Perinatal and environmental risk factors of childhood neuroblastoma

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Abstract

Background: Neuroblastoma is the most common extra-cranial tumor in children. Little is known about the etiology of neuroblastoma. The early age at onset and the embryonic nature suggest a role for perinatal exposures. In this work, we analyzed whether childhood neuroblastoma was associated with specific perinatal characteristics and environmental exposures around pregnancy. We assessed the following birth-related characteristics: gestational age, birth-weight and fetal growth, and the presence of congenital malformations. The maternal reproductive history before the index pregnancy and maternal intake of folic acid or vitamins/minerals before or during pregnancy was also assessed. With regards to environmental exposures related to parental habits, we focused on maternal use of household pesticides during pregnancy, parental smoking and maternal alcohol consumption.

Methods: We conducted a pooled analysis of two French national-based case-control studies. The mothers of 357 neuroblastoma case and 1,783 control children younger than 6 years, frequency-matched by age and gender, completed a telephone interview that focused on sociodemographic and perinatal characteristics, childhood environment and parental lifestyle. Unconditional logistic regression was used to estimate pooled odds ratios (OR) and 95% confidence intervals (CIs), including matching variables, study of origin and potential confounders. A meta-analysis of our findings with those of previous studies was also conducted with regards to maternal smoking and alcohol consumption during pregnancy. We used random effects, precision-based weighting to calculate the summary OR including our results.

Results: The first part of the thesis focused on perinatal characteristics. We observed that being born either small (OR 1.4 [95% CI 1.0-2.0]) or large (OR 1.5 [95% CI 1.1–2.2]) for gestational age and, among children younger than 18 months, having congenital malformations (OR 3.6 [95% CI 1.3–8.9]), were significantly associated with neuroblastoma. Inverse associations were observed with breastfeeding (OR 0.7 [95% CI 0.5–1.0]) and maternal use of any supplements containing folic acid, vitamins or minerals (OR 0.5 [95% CI 0.3–0.9]) during the preconception period.

The second part of the thesis showed that maternal use of any type of household pesticide during pregnancy was associated with neuroblastoma (OR 1.5 [95% CI 1.2–1.9]). The most

commonly used type of pesticides were insecticides and there was a positive association with their use alone (OR 1.4 [95% CI 1.1–1.9]) or with other pesticides (OR 2.0 [95% CI 1.1–3.4]). In the third part, our analyses showed that maternal smoking during pregnancy was slightly more often reported for the cases (24.1%) than for the controls (19.7%) (OR 1.3 [95% CI 0.9–1.7]; Paternal smoking in the year before child's birth was not associated with neuroblastoma as independent exposure (OR 1.1 [95%CI 0.9–1.4] but the association was stronger when both parents reported having smoked during pregnancy (OR 1.5 [95% CI 1.1–2.1]. Finally, in a meta-analysis of maternal smoking and neuroblastoma the summary OR from meta-analysis was 1.1 [95% CI 1.0–1.3].

Conclusions: Our findings support the hypothesis of a defective embryogenesis in neuroblastoma since fetal growth anomalies and congenital malformations were associated with an increased risk of neuroblastoma. This work also adds to the evidence of an association between neuroblastoma and some exposures during pregnancy, such as maternal use of household pesticides and maternal smoking, which are additional reasons why to advise pregnant women to limit these exposures in this period. Further investigations are needed to clarify the role of folic acid supplementation and breastfeeding, given their potential importance in neuroblastoma prevention.

Key words: neuroblastoma, childhood cancer, perinatal exposures, etiology, risk factors, case-control study.

Résumé

Contexte : Le neuroblastome est une tumeur embryonnaire qui se développe à partir du système nerveux sympathique. C'est la tumeur maligne solide extra-cérébrale la plus fréquente chez les enfants de moins d'un an. La cause du neuroblastome est encore inconnue dans la majorité des cas. Cependant, les caractéristiques embryonnaires de la tumeur ainsi que sa courte latence de survenue après la naissance suggèrent l'origine périnatale de ce cancer et l'importance d'étudier les expositions survenant pendant la grossesse et les premières années de vie de l'enfant. Dans ce travail de recherche, nous avons analysé le lien entre certains facteurs périnataux et des expositions pendant la grossesse et le risque neuroblastome chez l'enfant.

