

**Exposure to nitrogen oxide in the first trimester and risk of cardiovascular-related malformations: a dose-response meta-analysis of observational studies.**

Tie-Ning Zhang<sup>1</sup>, Da Li<sup>2</sup>, Qi-Jun Wu<sup>3</sup>, Jing Xia<sup>1</sup>, Ri Wen<sup>1</sup>, Xing-Chen Chen<sup>4</sup>, Ni Yang<sup>1</sup>, Yan-Ling Chen<sup>5</sup>, Yan-Hong Huang<sup>6</sup>, Chun-Feng Liu<sup>1</sup>.

**Supplementary Table S1. Characteristics of the studies included in the meta-analysis.**

<b>First author (ref), year, location</b>	<b>Study period</b>	<b>Study design</b>	<b>Gestational period</b>	<b>Exposure</b>	<b>Outcomes (No. of cases)</b>	<b>Risk estimates (95% CI)</b>
Hwang (27), 2015, China	2001-2007	Case-control study	First trimester	NO <sub>2</sub>	ASD (194)	0.98 (0.74-1.30)
					Conotruncal Defects (404)	0.81 (0.60-1.10)
					PDA (213)	1.02 (0.78-1.33)
					Pulmonary Artery and Valve (60)	0.77 (0.47-1.27)
					TOF (111)	1.11 (0.75-1.64)
					Transposition of the Great Arteries (70)	0.72 (0.44-1.16)
					VSD (193)	0.91 (0.68-1.22)
Farhi (33), 2014, Israel	1997-2004	Historical cohort-study	First trimester	NO <sub>x</sub>	Circulatory system (1,634)	1.01 (0.99-1.03)
					VSD (1,161)	1.02 (0.99-1.04)
Schembari (34), 2014, Spain*	1994-2006	Case-control study	Week 3-8	NO <sub>2</sub>	ASD (229)	1.20 (1.02-1.41)
					COA (69)	1.13 (0.90-1.41)
					CHD (823)	1.36 (1.06-1.75)
					TOF (49)	1.11 (0.75-1.64)
					VSD (351)	0.91 (0.68-1.22)

**Supplementary Table S1. (Continued)**

<b>First author (ref), year, location</b>	<b>Study period</b>	<b>Study design</b>	<b>Gestational period</b>	<b>Exposure</b>	<b>Outcomes (No. of cases)</b>	<b>Risk estimates (95% CI)</b>
Stingone (31),2014, America	1997-2006	Case-control study	Week 2-8	NO <sub>2</sub>	LVOTO (545)	1.07 (1.00-1.16)
					COA (215)	1.21 (1.07-1.36)
					Conotruncal (691)	1.05 (0.98-1.12)
					TOF (355)	1.03 (0.94-1.13)
					APVR (100)	0.95 (0.83-1.09)
					AVSD (53)	0.92 (0.76-1.11)
					RVOTO (459)	1.16 (1.07-1.25)
					Septal (944)	1.05 (0.99-1.12)
					ASD (461)	1.03 (0.95-1.12)
Agay-Shay (7), 2013, Israel	2000-2006	Registry-based cohort study	Week 3-8	NO <sub>2</sub>	Isolated atrial and ASD (534)	0.93 (0.80-1.08)
					Isolated PDA (147)	1.03 (0.77-1.38)
					Isolated VSD (493)	0.99 (0.84-1.15)
					Multiple CHD (569)	0.96 (0.83-1.11)
					VSD (395)	1.06 (0.99-1.15)

**Supplementary Table S1. (Continued)**

<b>First author (ref), year, location</b>	<b>Study period</b>	<b>Study design</b>	<b>Gestational period</b>	<b>Exposure</b>	<b>Outcomes (No. of cases)</b>	<b>Risk estimates (95% CI)</b>
Padula (30), 2013, America*	1997– 2006	Case-control study	First 2 months of pregnancy	NO	Aortic stenosis (23)	1.04 (0.81-1.34)
					COA (70)	1.02 (0.88-1.17)
					Dextro-transposition of the great arteries (54)	0.94 (0.79-1.11)
					Double outlet right ventricle – other (14)	0.91 (0.63-1.33)
					Heterotaxia (54)	0.97 (0.83-1.14)
					Hypoplastic left heart syndrome (53)	1.03 (0.87-1.21)
					Pulmonary atresia (50)	0.99 (0.84-1.18)
					Secundum ASD (123)	1.01 (0.90-1.14)
					TOF (88)	0.96 (0.83-1.10)
					Total anomalous pulmonary venous return (19)	0.95 (0.72-1.25)
					VSD-muscular (46)	0.95 (0.79-1.14)
VSD-perimembranous (88)	1.02 (0.89-1.17)					

