Ozone, NO2 and PM10 are associated with the occurrence of multiple sclerosis relapses. Evidence from seasonal multi-pollutant analyses

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Abstract

Background - Triggers of multiple sclerosis (MS) relapses are essentially unknown. PM_{10} exposure has recently been associated with an increased risk of relapses.

Objectives - We further explore the short-term associations between PM_{10}, NO_2, benzene (C_6H_6), O_3, and CO exposures, and the odds of MS relapses' occurrence.

Methods - Using a case-crossover design, we studied 424 MS patients living in the Strasbourg area (France) between 2000 and 2009 (1,783 relapses in total). Control days were chosen to be ±35 days relative to the case (relapse) day. Exposure was modeled through ADMS-Urban software at the census block scale. We consider single-pollutant and multi-pollutant conditional logistic regression models coupled with a distributed-lag linear structure, stratified by season ("hot" vs. "cold"), and adjusted for meteorological parameters, pollen count, influenza-like epidemics, and holidays.

Results - The single-pollutant analyses indicated: 1) significant associations between MS relapse incidence and exposures to NO_2, PM_{10}, and O_3, and 2) seasonality in these associations. For instance, an interquartile range increase in NO_2 (lags 0-3) and PM_{10} exposure were associated with MS relapse incidence (OR = 1.08; 95%CI: [1.03-1.14] and OR = 1.06; 95%CI: [1.01- 1.11], respectively) during the "cold" season (i.e., October-March). We also observed an association with O_3 and MS relapse incidence during "hot" season (OR = 1.16; 95%CI: [1.07-1.25]). C_6H_6 and CO were not significantly related to MS relapse incidence. However, using multi-pollutant models, only O_3 remained significantly associated with the odds of relapse triggering during "hot" season.

Conclusion - We observed significant single-pollution associations between the occurrence of MS relapses and exposures to NO_2, O_3 and PM_{10}, only O_3 remained significantly associated with occurrence of MS relapses in the multi-pollutant model.

Keywords: Multiple sclerosis; Relapse; Air pollution; Socioeconomic position.

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Written consent from subjects participating in the study was received prior to the study. Moreover, the study was approved by the ethics committee in accordance with the French law.

1. Introduction

Multiple Sclerosis (MS) is the most frequent neuro-inflammatory disease of the central nervous system (CNS), affecting almost 2.3 million people worldwide (1). The prevalence in France is about 1.5 individuals for 1,000 (2). In about 85% of cases, patients experience relapse(s) (1), that is, patients experience exacerbations of neurologic disabilities followed by partial or complete remission. Relapses characterize the relapsing-remitting MS course.

The etiology of MS remains unclear, yet clearly multifactorial (3). Genetic predispositions (*HLA-DRB1*, *IL2RA* and *IL7R* most associated genes) may represent only one quarter of estimated heritability of MS (4). Main environmental factors found to be influencing susceptibility to experience an MS onset are Epstein-Barr virus infection, tobacco consumption (either passive or active) and reduced levels of vitamin D increase or low sunlight exposure. In addition, women appear to be at greater risk than males (5–7). Relapses predisposing risk factors have been investigated to a lesser extent and associations reported are discordant, namely young age, short MS duration, low serum vitamin D levels, smoking, psychological and other stress, vaccination, infections, post-partum, breast feeding and assisted reproduction (3,8).

Relapses' incidence varies across seasons (9–17), suggesting a possible role of season-dependent factors such as meteorological variables (18) and ambient air pollution (19–21).

A few studies have investigated the role of air pollution in the development of MS, reporting mixed results (22–24). In Teheran, significant clustered patterns ($p<0.001$) and difference in exposure to sulfur dioxide (SO_2), PM_{10}, NO_2 and nitrogen oxides (NOx) were observed in MS cases compared to random controls (Heydarpour et al. 2014). Gregory et al. (2008) also suggested that PM_{10} might play a role in MS onset (Gregory et al. 2008). However, these studies suffered from a number of limitations (e.g. small sample size, imprecise exposure assessment etc.). Recently, Chen et al. 2017 observed, using a Cox proportional hazards model adjusted for individual features and latitude, that proximity living to heavy traffic was not associated with a higher incidence of MS (Chen et al. 2017). Concerning relapses, some studies investigated the link between air pollution and the odds of relapses triggering (17,20,21,25,26), MS-related hospitalization (19) and MS inflammatory activity (27). Associations were observed in several studies between PM_{10} exposure and MS relapse risk (19–21,27). Among studies that examined possible influence of other air pollutants such as

CO, O_3, SO_2 and NOx (17,20,25), one study reported exposure to acidic gas (NO_2, NO and SO_2) to be associated with risk of relapse (20). Overall, the authors did not take into consideration pertinent confounding factors such as meteorological parameters, infections etc.

There is a need to consider multi-pollutant models in environmental epidemiology, especially in studies relating MS occurrence to air pollutants' concentrations. The environmental health-related associations are complex to investigate in observational studies due to the high correlations and possible interactions between pollutants, as well as the seasonality of the pollutants' concentrations. To our knowledge, no study has yet considered multi-pollutant analyses to estimate the associations between air pollution and MS.

In the present study, we estimate the associations of PM_{10} and other ambient air pollutants (NO_2, benzene (C_6H_6), CO and O_3) using a multi-pollutant models to address the confounding issue due to the high correlations between pollutants originating from the same source.

2. Materials and Methods

2.1. Study design

We conducted a time-stratified case-crossover design to explore the associations between short-term air pollutants variations (i.e., PM_{10}, NO_2, C_6H_6, O_3, and CO) and multiple sclerosis relapses occurrences (28). This study design consists in within-subject comparisons by selecting for each patient his/her own control, i.e., the air pollutant exposure levels of the same patient will be compared between days of relapse onset (case) and days without any relapse (control). A time-stratified approach was chosen to define control days (29). That is, control days were chosen to be ±35 days relative to the case (relapse) day. This 35-days interval choice was motivated by the relapse clinical definition which confined a minimum of 30 days between two relapses. Every control day between the 30^{th} and 35^{th} day was excluded, justifying the reason why 158 case days only have a single control instead of two.