Matériel et méthodes : Les données sont issues des enquêtes ESCALE (2003-2004) et ESTELLE (2010-2011) menées par notre équipe de recherche. Les mères de 357 cas de neuroblastome issus du Registre National des Cancers de l'Enfant (RNCE) ainsi que 1753 témoins recrutés en population générale ont répondu à un entretien qui portait sur les caractéristiques périnatales de l'enfant, les expositions maternelles pendant la grossesse, antécédents médicaux familiaux et personnels de l'enfant ainsi que sur des variables contextuelles et socioéconomiques. La taille de notre échantillon a permis de réaliser des analyses stratifiées sur l'âge au diagnostic et le statut du proto-oncogène *MYCN*.

Résultats : Une première analyse sur l'association entre les caractéristiques périnatales et le risque de neuroblastome chez l'enfant a mis en évidence des associations positives avec la présence de malformations congénitales (OR 3.6 [95% CI 1.3–8.9] parmi les enfants de moins de 18 mois) et des altérations de la croissance fœtale tels que le retard de croissance intra-utérine ou la surcroissance fœtale (OR 1.4 [95% CI 1.0-2.0]) et (OR 1.5 [95% CI 1.1–2.2], respectivement). Le fait d'être allaité était inversement associé au risque de neuroblastome (OR 0.7 [95% CI 0.5–1.0]). Des associations inverses ont été également observées avec la supplémentation maternelle préconceptionnelle en acide folique (OR 0.5 [95% CI 0.3–0.9]). La difficulté pour concevoir ou l'utilisation d'assistance médicale à la procréation n'ont pas été associé au risque de neuroblastome dans notre étude.

Dans une deuxième partie de ce travail, nous avons analysé les expositions maternelles domestiques et professionnelles aux pesticides pendant la grossesse. Nos résultats suggèrent que l'utilisation domestique de pesticides pendant la grossesse pourrait augmenter le risque de neuroblastome chez l'enfant (OR 1.5 [95% CI 1.2–1.9]). Des associations positives ont été observées avec l'utilisation d'insecticides seulement (OR 1.4 [95% CI 1.1–1.9]), ou en

combinaison avec d'autres pesticides (OR 2.0 [95% CI 1.1–3.4]). L'exposition professionnelle de la mère pendant la grossesse était également associée au risque de neuroblastome.

La troisième partie de ce travail a porté sur l'analyse du tabagisme parental et la consommation maternelle d'alcool pendant la grossesse. La consommation maternelle de tabac était plus fréquente chez les mères des cas (24.1%) par rapport aux mères des témoins (19.7%) ; (OR 1.3 [95%CI 0.9–1.7]; OR à partir d'une méta-analyse 1.1 [95%CI 1.0–1.3].

Conclusion : Nos résultats portant sur les associations entre neuroblastome, surcroissance fœtale et malformations congénitales supportent l'hypothèse d'un rôle des altérations de l'embryogénèse dans la survenue des neuroblastomes de l'enfant. Ce travail contribue à l'évidence en faveur des associations entre neuroblastome et certaines expositions pendant la grossesse, notamment l'utilisation domestique de pesticides et le tabagisme maternelle. Nos résultats soulignent l'importance des recommandations visant à réduire l'exposition aux pesticides et le tabagisme maternel pendant la grossesse. Des nouvelles études sont nécessaires afin d'éclaircir le rôle de l'allaitement, ainsi que la supplémentation préconceptionnelle en acide folique dans la survenue de neuroblastome, vu son rôle potentiel dans la prévention de ce cancer.

Mots clés: neuroblastome, facteurs de risque, pesticides, tabac, alcool, facteurs périnataux, acide folique, allaitement

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List of abbreviations

AGA Appropriate for gestational age

ALK Anaplastic Lymphoma Kinase gene

BARD1 BRCA Associated RING Domain 1

CI Confident Interval

ENP Perinatal National Surveys

ESCALE Etude Sur les Cancers et les Leucémies de l'Enfant

ESTELLE Etude Sur les Tumeurs Embryonnaires, Leucémies et Lymphomes de l'Enfant

EPICEA Equipe d'épidémiologie des cancers de l'Enfant et de l'Adolescent

IARC The International Agency for Research on Cancer

ICCC-3 International Classification of Childhood Cancer Third edition

LGA Large for gestational age

MYCN v-myc myelocytomatosis viral related oncogene, neuroblastoma derived

N-MYC MYCN proto-oncogene protein

OR Odds ratio

PHOX2B Paired-like homeobox 2b

RNCE French National Registry of Childhood cancers

SFCE Société Française de lutte contre les Cancers de L'Enfant et de l'Adolescent