**Supplementary Table S1. (Continued)**

<b>First author (ref), year, location</b>	<b>Study period</b>	<b>Study design</b>	<b>Gestational period</b>	<b>Exposure</b>	<b>Outcomes (No. of cases)</b>	<b>Risk estimates (95% CI)</b>
				NO <sub>2</sub>	Aortic stenosis (26)	0.86 (0.59-1.25)
					Atrioventricular septal defects (14)	1.00 (0.59-1.71)
					COA (78)	1.10 (0.87-1.40)
					Dextro-transposition of the great arteries (63)	1.25 (0.96-1.62)
					Double outlet right ventricle – TGA (17)	1.19 (0.79-1.81)
					Heterotaxia (61)	0.99 (0.77-1.26)
					Hypoplastic left heart syndrome (61)	0.90 (0.69-1.18)
					Pulmonary atresia (59)	0.95 (0.73-1.24)
					Secundum ASD (140)	0.92 (0.78-1.09)
					Single ventricle or other complex (21)	0.86 (0.59-1.25)
					TOF (100)	1.05 (0.83-1.32)
					Tricuspid atresia (18)	0.88 (0.53-1.46)
					VSD-conov (19)	1.02 (0.68-1.54)
					VSD-muscular (48)	0.90 (0.69-1.16)
					VSD-perimembranous (98)	0.90 (0.71-1.14)

**Supplementary Table S1. (Continued)**

<b>First author (ref), year, location</b>	<b>Study period</b>	<b>Study design</b>	<b>Gestational period</b>	<b>Exposure</b>	<b>Outcomes (No. of cases)</b>	<b>Risk estimates (95% CI)</b>
Dadvand (32), 2011, United Kingdom*	1993–2003	Case-control study	Week 3-8	NO	Pooled-cases (2,140)	1.07 (1.01-1.13)
					ASD (274)	1.11 (0.95-1.30)
					COA (125)	1.08 (0.85-1.39)
					TOF (126)	1.25 (1.05-1.50)
					VSD (1,154)	1.20 (1.13-1.29)
				NO <sub>2</sub>	Pooled-cases (2,140)	0.90 (0.77-1.05)
					ASD (274)	0.97 (0.63-1.46)
					COA (125)	0.80 (0.43-1.47)
					TOF (126)	0.94 (0.49-1.80)
					VSD (1,154)	0.90 (0.73-1.11)
Hansen (28), 2009, Australia*	1998-2004	Case-control study	Week 3-8	NO <sub>2</sub>	Aortic artery and valve defect (63)	1.58 (0.40-6.15)
					ASD (127)	1.24 (0.53-2.93)
					Conotruncal Defects (63)	0.30 (0.07-1.34)
					Endocardial cushion and mitral valve defect (33)	3.04 (0.51-18.06)
					Pulmonary artery and valve defect (64)	1.10 (0.29-4.15)
					VSD (222)	0.77 (0.21-2.85)

**Supplementary Table S1. (Continued)**

<b>First author (ref), year, location</b>	<b>Study period</b>	<b>Study design</b>	<b>Gestational period</b>	<b>Exposure</b>	<b>Outcomes (No. of cases)</b>	<b>Risk estimates (95% CI)</b>
Strickland (29), 2009, America*	1986-2003	Retrospective cohort study	Week 3-7	NO <sub>2</sub>	ASD, secundum (379)	1.28 (0.87-1.88)
					COA (275)	1.21 (0.79-1.83)
					Conotruncal defect (661)	0.91 (0.69-1.21)
					Hypoplastic left heart syndrome (175)	0.85 (0.49-1.47)
					Left ventricular outflow tract defect (558)	1.02 (0.75-1.38)
					Patent ductus arteriosus (219)	1.52 (0.92-2.51)
					Pulmonary stenosis, valvar (312)	1.02 (0.67-1.54)
					Right ventricular outflow tract defect (421)	1.04 (0.73-1.47)
					TOF (299)	0.90 (0.59-1.37)
					Transposition of the great arteries (165)	0.68 (0.38-1.21)
					VSD, muscular (1,108)	1.16 (0.93-1.46)
VSD, perimembranous (546)	1.21 (0.90-1.65)					

\* For dose-response, original data of study was converted into “10 ppb increment” as displayed.

