Ammonia, ammonium and the risk of asthma
- A register-based case-control study in Danish children

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Online Data Supplement

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Information on ammonia and ammonium

Physical and chemical properties

Gaseous ammonia (NH_3) is a precursor to ammonium (NH_4^+) that is formed in a reaction between NH_3 and acid aerosols forming ammoniumbisulphate and ammoniumsulphate ($NH_3+H_2SO_4 \rightarrow NH_4HSO_4$; $NH_4HSO_4+H_2SO_4 \rightarrow (NH_4)_2SO_4$). However, NH_4^+ is also formed when NH_3 and gaseous acids are reacting to form ammonium nitrate and ammonium chloride (NH_3+HNO_3 -> NH_4NO_3; NH_3+HCl -> NH_4Cl) (1). A measure for the total concentration of these pollutants (NH_x) is often applied in estimation of atmospheric emissions ($NH_3 + NH_4^+ = NH_x$). The availability and formation of inorganic NH_4^+ in the atmosphere is essentially dependent on the presence of the acidic species and NH_3. Thus, atmospheric concentrations of NH_4^+ particles vary considerably according to geographic location. In a worldwide study, NH_4^+ was estimated to be 2 µg/m^3 in Ilorin (Nigeria) and 17 µg/m^3 in Kanpur (India) for $(NH_4)_2SO_4$), and 0.2 µg/m^3 in Atlanta (USA) and 6.7 µg/m^3 in Kanpur for (NH_4NO_3)(2). The lifetime of NH_3 is relatively short, allowing NH_3 to transfer only within distances of 10 to 100 km, whereas NH_4^+ may be transferred much longer distances ranging from 100 to 1000 km (3,4). Therefore, valid exposure assessment resolution of NH_3 and NH_4^+ emissions should be obtained on both a regional and local scale (5,6); however, information on local emissions is rarely available (7).

Emission sources

The agricultural sector contributes approximately 90% of atmospheric NH_3 gas(8) due to evaporation from barns and storages as well as in connection with manure application to the fields. Therefore, this sector is also responsible for the vast majority of atmospheric NH_4^+ owing to the before-mentioned gas-to-particle conversion (1,9). Other minor emission sources for atmospheric NH_3 comprise: transportation (related to the use of catalytic converters producing small amounts of NH_3 when reducing nitrogen oxides), chemical industry, sewage works, waste disposal, combustion and animals other than livestock (10,11).

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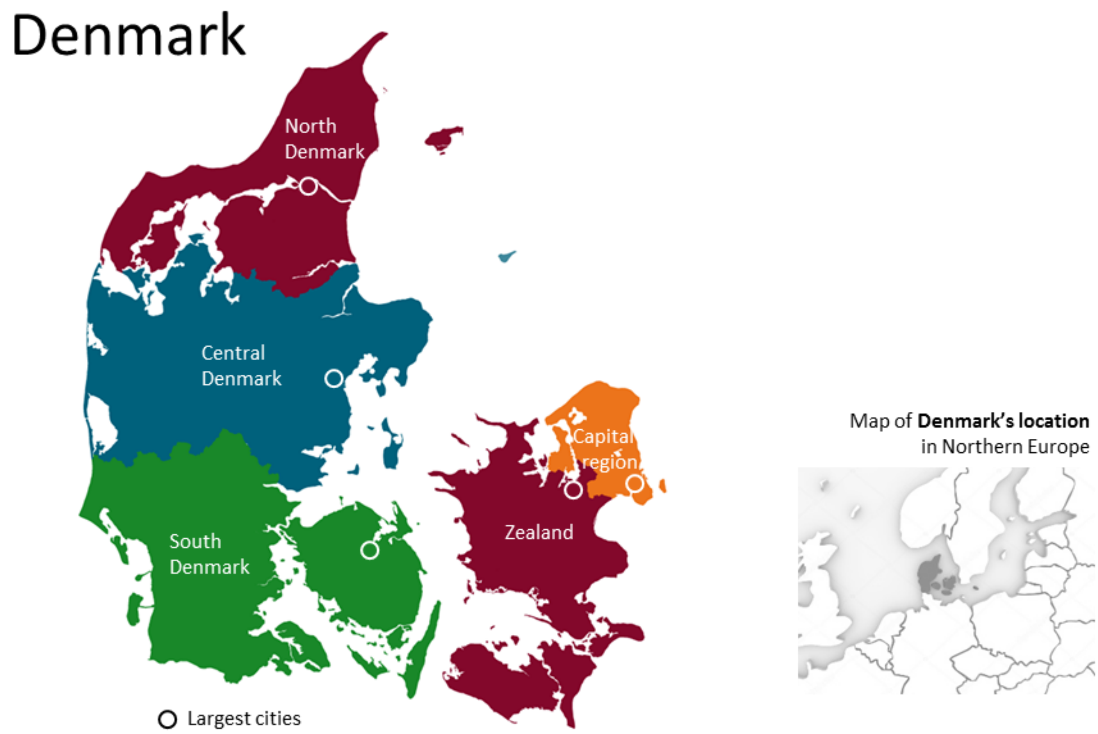