SGA Small for gestational age

Publications proceeding during candidature

Rios P, Bailey HD, Poulalhon C, Valteau-Couanet D, Schleiermacher G, Bergeron C, Petit A, Defachelles A-S, Bertozzi A-I, Sirvent N, Thomas C, Ducassou S, Munzer C, Orsi L, Lacour B, Clavel J. Parental smoking, maternal alcohol consumption during pregnancy and the risk of neuroblastoma in children. The ESCALE and ESTELLE French studies and a metaanalysis. 2019 Int J Cancer 10.1002/ijc.32161.

Rios P, Bailey HD, Lacour B, Valteau-Couanet D, Michon J, Bergeron C, Boutroux H, Defachelles A-S, Gambart M, Sirvent N, Thebaud E, Ducassou S, Orsi L, Clavel J. Maternal use of household pesticides during pregnancy and risk of neuroblastoma in offspring. A pooled analysis of the ESTELLE and ESCALE French studies (SFCE). Cancer Causes Control. 2017;28(10):1125-32

Rios P, Bailey HD, Orsi L, Lacour B, Valteau-Couanet D, Levy D, Corradini N, Leverger G, Defachelles A-S, Gambart M, Sirvent N, Thebaud E, Ducassou S, Clavel J. Risk of neuroblastoma, birth-related characteristics, congenital malformations and perinatal exposures: A pooled analysis of the ESCALE and ESTELLE French studies (SFCE). Int J Cancer. 2016;139(9):1936-48

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Preface

This thesis involves the analysis of data collected about childhood neuroblastoma in the ESCALE and ESTELLE French studies.

The general aim was to investigate whether specific birth-related characteristics and environmental exposures around pregnancy were associated with neuroblastoma.

Chapter 1 gives an overview of the descriptive epidemiology of neuroblastoma using data from the National Registry of Childhood Cancer (RNCE) and describes the burden of disease and overall survival. It also contains what is known about the disease, the hypothesis of a prenatal origin and a review of the literature about the suspected risk factors of neuroblastoma. The exposures were chosen based on previous research that suggested a biologically plausible association with neuroblastoma.

The majority of the contents of these chapters has been published in three papers that explored:

1. Birth related characteristics, congenital malformations and perinatal exposures
2. Maternal use of household pesticide during pregnancy
3. Parental smoking and maternal alcohol consumption during pregnancy

To avoid repetition and to increase readability, the content of the published papers has now been edited for inclusion in relevant sections of the thesis, and the complete papers are included as appendices.

Chapter 2 gives a description of the general study methods including recruitment of the study population and data collection methods.

Chapter 3 describes the main results of this study.

Chapter 4 contains a detailed discussion of the findings.

Introduction and background

1 Introduction

Childhood cancers are the second leading causes of death in children aged 1 to 14 years. Each year in France, about 1700 children younger than 15 years are diagnosed with cancer. The incidence of the disease has been stable since 2000[1]. Neuroblastoma is the most common extra-cranial solid tumor in childhood and it is the most frequently diagnosed neoplasm during infancy. Compared with other childhood cancers such as leukemia and lymphoma, the sporadic occurrence of neuroblastoma has made this disease particularly challenging to study so literature is sparse.

1.1 Descriptive epidemiology of neuroblastoma

In the International Classification of Disease for Oncology (ICD-O-3)[2], neuroblastoma are classified into the two morphology codes 95003 (neuroblastoma, the most frequent) and 94903 (ganglioneuroblastoma, the best differentiated form of neuroblastoma). Both are grouped in the category IV (Tumors of the sympathetic nervous system), subgroup IV.a of the International Classification for Childhood Cancers (ICCC)[3].

In this chapter, the epidemiological features of neuroblastoma are described using the 2000-2013 data extracted from the French National Registry of Childhood Cancer (RNCE) *(http://rnce.inserm.fr)*. The RNCE registers 130 to 150 new cases of neuroblastoma aged less than 15 years every year.