Time-invariant or long-term varying confounders, such as individual characteristics and behaviors, are controlled through within-subject comparisons. This approach also permits to tackle time trends such as seasonality, between days of the week variation, and the temporal autocorrelation (29).

2.2. Study setting

The Strasbourg Metropolitan Area (SMA), located in North-Eastern France, aggregates 28 municipalities, 21 of which are rural and seven urban. The SMA sprawls over 316 km² with 450,000 inhabitants. It is subdivided into 186 French census blocks, defined as a sub-municipal division and designed by French National Institute for Statistics and Economic Studies (INSEE). Census blocks are devised according to land use, homogeneity of population size and socio-economic features. They are the smallest spatial unit in France for which socio-economic data are made available due to French confidentiality regulations. In average, a census block is 2,000 inhabitants (ranging from 2 to 4,885), with a surface from 0.05 km² up to 19.6 km².

2.3. Study population and environmental data

2.3.1. Patients' and relapses' inclusion criteria

Patients' data were provided by the multiple sclerosis network alSacEP based in Alsace North-Eastern region, since 2006. All patients were managed through the European Database for Multiple Sclerosis (EDMUS) using a standardized definition and management of patients' data (30).

Study period was January 1, 2000 to December 31, 2009. Inclusion criteria for patients were: i) clinical definition fitting McDonalds' MS criteria; *ii*) first symptoms of MS occurred before December 31th, 2009; *iii*) patients were affected with relapsing-remitting and secondary progressive forms; *iv*) residence address within Strasbourg Metropolitan Area.

When the day of relapse occurrence was doubtful (uncertain or unknown), the relapse (*i.e.*, case day) was excluded, leading to additional patients exclusions.

The French Authority for Data Confidentiality (CNIL) approved the present protocol (DR-2015-504).

2.3.2. Air pollution data

Air pollution concentrations of PM_{10}, NO_2, C_6H_6, O_3, and CO were estimated throughout the study period on an hourly basis at the census block scale. The deterministic ADMS-Urban air dispersion model was used considering different parameters, namely background pollution

measurements, emissions inventories, meteorological data but also land use or surface roughness (Atmospheric Dispersion Modeling System) (31). Details of parameters and model performance have been previously discussed (Havard et al. 2009; Bard et al. 2014). Previous works have shown that air pollution assessment performance proved excellent results (35): coefficients for the modeled and effectively measured ambient concentrations were highly correlated 0.87 for NO_2, 0.73 for PM_{10} and 0.84 for O_3.

We did not assess the plausible role of sulfur dioxide (SO_2) which was suggested in the recent literature (24) because i) Strasbourg Metropolitan Area concentrations are low ($\leq$11 $\mu g/m^3$) in the Alsace region, and ii) it originates from a single location, which altogether decrease the preciseness of the modelling. Concentrations of $PM_{2.5}$, which represents a substantial proportion of PM_{10} (36), were not measured routinely during the study period. PM_{10} is a proxy measure of $PM_{2.5}$. The potential interventions to reduce PM_{10} and $PM_{2.5}$ involve similar sources. However, benzene, rarely measured by air pollution monitoring systems, was properly measured and modeled in our study setting.

2.3.3. Established or likely confounding variables

According to the literature on the link between air pollution and different health outcomes occurrence and especially MS, we considered different time-varying confounders in our study. Meteorological parameters (daily temperature, relative humidity and atmospheric pressure) were obtained from the French meteorological service (Météo France). Daily pollen counts were provided by the National Network of Aerobiological Surveillance (37). Weekly influenza-like case count was given by the "Sentinelles" network (38) of the French National Institute of Health and Medical Research (INSERM). We considered holidays, which could potentially influence industrial activities and road traffic, as well as stress level, fatigue or being at home or not.

2.4. Data analysis

Associations between exposures to air pollutants and the occurrence of relapses were estimated by fitting distributed-lag linear models within conditional logistic regressions. We examined how the associations between lagged exposures and the outcomes varied across lags. This methodology, previously developed for the analysis of time-series data (39), was performed here in the context of case-crossover data.

Seasonal variations occur for both air pollutants concentrations and MS incidence (12). Therefore, we fitted regression models separately for "hot" (April 1st to September 30th) and "cold" seasons (October 1st to March 31th). We estimated odds ratios corresponding to an interquartile range (IQR) increment of concentration ($\mu g/m^3$). An association was considered significant if the p-value was less or equal to 0.05. All statistical analyses were performed using R software (v. 3.2.3) (40) and the "*dlnm*" R package (41).

An unconstrained (i.e. nonparametric) lag structure was used for the initial analysis. Then, we considered another choice of distributed-lag function that assumes a constant lag effect within days. The constant lag modeling prevents overfitting of the data. This assumption is equivalent to fitting a constrained model that includes consecutive daily exposure moving average. Because some pollutants were highly correlated, we fitted multi-pollutant models to determine which pollutant(s) explained the single-pollutant associations. The collinearity between the independent variables of the multi-pollutant models was assessed with the Generalized Variance Inflation Factor ($GVIF^{(1/[2df])}$)(42). To assess collinearity, values of GVIF were compared to the threshold of 10, which was considered as a maximum value according to the literature (43). Each model was adjusted on all lagged (including lag 0) daily concentrations and daily temperature to also take into account the exposure correlation between the lags.

3. Results

3.1. Individual demographic and clinical patients' characteristics

We obtained carefully verified data for 1,783 relapses and selected 3,408 control days from 424 patients. Data were analyzed separately for "cold" and "hot" seasons (*i.e.*, 888 case days and 1,703 control days during the "hot" season and 895 case days and 1,705 controls during the "cold" season) over the 2000-2009 study period. All of them were living in 145 French census blocks of the Strasbourg Metropolitan Area (SMA) at some time over the study period.

Patients' characteristics are presented in Table 1. Sex ratio (Female:Male) was 2.93 and patient's mean age at MS clinical onset was 30.5 (±10.0) years old. Throughout the study period, patients experienced in average 4.2 relapses (2.11 in cold season and 2.09 in hot season).