Region	Largest city	Population (2018)	Area, km^2	Population density per km^2	Annual income per person, dkr. (2014)	Annual expenditures per household, dkr. (2015)
North Denmark	Aalborg	589,148	7,874	75	160,000	295,820
Central Denmark	Aarhus	1,313,596	13,000	101	158,000	278,983
South Denmark	Odense	1,220,763	12,191	100	157,000	295,820
Capital region	Copenhagen	1,822,659	2,546	716	165,000	347,106
Zealand	Roskilde	835,024	7,218	116	160,000	327,741

Data are derived from Denmarks Statistics

Figure E1 Location and characteristics of the Danish regions

Table E1 Study population characteristics and risk factors for asthma incidence among covariates.

	Cases N = 12 935	Controls N = 322 694	HR (95% CI)
Sex			
Female	5 010 (39%)	124 931 (39%)	-
Male	7 925 (61%)	197 763 (61%)	-
Year of birth			
Year 2000	79 (1%)	1 963 (1%)	-
Year 2001	241 (2%)	6 030 (2%)	-
Year 2002	418 (3%)	10 445 (34%)	-
Year 2003	708 (5%)	17 654 (5%)	-
Year 2004	1 529 (12%)	38 113 (12%)	-
Year 2005	1 970 (15%)	49 135 (15%)	-
Year 2006	2 039 (16%)	50 865 (16%)	-
Year 2007	1 754 (14%)	43 739 (14%)	-
Year 2008	1 630 (13%)	40 686 (13%)	-
Year 2009	1 263 (10%)	31 536 (10%)	-
Year 2010	895 (7%)	22 325 (7%)	-
Year 2011	409 (3%)	10 203 (3%)	-
Age of diagnosis			
Age 1	7 078 (55%)	176 579 (55%)	-
Age 2	2 497 (19%)	62 250 (19%)	-
Age 3	1 398 (11%)	34 897 (11%)	-
Age 4	1 076 (8%)	26 858 (8%)	-
Age 5	886 (7%)	22 110 (7%)	-
Year of diagnosis			
Year 2006	2 321 (18%)	57 908 (18%)	-
Year 2007	2 024 (16%)	50 480 (16%)	-
Year 2008	2 008 (16%)	50 059 (16%)	-
Year 2009	1 772 (14%)	44 196 (14%)	-
Year 2010	1 983 (15%)	49 518 (15%)	-
Year 2011	1 505 (12%)	37 543 (12%)	-
Year 2012	1 322 (10%)	32 990 (10%)	-

Table E1 Continued

Region			
North Denmark	1 200 (9%)	34 894 (11%)	1.17 (1.09 -1.25)
Central Denmark	3 049 (24%)	79 165 (25%)	1.31 (1.24 - 1.38)
South Denmark	4 043 (31%)	68 615 (21%)	2.00 (1.90 -2.10)
Zealand	1 953 (15%)	48 658 (15%)	1.36 (1.29 - 1.45)
Capital region	2 690 (21%)	91 360 (28%)	1.0
Education* (mother)			
Primary school	2606 (20%)	45 507 (14%)	1.71 (1.24 -2.36)
Short education	5915 (46%)	146 087 (45%)	2.22 (2.07 - 2.40)
Medium education	3366 (26%)	91 157 (28%)	1.57 (1.47 - 1.68)
Long education	1008 (8%)	39 033 (12%)	1.0
Income# (father)			
1st Quintile	334 (3%)	7710 (2%)	1.03 (0.53 -1.99)
2nd Quintile	651 (5%)	14 218 (4%)	1.25 (1.11 -1.40)
3rd Quintile	1817 (14%)	36 163 (11%)	1.32 (1.21 - 1.43)
4th Quintile	4526 (35%)	103 329 (32%)	1.45 (1.37 -1.53)
5th Quintile	5598 (43%)	161 022 (50%)	1.0

Definition of abbreviations: N, number; HR, hazard ratio; 95% CI, 95% confidence interval.

*Description of the Danish education system in short. Primary school: primary and lower secondary education. Short education: 2-3 years (without upper secondary education). Medium education: bachelor's programme, professional bachelor's programme, academy profession programme (requires completion of upper secondary education). Long education: master's programmes (candidatus) and PhD.

#Quintiles are based on the income of the general population.