1.1.1 Incidence

According to ICD-O-3 categories, 11% of the French cases are ganglioneuroblastoma and 89% neuroblastoma. Almost half of the tumors are located in the adrenal glands, 20% in other abdominal sites, 16% in mediastinum and the remaining 15% are distributed in pelvis, cervical or lumbar chains of the sympathetic nervous system. Almost half of the cases have distant metastases at diagnosis. The proportion of infants (under one year) with metastases is less but nearly a quarter are stage 4S (stage 4S; S=special). These infants have small primary tumors with metastasis in liver or skin, and with less than 10% marrow involvement.

Amplification of *MYCN* oncogene is present in less than 10% of infant neuroblastoma, but in more than 20% of neuroblastoma after the age of one year.

The annual age-standardized incidence rate in France is around 14 cases per million of children aged 0-14 years (Table 1). Neuroblastoma is mostly diagnosed before the age of 5 years (85%) and 40% occur in infancy. It is very uncommon after the age of 10 years. Annual incidence rates range from 73 cases per million in infancy to 1 case per million after the age of 10 years. One out of ten cases of neuroblastoma are neonates (<28 days). Most of them (65%) are diagnosed before birth by routine ultrasonography.

An increase in incidence has been reported for Europe as a whole over the earlier period 1978 to 1997 with an average change of 1.5% per year[4]. In the US the incidence rates were stable in metropolitan areas over the period 1973-2003, while raising by 2% in non-metropolitan areas[5].

The incidence rate increase could be partly explained by the changes in registry coverage and registration methods over the period. The highest incidence rates have been reported in countries with greater medical surveillance such as Western Europe, US, Canada, Japan, and Australia which may reflect better diagnostic facilities[6]. Incidence rates reported by low and medium resource countries are generally lower[7], [8]. A recent study revealed a statistically significant positive association between human development index and neuroblastoma incidence rates[9], which may reflect true risk factors as well as differences in diagnosis and registration practices.

Because neuroblastomas frequently produce increased levels of catecholamines, the metabolites of which are detectable in the urine, active screening programs have been implemented in several countries in the past, including Japan, Germany and Canada. The rationale for mass screening around 6 to 12 months of life assumed that high-risk neuroblastomas, which are more frequent after 18 months, were low-risk neuroblastomas that had then become of worse prognosis. Eventually, screening led to an increase in incidence rates among infants, some of whom would have never developed symptomatic disease, but did not decrease high-risk neuroblastoma and thus did not improve survival. [10]–[12]

Ethnic differences have been suggested in previous periods. Nevertheless, annual incidence rate in black children in the USA is now 10.2 per million for 2001-2010, closer to that of white children while very low rates are reported in the mostly black population of Sub-Saharan Africa[8].

Table 1: Number of cases of neuroblastoma and annual incidence rates in France (2000-2013), by age groups, by MYCN status and tumor extension (source: RNCE)

	Age groups (years)				Total
	< 1	**1-4**	**5-9**	**10-14**	
Incidence rate					
IR (/million*year)	73.1	22.2	4.0	1.1	12.5
ASR (/million*year)					14.1
Mean annual numbers	55	67	15	4	141
	39.0%	47.5%	10.6%	2.8%	100.0%
***MYCN* amplification**					
Amplified	7.2%	26.4%			16.6%
Non amplified	78.1%	59.8%			67.8%
Unknown	14.7%	13.8%			15.6%
Tumoral extension					
Non metastatic	61.7%	44.8%	49.1%	44.3%	51.8%
Metastatic not 4S	24.4%	54.9%	50.5%	55.7%	42.6%
Metastatic 4S	13.9%	0.2%			5.6%

IR: Incidence rate; ASR: age-standardized rate

1.1.2 Survival

The overall survival rates in France were 92% at 1 year and 75% at 5 years after diagnosis in 2000-2010 (Table 2). Survival varies strongly with age, with best prognosis in children aged under 18 months. An age of 18 months or more, presence of metastases, *MYCN* amplification and some tumoral genomic profiles are factors with poor prognosis (see section1.2 Clinical and biological features of neuroblastoma). The particular clinical phenotype of neuroblastoma 4S occurs in about 5-10% of cases and almost always regress[13].

Treatment protocols have evolved over the last 40 years to better account for poor prognostic factors and to intensify the treatment of high-risk groups[6], [14], [15]. However, recent data shows a stagnation and even a significant fall in survival for neuroblastoma in Central Europe[16] and USA[17], while survival has increased in France, reaching 78.7% for the period 2010-2014[1].