Most of the patients included were affected with relapsing-remitting MS form (83.0%). The others were affected with secondary progressive form (17.0%).

3.2. Environmental data and flu-like infections

A description of environmental data is detailed in Table 2. Over the study period, mean concentrations of PM_{10}, NO_2, C_6H_6, and CO in the SMA were higher during "cold" than "hot" season. By contrast, mean concentrations of O_3 were higher during "hot" than "cold" seasons (respectively 86.8±30.9 µg/m^3 and 37.3±20.2 µg/m^3, p<001). As expected, pollutants were highly correlated (Table 3, Figure 1). Concentrations of PM_{10}, NO_2, CO and benzene were highest in the center of the CUS and concentrations of O_3 were highest in the periphery (Figure 1). We observed seasonal variations in pollen counts (higher in the "hot" season) and flu-like infections (higher in the "cold" season) (Table 2).

3.3. Relation between air pollutants and the occurrence of relapses

3.3.1. Unconstrained distributed-lag single pollutant models

Figure 2 presents the associations between air pollutants concentrations (for every lag of the week preceding the relapse) and the occurrence of relapses, separately for "hot" and "cold" seasons. We observed significant negative and positive associations on several distinct days, when adjusting individually on lagged daily air pollutants concentrations, lagged daily maximum temperature, day of relapse maximum relative humidity, maximum atmospheric pressure, as well as pollen count, influenza-like epidemics, and holidays.

We observed a significantly increased risk (about 40%) with NO_2 exposure at lag 1 in "hot" season, but no association during "cold" season. For PM_{10}, we found an increased risk at lag 1 in "hot" season (OR = 1.26 [1.03-1.54]) whereas lag 2 was significant in "cold" season (OR= 1.28 [1.05-1.55]). Exposure to benzene was significantly associated with 30% excess risk in the “cold” season only, at lag 2 (OR = 1.32 [1.05-1.67]). We found that CO exposure increased the odds of MS relapse to a lesser extent in both "cold" (20%, lag 3) and "hot" season (30%, lag 2). The strongest association (roughly 60%) was observed with O_3 exposure during "hot" season at lag 2. However, we noticed significant inverse associations at lag 3 in "hot" season for NO_2 (OR = 0.82 [0.68-0.99]) and for benzene (OR = 0.81 [0.67-0.98]), which motivated the multi-pollutant approach presented in Section 3.3.3.

3.3.2. Constrained distributed-lag single pollutant models

We also assumed a constant lag effect within days (Table 4 and Figure 3). Finally, we checked whether the associations we observed were still significant when considering a longer period (i.e. lags 0-6, one-week period) (Table 4). As compared to the unconstrained models, we observed a much lower increased risk (less than 10%) for both NO_2 and PM_{10} and only in "cold" season for lag 0-3 (respectively, OR = 1.08 [1.03-1.14] and OR = 1.06 [1.01-1.11]) (Table 4 and Figure 3). In contrast, O_3 exposure was significantly associated with an excess MS relapse risk of 16% in "hot" season only, lag 0-3 (OR = 1.16 [1.07-1.25]). Exposure to C_6H_6 yielded borderline significant excess risk estimates, during "cold" season, lag 0-3 (OR = 1.05 [1.00-1.10]) and inversely in "hot" season (OR = 0.98 [0.94-1.02]). We found no association with CO exposure. Associations with PM_{10} and NO_2 exposures were no longer significant when one week of exposure (lag 0-6) was considered (respectively, OR = 1.02 [0.99-1.05]; and OR = 1.03 [1.00-1.07]) (Table 4). However, associations with O_3 remained significant although weaker when one week of exposure was considered (OR = 1.13 [1.07-1.19]).

3.3.3. Constrained distributed-lag multiple-pollutant models

Table 5 displays the multi-pollutant analyses results using the same constrained adjusted models as above. We estimated the associations between the odds of relapses using the all-pollutant model ($M_{Full/p=5}$) and PM_{10} exposure during "cold" season. The estimate was no longer significant (OR = 1.02 [0.95-1.11]) as compared to the results from single pollutant models (Table 4). The NO_2-MS relapse associations were borderline significant (OR = 1.08 [1.00-1.18]). In the "hot season", only the PM10-MS relapse association remained borderline significant (OR = 1.07 [1.00-1.15]).

When the model was fitted with all the pollutants except NO_2, the risk of relapses with PM_{10} exposure became significant in "hot" season (OR = 1.08 [1.03-1.13]) as compared to the single pollutant analysis (Table 4) where no association was observed. An equivalent magnitude of risk was observed from single to multi-pollutant models with O_3 exposure. When the model was run excluding PM_{10}, the risk of relapse associated with NO_2 exposure in "cold" season was very close to the single pollutant results. While benzene and CO exposures in “hot” season were not associated with risk in the single pollutant analyses, we observed inverse significant associations using multi-pollutant models: for benzene, excluding NO_2, OR = 0.96 [0.93-0.99]; for CO, excluding O_3, OR = 0.89 [0.81-0.99].

Collinearity between pollutant variables across multi-pollutant models was considered as moderate (all $GVIF^{(1/[2df])} < 10$, and most of them < 2) (Table 6).

4. Discussion

We have shown significant associations between short-term exposure to ambient air pollutants (NO_2, PM_{10} and O_3) and the occurrence of MS relapses in a French population-based study spanning 10 years (2000-2009) (Table 4). We confirmed our previous results (21) on PM_{10}-associated risk using a more sound statistical approach. In particular, our model-adjustments were more precise, *i.e.*, we consider all lagged (including day 0 to 3 instead of 1 to 3) of daily concentrations and daily temperature instead of the single day of relapse, and therefore took into account the correlation between the lag exposures. We estimated odds ratios corresponding to an interquartile range (IQR) increment of concentration (µg/m3) instead of a one-unit increment of ln PM_{10}. These differences in methodology might explain the variation of magnitude observed between both studies (present analysis OR = 1.06 [1.02-1.11]: previous OR = 1.40 [1.08-1.81]) (21). We investigated the relation between air pollutants and the occurrence of relapses on each day of a one-week period before onset, which is supposed to be the maximum air pollution effect (19). When fitting the models considering one week of exposure to air pollutants, we observed no association beyond lag 3 (except a “protective” effect for CO at lag 5 in "hot" season) (Figure 2). These observations yield to conduct analysis considering only 3 days (instead of 6) before the occurrence of relapses and we did not observe any major difference (results not shown). Our results are in line with those of the literature (19,20,27) which reported a short term association between PM_{10} and the odds of relapse (19,27).