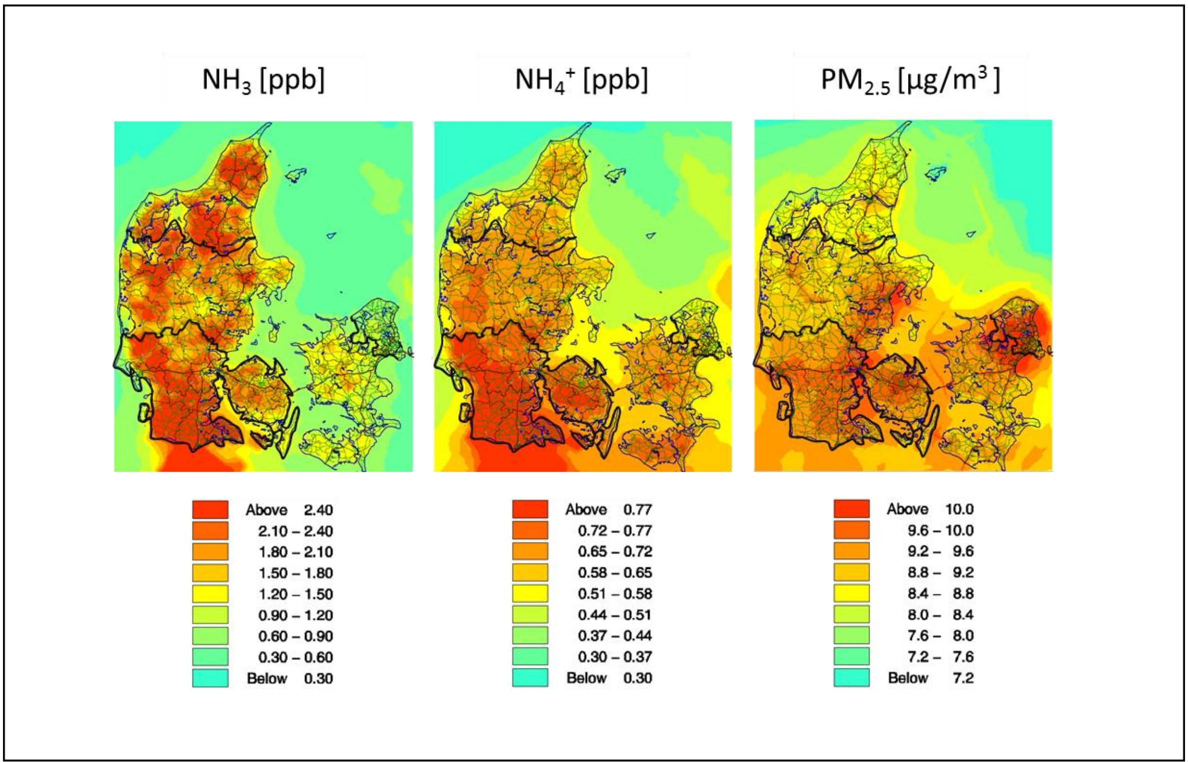

Figure E2 Study area and annual mean concentrations of NH_3, NH_4^+ and $PM_{2.5}$ in 2008

Table E2 Effects of NH_3, NH_4^+, NH_x and $PM_{2.5}$ concentrations on the risk of asthma for different exposure time-windows.

	3 months		6 months		12 months	
	HR	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**
Pollutants						
NH_3	1.74	(1.60 - 1.89)	1.67	(1.56 - 1.79)	1.59	(1.50 - 1.68)
NH_4^+	2.33	(2.04 - 2.65)	2.56	(2.29 - 2.86)	2.36	(2.17 - 2.58)
NH_x	1.82	(1.68 - 1.96)	1.78	(1.67 - 1.90)	1.71	(1.61 - 1.81)
$PM_{2.5}$	0.96	(0.86 - 1.06)	1.05	(0.96 - 1.15)	1.02	(0.94 - 1.10)

Definition of abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; NH_3, ammonia; NH_4^+, ammonium; NH_x, NH_3 + NH_4^+; $PM_{2.5}$, particulate matter less than 2.5 μm.

Conditional logistic regression results are presented as HRs and 95% CIs describing the effects of NH_3, NH_4^+, NH_x and $PM_{2.5}$ on the risk of asthma treating the pollutants as trend variables for exposure averages of 3, 6, and 12 months prior to cases were first diagnosed with asthma measuring the risk of the highest exposed children (10^{th} decile) compared to the lowest exposed children (1^{st} decile). Analyses were adjusted for sex, date of birth, age, and calendar year.

Table E3 Effects of NH_3, NH_4^+, NH_x and $PM_{2.5}$ concentrations on the risk of asthma in one year old children.