Abbreviations: APVR, anomalous pulmonary venous return; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHD, congenital heart defect; CI: confidence interval; COA, coarctation of the aorta; LVOTO, left ventricular outflow tract obstructions; RVOTO, right ventricular outflow tract obstructions; TOF, tetralogy of Follot; PDA, patent ductus arteriosus; VSD, ventricular septal defect.

**Supplementary Table S2. Adjusted factors of the studies included in the meta-analysis.**

<b>First author (ref), year, location</b>	<b>Adjusted factors</b>
Hwang (27), 2015, China	Maternal age, diabetes mellitus, plurality, gestational age, SES, and season of conception.
Farhi (33), 2014, Israel	Maternal age, maternal ethnicity, maternal country of birth, maternal education, plurality, season of birth and infant gender.
Schembari (34), 2014, Spain	Maternal age, conception season, year of birth/termination, socioeconomic index.
Stingone (31), 2014, America	Maternal race, age, educational attainment, household income, maternal smoking status, and alcohol consumption during early pregnancy, nativity, and site-specific heart defect ratio.
Agay-Shay (7), 2013, Israel	Multi-births, maternal age, maternal marital status, maternal origin of birth, infant's sex, religion, year of birth, season of conception, ward-base SES.
Padula (30), 2013, America	Maternal race/ethnicity, education and vitamin use (for the month prior to and/or first 2 months of pregnancy).
Dadvand (32), 2011, United Kingdom	Year of birth, SES, degree of urbanity, and season of conception.
Hansen (28), 2009, Australia	Neonate sex.

**Supplementary Table S2. Adjusted factors of the studies included in the meta-analysis. (Continued)**

<b>First author (ref), year, location</b>	<b>Adjusted factors</b>
Strickland (29), 2009, America	Week of year, and cubic spline for day of follow-up.

Abbreviations: SES, socioeconomic status.

**Supplementary Table S3. Methodological quality of case-control studies included in the meta-analysis\***

First author (reference), publication year	Case definition adequate	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cases and controls on the basis of the design or analysis <sup>†</sup>	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate <sup>‡</sup>
Hwang (27), 2015	*	*	*	*	**	*	*	*
Schembari (34), 2014	*	*	*	*	**	*	*	-
Stingone (31), 2014	*	*	*	*	**	*	*	*
Padula (30), 2013	*	*	*	*	-	*	*	*
Dadvand (32), 2011	*	*	*	*	*	*	*	-
Hansen (28), 2009	*	*	*	*	-	*	*	-

\* A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor. The definition/explanation of each column of the Newcastle-Ottawa Scale is available from ([http://www.ohri.ca/programs/clinical\\_epidemiology/oxford.asp](http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)).

<sup>†</sup> A maximum of 2 stars could be awarded for this item. Studies that controlled for maternal age, whereas studies that controlled for socioeconomic status received an additional star.

<sup>‡</sup> A case-control study with a non-response rate <10% was assigned one star.

**Supplementary Table S3. Methodological quality of cohort studies included in the meta-analysis \***

<b>First author (reference), publication year</b>	<b>Representativeness of the exposed cohort</b>	<b>Selection of the unexposed cohort</b>	<b>Ascertainment of exposure</b>	<b>Outcome of interest not present at start of study</b>	<b>Control for important factor or additional factor †</b>	<b>Assessment of outcome</b>	<b>Follow-up long enough for outcomes to occur ‡</b>	<b>Adequacy of follow-up of cohorts§</b>
Farhi (33), 2014	*	*	*	*	*	*	-	-
Agay-Shay (7), 2013	*	*	*	*	**	*	-	-
Strickland (29), 2009	*	*	*	*	-	*	-	-

\* A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor. The definition/explanation of each column of the Newcastle-Ottawa Scale is available from ([http://www.ohri.ca/programs/clinical\\_epidemiology/oxford.asp](http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)).

† A maximum of 2 stars could be awarded for this item. Studies that controlled for maternal age, whereas studies that controlled for socioeconomic status received an additional star.

‡ A cohort study with a follow-up time >1 year was assigned one star.

§ A cohort study with a follow-up rate >80% was assigned one star.