Since air pollutants are highly correlated and that correlations vary across season (Table 3) due to complex reactions such as photochemical reactions which necessitate sunlight, we explored the impact of air pollution testing multi-pollutant models (Table 5). Several associations decreased in magnitude when adding pollutants in the model, It might be due to the relation between pollutants, such as collinearity (44). Only O_3 was systematically significantly associated with MS risk during "hot" season, using one, four, or five pollutants in the model. However, variations observed by single pollutant to multi-pollutant models were very limited in size. O_3 showed the largest association with the odds of MS relapse. In this study, we provide pollutant-specific comparisons. Results of PM_{10} in the multi-pollutant

models were partly confounded by NO_2 and vice versa. Indeed, the both pollutants are highly correlated for both seasons (around 0.70). We did not conduct any bi-pollutant analyses because we considered that it did not reflect actual exposures and that adding statistical tests might lead to observe false positives which could alter the interpretation. Collinearity may be an important issue in multi-pollutant models, so that we have calculated the $GVIF^{\wedge(1/(2df))}$ for each pollutant included in the multi-pollutant distributed-lag models. As coefficient values were low (<5), all multi-pollutant associations were reported.

Air pollutants' concentrations exhibit seasonal patterns, leading us to conduct analyses according to season ("cold": from October to March; "hot": from April to September) rather than fitting models adjusting for season as in the Italian study (19). Farez et al. (2015) observed that melatonin, which is a neuro-hormone regulated by seasonal variation in sunlight level and especially night length, was inversely correlated with relapses incidence (45). Yet, some studies have reported associations between UV level and MS exacerbations (16,17), suggesting a possible role of erythemal ultraviolet radiation in the production of serum vitamin D production that might influence relapse incidence (17). However, Hardin et al. (2017) reported highest risks in early summer when sunshine duration is elevated and lowest risks at the end of summer (12), suggesting that vitamin D might not be the only environment factor incriminated in MS relapses (e.g., interactions etc.). Our results seem to corroborate seasonal changes in MS activity, as associations with air pollutants varied across season, so as established or likely confounding variables we accounted in our models (meteorological, infections and allergy variables). Indeed, we observed differences in risks between air pollutants concentrations and risk of relapses according to "hot" and "cold" seasons, yet this categorization is only an uncertain proxy of UV exposure. Moreover, PM_{10} and NO_2 concentrations were associated with MS relapses occurrence only in "cold" months, while O_3 was associated in "hot" months. Seasonal changes in air pollutants concentrations, the type of area (e.g., topography, level of traffic etc.) and the seasonal possible individual air pollutant effect on MS activity could explain mixed results concerning seasonal pattern in relapses rate observed in the literature (12). We adjusted on holidays since they may influence industrial activities and road traffic but also and particularly the presence of patients at home. We have previously shown that holidays suggested a "protective" association during "cold" months (21). This result could be related with a drop in stress-related work (46) or a leave for areas

featuring different exposure patterns. In addition, during summer, patients are more likely to stay outside and might be more exposed to ozone.

Limitations - We considered patients' living address only, which can be a limitation as much they could spend a part of their time out of home (e.g., commuting, job site, leisure...). However, patients affected with MS generally see their mobility reduced along with the course of the disease. Therefore, there are expected to spend more time at home with time (47). We did not adjust for sunlight exposure, which is reported to be related to MS incidence (12).

Strengths - This study has several strengths. First, we conducted multi-pollutant models to determine pollutant-specific associations despite the high collinearity between pollutants. Second, a state-of the-art case ascertainment through the systematic reporting of patients followed up within the EDMUS database (30). Because the data collection started before the study period, most of the relapses were prospectively collected and dates of relapses occurrence were set by neurological experts. We also used a robust and accurate exposure assessment at the census blocks scale, limiting exposure misclassification to the extent possible. Finally, we adjusted models with a number of confounders known to be season-dependent.

The role of air pollution in the pathogenesis of MS remains to be fully elucidated. Recently, Esmaeil Mousavi et al. (2017) formulated the assumption that air pollution might impact MS incidence and activity through biological mechanisms inducing neuroinflammatory-oxidative cascades reactions, decrease of immunological self-tolerance and neurodegeneration (i.e. axonal deterioration and neuronal loss) that finally conduct to autoimmunity (48). For instance, some of those mechanisms enrolled could be the blood brain barrier breakdown, a mitochondrial dysfunction, an overproduction of free radicals or the expression of inflammatory factors. Moreover, showing a significant association between PM_{10} concentrations before magnetic resonance imaging (MRI) examination and MRI Gadolinium-enhancing lesions, an Italian study provided recent additional evidence that ambient air pollution might be a determinant for MS inflammatory relapses triggering (27). Epigenetic changes in autoimmune disease also occur, especially changes in DNA methylation (49).

5. Conclusion

Using a precise exposure spatio-temporal model and a clinically-diagnosed outcome from a fairly exhaustive multiple sclerosis registry, we reported associations between exposures to air pollutants (NO_2, PM_{10} and O_3) and the risk of MS relapses, when pollutants were assessed individually. In a multi-pollutant model, only O_3 was significantly associated with the risk of MS triggering. When assessing the link between exposure to PM_{10} and MS relapses, we recommend adjusting for NO_2 level and vice versa. Taken together, these findings enhanced our understanding of the plausible association between air pollution exposure and MS relapses but further research is needed to confirm this hypothesis. Our observation of an association with PM_{10} is in line with the results of the few studies published so far. Yet, the association we observed with NO_2 and O_3 is to our knowledge unprecedented.