	Base adjustment*		2nd adjustment†		3rd adjustment‡		4th adjustment§	
	HR	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**
Pollutants								
NH_3	2.03	(1.81 - 2.28)	1.76	(1.57 - 1.97)	0.76	(0.65 - 0.90)	0.72	(0.61 - 0.85)
NH_4^+	2.13	(1.79 - 2.54)	1.95	(1.64 - 2.31)	1.07	(0.84 - 1.36)	0.99	(0.78 - 1.27)
NH_x	2.18	(1.95 - 2.43)	1.89	(1.69 - 2.11)	0.88	(0.76 - 1.02)	0.83	(0.71 - 0.97)
$PM_{2.5}$	0.81	(0.71 - 0.93)	0.94	(0.81 - 1.08)	0.87	(0.71 - 1.07)	0.92	(0.75 - 1.14)

For definition of abbreviations, see Table E2.
* Base adjustment: Adjustment for sex, date of birth, age and calendar year.
† 2nd adjustment: base adjustment + socio economic status.
‡ 3rd adjustment: base adjustment + region.
§ 4th adjustment: base adjustment + region + socio economic status.

Table E4 Effects of NH_3, NH_4^+, NH_x and $PM_{2.5}$ concentrations on the risk of asthma in 2-3 year old children.

	Base adjustment*		2nd adjustment†		3rd adjustment‡		4th adjustment§	
	HR	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**
Pollutants								
NH_3	1.62	(1.39 - 1.88)	1.45	(1.24 - 1.68)	0.74	(0.59 - 0.93)	0.70	(0.56 - 0.88)
NH_4^+	2.35	(1.85 - 2.99)	2.17	(1.71 - 2.74)	0.75	(0.54 - 1.05)	0.71	(0.51 - 0.99)
NH_x	1.70	(1.48 - 1.96)	1.52	(1.32 - 1.76)	0.76	(0.62 - 0.93)	0.72	(0.59 - 0.89)
$PM_{2.5}$	1.10	(0.91 - 1.34)	1.23	(1.01 - 1.49)	0.70	(0.53 - 0.92)	0.72	(0.55 - 0.95)

For definition of abbreviations, see Table E2.
* Base adjustment: Adjustment for sex, date of birth, age and calendar year.
† 2nd adjustment: base adjustment + socio economic status.
‡ 3rd adjustment: base adjustment + region.
§ 4th adjustment: base adjustment + region + socio economic status.

Table E5 Effects of NH_3, NH_4^+, NH_x and $PM_{2.5}$ concentrations on the risk of asthma in 4-5 year old children.

	Base adjustment*		2nd adjustment†		3rd adjustment‡		4th adjustment§	
	HR	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**	**HR**	**(95% CI)**
Pollutants								
NH_3	1.18	(0.96 -1.44)	1.14	(0.93 - 1.39)	0.96	(0.69 - 1.31)	0.96	(0.69 - 1.31)
NH_4^+	2.48	(1.77 - 3.48)	2.41	(1.72 - 3.37)	0.86	(0.54 - 1.39)	0.84	(0.52 - 1.35)
NH_x	1.27	(1.05 - 1.54)	1.23	(1.02 - 1.49)	0.97	(0.73 - 1.29)	0.96	(0.72 - 1.29)
$PM_{2.5}$	1.59	(1.21 - 2.11)	1.64	(1.24 - 2.17)	0.82	(0.56 - 1.21)	0.81	(0.56 - 1.19)

For definition of abbreviations, see Table E2.
* Base adjustment: Adjustment for sex, date of birth, age and calendar year.
† 2nd adjustment: base adjustment + socio economic status.
‡ 3rd adjustment: base adjustment + region.
§ 4th adjustment: base adjustment + region + socio economic status.

Table E6 Effects of NH_3, NH_4^+, NH_x and $PM_{2.5}$ concentrations on the risk of asthma stratified by region.

	NH_3	**NH_4^+**	**NH_x**	**$PM_{2.5}$**
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Pollutants				
North Denmark	0.68 (0.56, 0.84)	0.93 (0.71, 1.22)	0.75 (0.62, 0.90)	0.76 (0.58, 0.99)
Central Denmark	0.83 (0.71, 0.98)	0.94 (0.76, 1.15)	0.86 (0.74, 0.99)	0.76 (0.63, 0.92)
South Denmark	0.71 (0.61, 0.83)	0.98 (0.8, 1.2)	0.73 (0.63, 0.85)	0.79 (0.63, 0.92)
Capital region	0.72 (0.56, 0.92)	0.99 (0.81, 1.23)	0.82 (0.65, 1.02)	0.72 (0.59, 0.86)
Zealand	0.82 (0.68, 0.99)	0.93 (0.71, 1.17)	0.85 (0.70,1.03)	0.74 (0.6, 0.92)
Test for interaction*	P=0.18	P=0.93	P=0.31	P=0.87

For definition of abbreviations, see Table E2.

Analyses are adjusted for: sex, date of birth, age and calendar year.

*log likelihood test for interaction between the effect of each pollutant and region. For each pollutant the effect was not modified significantly by region (p=0.18, p=0.93, p=0.31, p=0.87, respectively).