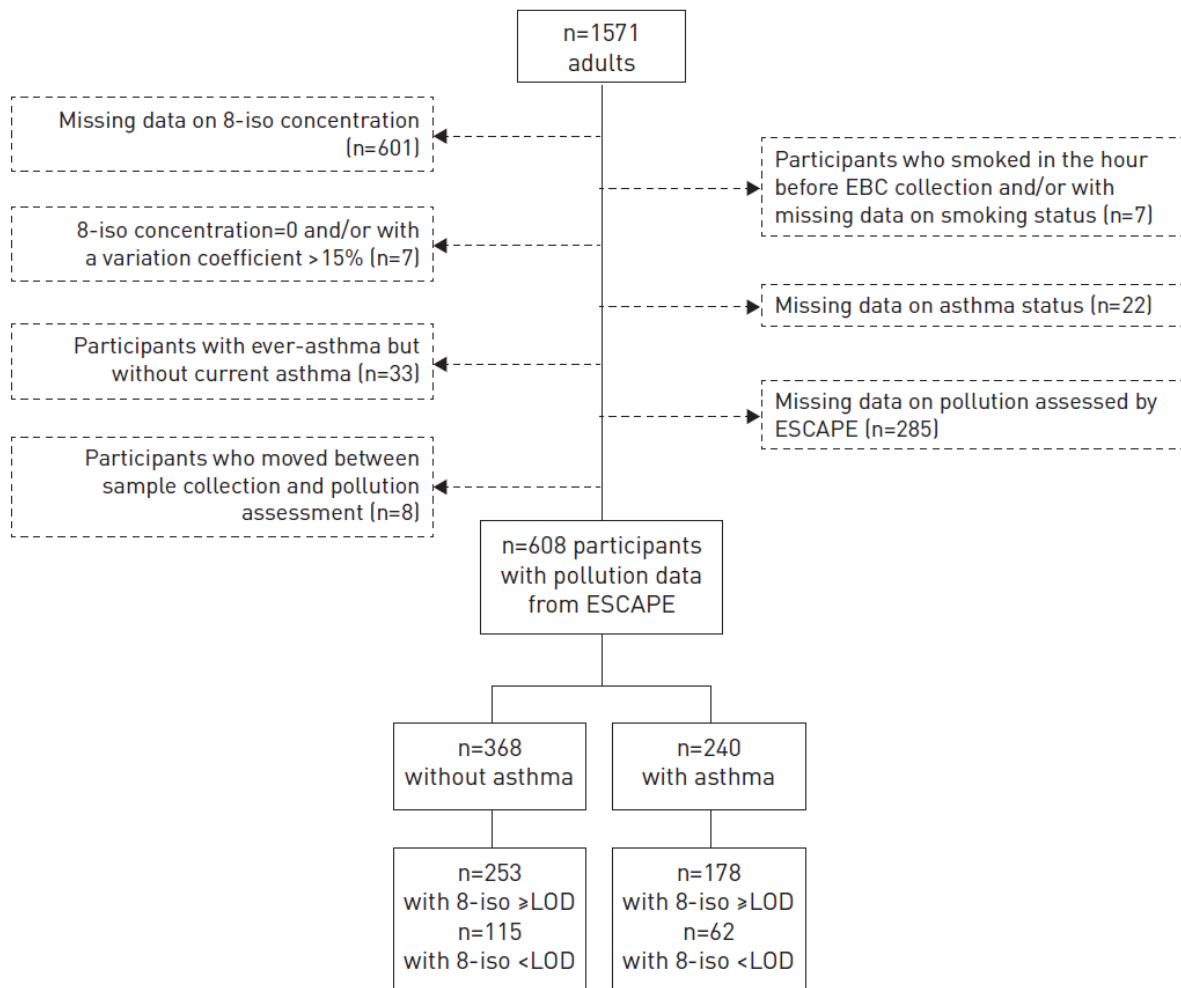


Figure 1

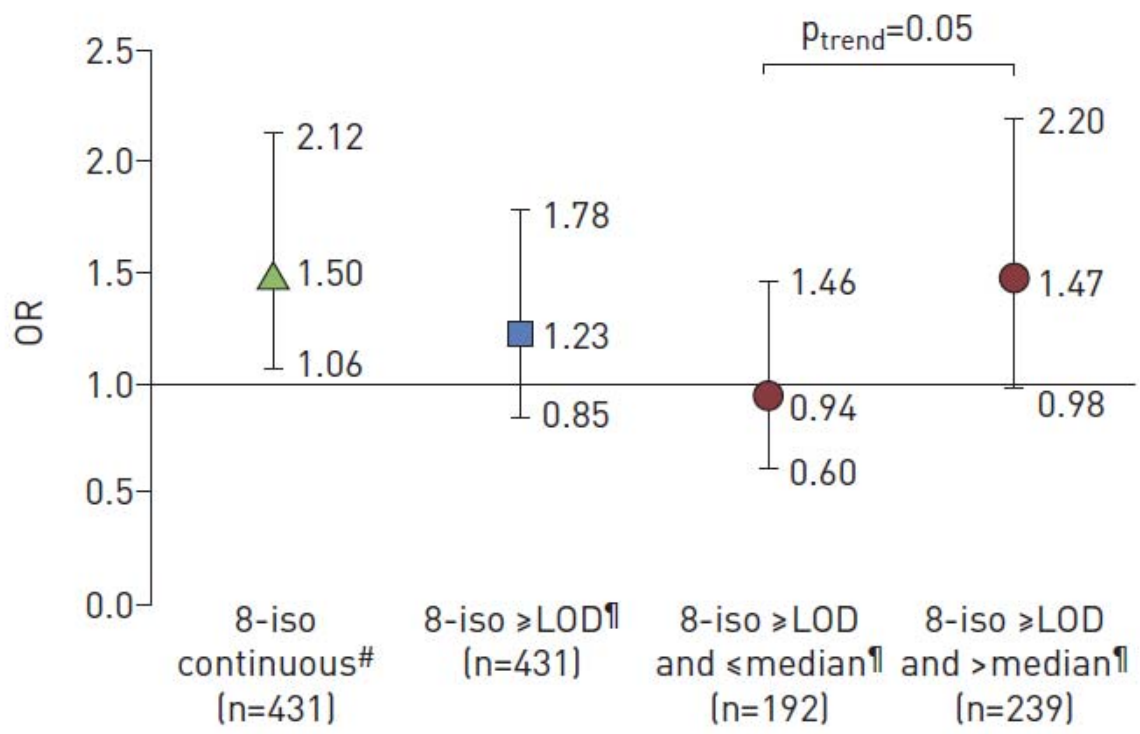


Figure 2