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Conflicts of interest

The authors declare no conflicts of interest.

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523 Table 1. Baseline characteristics of the 424 patients included in the study.

	Multiple Sclerosis patients N = 424
Demographic characteristics	
Gender	
Males	108 (25.5%)
Females	316 (74.5%)
Clinical characteristics	
Mean age at MS onset (± sd), years	(30.5±10.0)
Mean follow-up duration (± sd), years	(6.6±3.5)
Mean relapses per patient (between 2000 and 2009)	(4.2±4.7)
MS form (at last information)	
Relapsing-Remitting	352 (83.0%)
Secondary Progressive	72 (17.0%)

524 sd: standard deviation

525

Table 2. Baseline characteristics of daily air pollutants concentrations, meteorological features, pollen and infections across Strasbourg (France) Metropolitan Area, 145 census blocks (2000-2009).

	"Hot" season[a]							"Cold" season[a]						
Parameters[b]	**Mean**	**Sd[c]**	**Min.**	**Q_1**	**Median**	**Q_3**	**Max.**	**Mean**	**Sd**	**Min.**	**Q_1**	**Median**	**Q_3**	**Max.**
Air pollutants (µg/m^3)														
PM_{10}	18.94	6.78	4.68	13.88	17.97	22.98	51.59	23.70	12.69	2.55	14.52	21.59	30.12	101.50
NO_2	29.30	11.66	4.06	20.95	28.16	36.45	92.31	38.66	13.90	3.32	28.88	38.60	48.02	95.90
C_6H_6	1.10	0.59	0.14	0.71	0.97	1.34	4.67	2.23	1.24	0.19	1.33	2.02	2.91	10.58
CO	577.7	58.54	501.9	536.8	565.1	603.7	998.3	608.1	84.26	502.7	546.4	585.9	647.8	1175.0
O_3	86.85	30.89	15.12	65.49	83.44	105.20	221.00	37.26	20.19	2.44	20.47	35.66	51.45	107.20
Meteorological features														
Maximum temperature (°C)	22.61	5.56	5.10	19.10	22.80	26.50	38.50	9.45	6.02	-10.30	5.38	9.60	13.52	28.50
Maximum atmospheric pressure (hPa)	1010.01	10.41	979.10	1001.00	1011.00	1019.00	1031.00	1013.00	13.03	970.30	1003.00	1013.00	1023.00	1043.00
Maximum relative humidity (%)	91.99	5.49	59.0	90.00	93.00	96.00	100.00	93.86	4.35	72.00	92.00	95.00	97.00	100.00
Allergy														
Pollen count (daily mean number of grains/m^3)	130.30	210.10	0.00	21.16	69.84	158.10	2368.00	40.04	130.55	0.00	0.00	0.00	15.64	1519.00
Infections														
Influenza-like epidemics (Nb of cases per week)	48.73	107.87	0.00	0.00	0.00	59.00	851.00	453.50	587.02	0.00	112.00	254.00	535.00	3486.00

[a]"Hot" season (April to September) and "Cold" season (October to March);
[b]Estimations for the 145 census blocks of Strasbourg Metropolitan Area;
[c]Sd: Standard deviation;
Wilcox.test (hot vs. cold): Significant ($p<0.001$) for all the PM_{10}, NO_2, C_6H_6, O_3 and CO pollutants.

Table 3. Air pollutants daily mean concentrations value correlation coefficient (r), Strasbourg *(France)* Metropolitan Area, 145 census blocks (2000-2009).

		PM_{10}		NO_2		C_6H_6		CO		O_3	
		"cold"	"hot"	"cold"	"hot"	"cold"	"hot"	"cold"	"hot"	"cold"	"hot"
PM_{10}	"cold"	1									
	"hot"		1								
NO_2	"cold"	0.71		1							
	"hot"		0.70		1						
C_6H_6	"cold"	0.72		0.64		1					
	"hot"		0.50		0.59		1				
CO	"cold"	0.55		0.72		0.59		1			
	"hot"		0.46		0.59		0.57		1		
O_3	"cold"	-0.39		-0.48		-0.54		-0.48		1	
	"hot"		0.29		-0.06		-0.18		-0.21		1

Correlation coefficient (r): Pearson'r

Table 4. Estimated risk for associations between exposure to air pollutants concentrations (lags 0-3 days) and risk of MS relapse triggering, according to season.

		"Hot" season (n = 2,594)[a]		**"Cold" season (n = 2,597)[a]**	
	Parameter (µg/m^3)[d]	OR[b]	$CI_{95\%}$[c]	OR	$CI_{95\%}$
PM_{10}	Lags 0-3	1.04	[0.99-1.09]	**1.06**	**[1.01-1.11]**
	Lags 0-6 (one week)	1.03	[1.00-1.06]	1.02	[0.99-1.05]
NO_2	Lags 0-3	1.01	[0.96-1.07]	**1.08**	**[1.03-1.14]**
	Lags 0-6 (one week)	1.02	[0.98-1.05]	1.03	[1.00-1.07]
C_6H_6	Lags 0-3	0.98	[0.94-1.02]	1.05	[1.00-1.10]
	Lags 0-6 (one week)	0.99	[0.96-1.02]	1.02	[0.99-1.05]
CO	Lags 0-3	0.96	[0.90-1.02]	1.04	[0.98-1.10]
	Lags 0-6 (one week)	0.97	[0.93-1.01]	1.02	[0.99-1.06]
O_3	Lags 0-3	**1.16**	**[1.07-1.25]**	0.96	[0.90-1.03]
	Lags 0-6 (one week)	**1.13**	**[1.07-1.19]**	0.98	[0.94-1.02]

[a]"Hot" season (April to September) and "Cold" season (October to March) / n = Number of cases and control days; [b] Odds-ratio (lags 0 to 3 days and lags 0 to 6 days, constant lag effect). OR corresponds to an interquartile increment of concentration; [c] 95% Confidence Interval. Multivariate conditional logistic regression models were adjusted on all lagged daily air pollutants concentrations, daily maximum temperature, day of relapse maximum relative humidity, maximum atmospheric pressure, pollen count, influenza-like epidemics, and holidays.

1 Table 5. Multi-pollutant analysis.

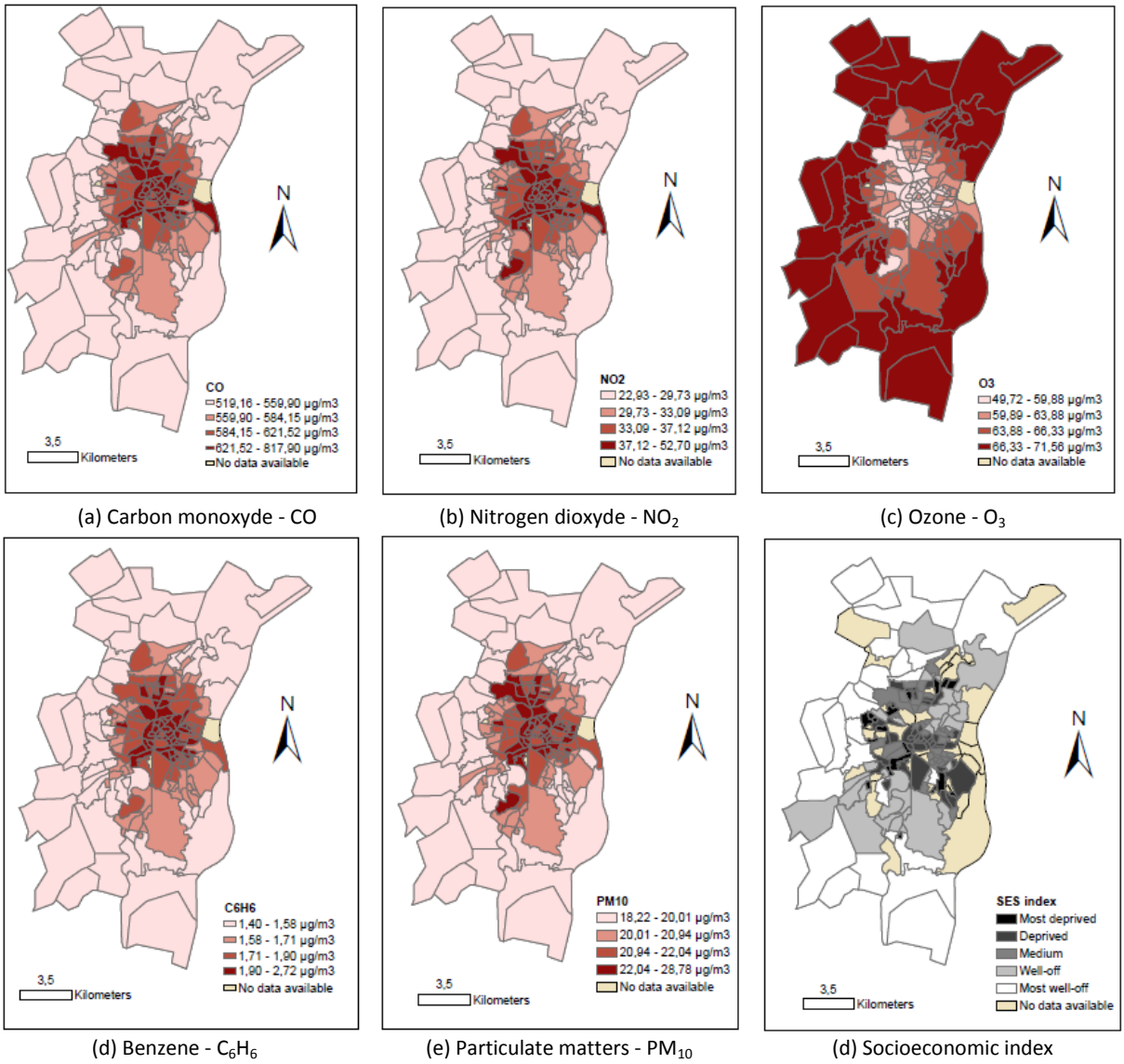
	PM_{10}				**NO_2**				**C_6H_6**				**CO**				**O_3**			
	"Hot"[a]		"Cold"[a]		"Hot"		"Cold"		"Hot"		"Cold"		"Hot"		"Cold"		"Hot"		"Cold"	
	OR[b]	$CI_{95\%}$[c]	OR[b]	$CI_{95\%}$[c]	OR	$CI_{95\%}$	OR	$CI_{95\%}$	OR	$CI_{95\%}$	OR	$CI_{95\%}$	OR	$CI_{95\%}$	OR	$CI_{95\%}$	OR	$CI_{95\%}$	OR	$CI_{95\%}$
Multi-pollutant (p=4)[d]																				
$M_{noPM10\ /\ p=4}$	-		-		1.07 [0.98-1.17]		**1.10 [1.02-1.18]**		0.97 [0.92-1.02]		1.02 [0.96-1.08]		0.94 [0.84-1.04]		0.97 [0.89-1.05]		**1.13 [1.04-1.23]**		1.01 [0.93-1.10]	
$M_{noNO2\ /\ p=4}$	**1.08 [1.03-1.13]**		1.06 [0.99-1.14]		-		-		**0.96 [0.93-0.99]**		1.01 [0.94-1.08]		0.95 [0.90-1.00]		1.00 [0.92-1.08]		**1.12 [1.01-1.24]**		0.99 [0.92-1.07]	
$M_{noC6H6\ /\ p=4}$	1.05 [0.98-1.13]		1.03 [0.96-1.10]		1.01 [0.91-1.11]		1.09 [1.00-1.18]		-		-		0.93 [0.84-1.04]		1.01 [0.93-1.09]		**1.12 [1.04-1.22]**		0.96 [0.89-1.04]	
$M_{noCO\ /\ p=4}$	1.07 [1.00-1.15]		1.02 [0.94-1.10]		0.98 [0.91-1.06]		1.07 [0.99-1.16]		0.95 [0.90-1.00]		1.00 [0.94-1.08]		-		-		**1.14 [1.05-1.23]**		1.02 [0.94-1.11]	
$M_{noO3\ /\ p=4}$	**1.09 [1.01-1.17]**		1.02 [0.95-1.11]		1.04 [0.95-1.15]		1.08 [1.00-1.18]		0.95 [0.90-1.00]		1.00 [0.94-1.08]		**0.89 [0.81-0.99]**		0.96 [0.89-1.04]		-		-	
All-pollutant (p=5)[e]																				
$M_{Full\ /\ p=5}$	1.07 [1.00-1.15]		1.02 [0.95-1.11]		1.02 [0.92-1.13]		1.08 [1.00-1.18]		0.95 [0.90-1.01]		1.00 [0.94-1.08]		0.94 [0.85-1.05]		0.96 [0.89-1.04]		**1.12 [1.03-1.22]**		1.01 [0.93-1.10]	

2 [a]"Hot" season (April to September) and "Cold" season (October to March) / n = Number of cases and control days; [b] Odds-ratio concentrations (lags 0 to 3 days, constant lag effect). OR
3 corresponds to an interquartile increment of concentration; [c] 95% Confidence Interval. Multivariate conditional logistic regression models were adjusted on all lagged daily air pollutants
4 concentrations, daily maximum temperature, day of relapse maximum relative humidity, maximum atmospheric pressure, pollen count, influenza-like epidemics, and holidays.
5 [d]Multi-pollutant models (p = 4: four pollutants included in the model).
6 [e]All-pollutant models (p = 5: all five pollutants included in the model).

Table 6. Generalized Variance Inflation Factor values for pollutants by multi-pollutant models.

		GVIF$^{(1/[2df])}$ [a]	
		"Hot"[b]	"Cold"[b]
Multi-pollutant (p=4)[c]			
$M_{noPM10\ /\ p=4}$	NO_2	1.93	1.74
	C_6H_6	1.39	1.58
	CO	1.80	1.66
	O_3	2.26	2.06
$M_{noNO2\ /\ p=4}$	PM_{10}	1.68	2.07
	C_6H_6	1.44	1.87
	CO	1.43	1.58
	O_3	2.25	2.05
$M_{noC6H6\ /\ p=4}$	PM_{10}	1.91	1.92
	NO_2	2.26	1.91
	CO	1.79	1.68
	O_3	2.29	2.05
$M_{noCO\ /\ p=4}$	PM_{10}	1.98	2.24
	NO_2	1.81	1.79
	C_6H_6	1.45	1.87
	O_3	2.14	1.98
$M_{noO3\ /\ p=4}$	PM_{10}	1.96	2.27
	NO_2	2.25	1.90
	C_6H_6	1.46	1.85
	CO	1.68	1.61
All-pollutant (p=5)[d]			
$M_{Full\ /\ p=5}$	PM_{10}	1.98	2.27
	NO_2	2.28	1.92
	C_6H_6	1.45	1.87
	CO	1.80	1.68
	O_3	2.29	2.06

[a] $GVIF^{(1/[2df])}$: Generalized Variance Inflation Factor; Coefficient used to assess collinearity.
[b] "Hot" season (April to September) and "Cold" season (October to March).
[c] Multi-pollutant models (p=4: four pollutants included in the model).
[d] All-pollutant models (p=5: all five pollutants included in the model).



(a) Carbon monoxyde - CO (b) Nitrogen dioxyde - NO_2 (c) Ozone - O_3

(d) Benzene - C_6H_6 (e) Particulate matters - PM_{10} (d) Socioeconomic index

Figure 1. Ambient air pollution concentrations and SES index across Strasbourg Metropolitan Area census blocks (2000-2009).

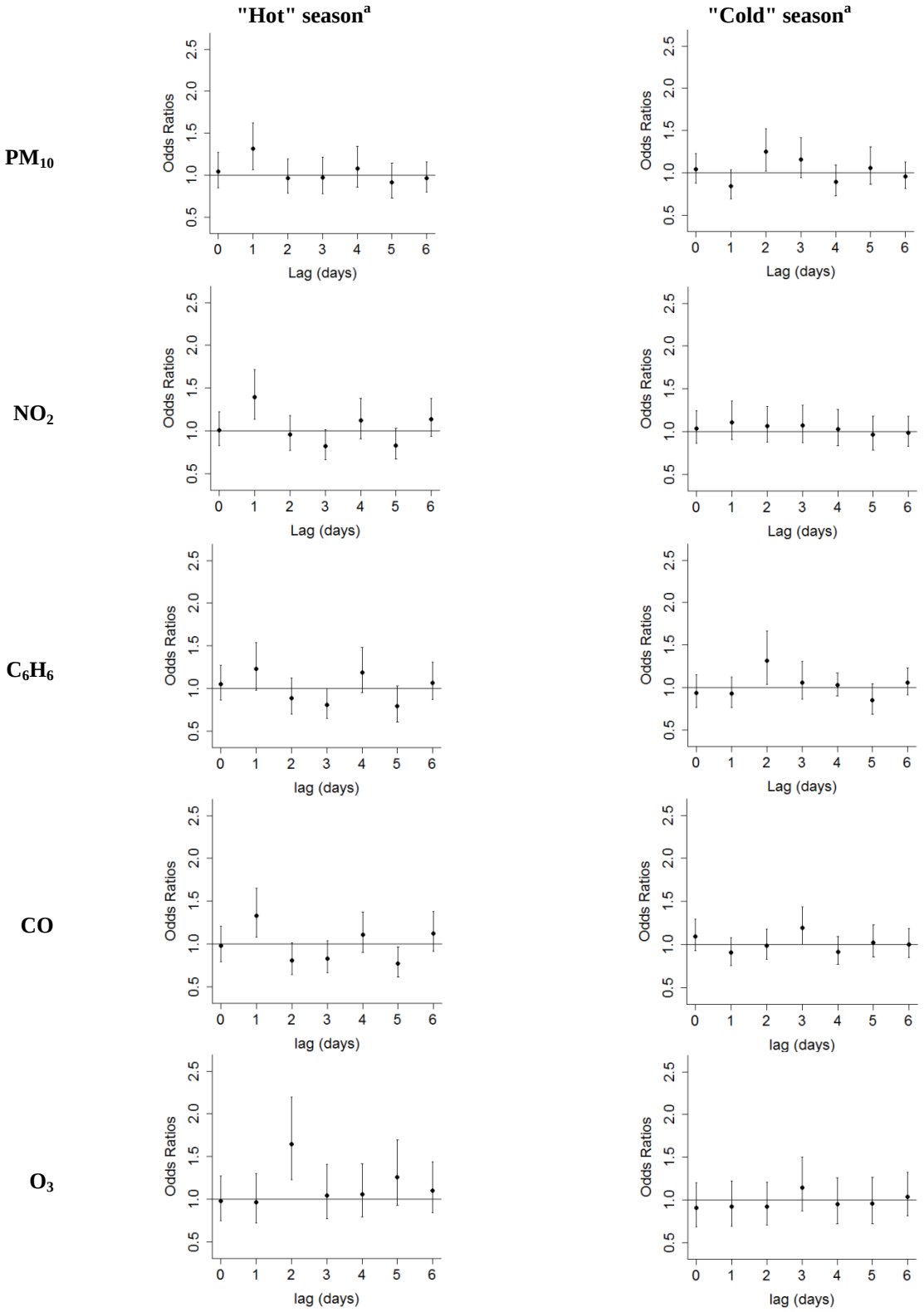


Figure 2. Associations between exposure to air pollutants concentrations (lags 0-6 days before relapse onset, inconstant lag effect) and risk of MS relapse triggering, according to season.

[a]"Hot" season (April to September) and "Cold" season (October to March); Odds-ratio (lags 0 to 6 days, inconstant lag effect). OR corresponds to an interquartile increment of concentration and are represented with their 95% Confidence Interval. Multivariate conditional logistic regression models were adjusted on all lagged daily air pollutants concentrations, daily maximum temperature, day of relapse maximum relative humidity, maximum atmospheric pressure, pollen count, influenza-like epidemics, and holidays.

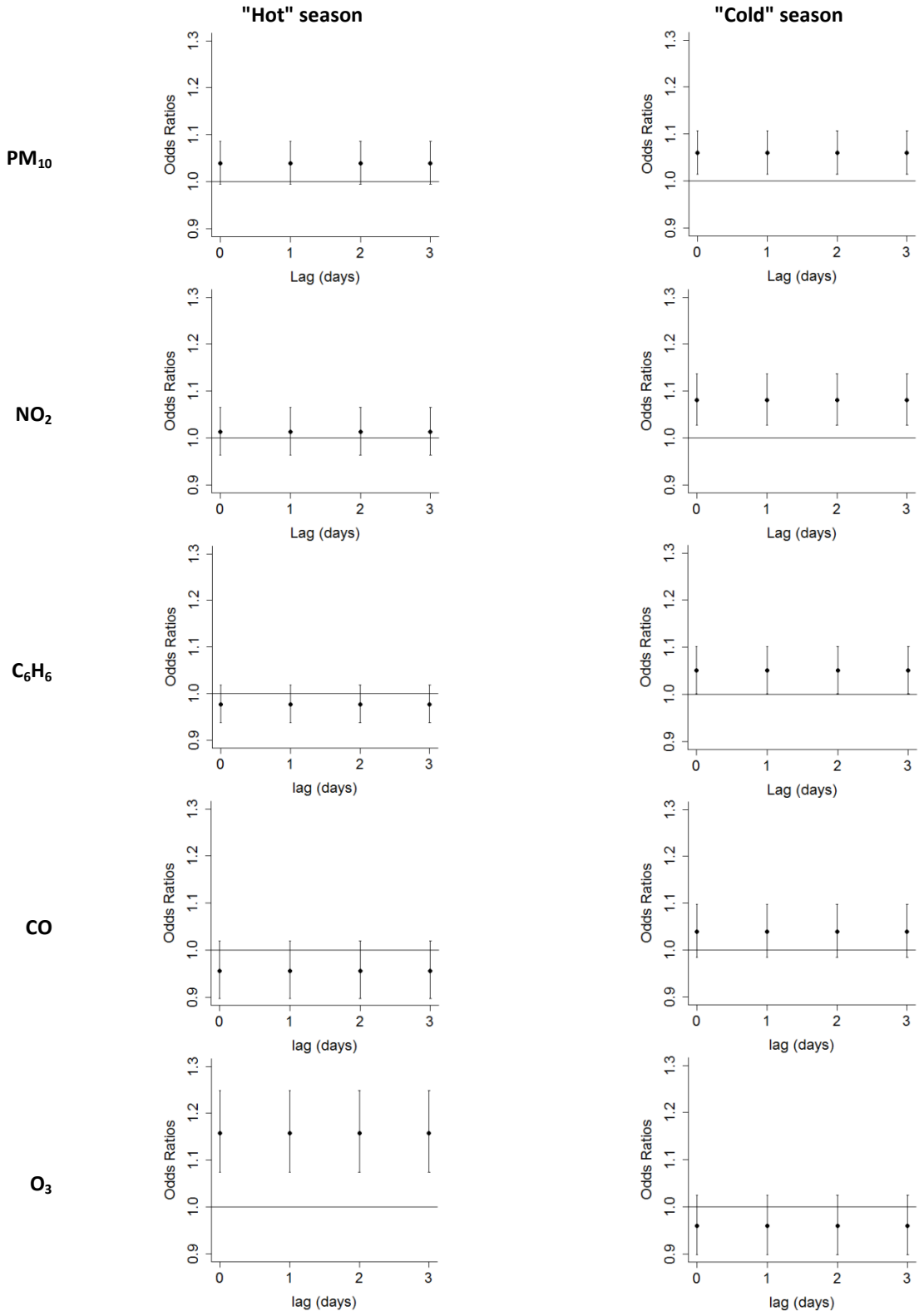


Figure 3. Associations between exposure to air pollutants concentrations (lags 0-3 days before relapse onset, constant lag effect) and risk of MS relapse triggering, according to season.