There are still survival disparities between countries, even within Europe[16]. A 5-year overall survival estimate of 59% was recently reported for Southern and Eastern European countries[18] compared to 77% in the USA. Disparities could be attributed, at least partly, to

[1] http://nce.vjf.inserm.fr/index.php/fr/statistiques/survie/variations-temporelles-du-taux-de-survie-a-5-ans-entre-2000-et-2014-par-groupe-diagnostique

differences in detection or registration since more elaborate healthcare systems may capture more low-risk cases, including for example, spontaneously regressive cases.
Finally, relapse and long-term outcomes in survivors also depend on the clinical and biological features of neuroblastoma. Nearly 6% of patients die later than 5 years after diagnosis, usually from disease recurrence or second malignant neoplasms. Relapse occurs in more than 50% of children with high-risk neuroblastoma and 20% of intermediary-risk[19].

Cohorts of survivors have shown that children who have had a neuroblastoma were likely to have chronic health conditions, particularly after multimodality therapy. In the Childhood Cancer Survivor Study cohort in the USA, nearly a third of the survivors developed neurological complications and about 8% developed endocrine, sensory, and musculoskeletal complications [20].

Table 2: Five-year overall survival by age, by MYCN status and tumoral extension (RNCE, France, 2000-2010)

	5 years survival [95% CI]		**Total**
	< 1 year old	**≥ 1 year old**	
Total	90.3 [88.0-92.2]	66.0 [63.2-68.7]	75.3 [73.3-77.2]
MYCN			
Amplified	40.0 [27.0-52.7]	44.1 [38.0-50.0]	43.3 [37.8-48.7]
Non amplified	95.1 [93.1-96.6]	71.9 [68.4-75.2]	82.4 [80.2-84.4]
Unknown	89.4 [82.1-93.8]	71.7 [64.6-77.6]	78.1 [72.9-82.4]
Tumor extension			
Non metastatic	96.5 [94.3-97.8]	89.7 [86.6-92.2]	93.0 [91.1-94.4]
Metastatic not 4S	71.3 [61.4-79.1]	45.2 [41.2-49.2]	48.7 [44.9-52.4]
Metastatic 4S	84.6 [78.6-89.0]	48.6 [19.2-73.0]	82.5 [76.5-87.1]

1.2 Clinical and biological features of neuroblastoma

Neuroblastoma is an embryonic and neuroendocrine tumor that arises in the developing sympathetic nervous system. The tumor can develop from any neural crest element, which results in tumors at any site in the sympathetic nervous system (adrenal glands and/or sympathetic ganglia). Around 80% of cases develop in the abdomen, with more than 50 % of tumors occurring in the medulla of the adrenal glands. Other locations include the paraspinal sympathetic ganglia of the neck, chest or pelvis.

The clinical signs and symptoms of neuroblastoma vary greatly depending on size, location, and spread of the tumor. The most common presentation of neuroblastoma is a painless

abdominal mass and often the diagnosis is made following an incidental finding. When present, symptoms can be related to the mass effect from the primary tumor, metastatic disease, or paraneoplastic syndromes. Large tumors can cause abdominal pain and distension, damage of the cervical ganglions or spinal cord compression. Metastatic disease is present at diagnosis for overall 50% of cases and the most frequent locations are regional lymph nodes, bone marrow and bone. Finally, in rare cases neuroblastoma can be associated with syndromes like opsoclonus-myoclonus syndrome or profuse watery diarrhoea as result of hypersecretion of vasoactive intestinal peptide.

The diversity on neuroblastoma clinical behaviour correlates closely with a number of clinical and biologic features. Remarkable efforts have been made in the last decades in developing risk-group assignment in order to provide to each child with neuroblastoma the optimal treatment regimen[21]. Children with low-risk disease may be observed or undergo surgery. Those with intermediate-risk disease may receive chemotherapy and undergo surgical resection, while those with high-risk disease receive intensive multimodality therapy (that includes chemotherapy, surgery, radiation, and immunotherapy).

The International Neuroblastoma Risk Group classification [22] considers the following factors as the most statistically and clinically relevant factors:

- Stage of the tumor at diagnosis
- Age at diagnosis (less than 18 months / 18 months or more)
- Histologic category and grade of tumor differentiation
- Status of *MYCN* oncogene (amplified or not amplified)
- Other genetic characteristics of the tumor like Chromosome 11q status and DNA ploidy

Besides being relevant as risk stratification and treatment factors, the stage and age at diagnosis[23], as well as the status of *MYCN* oncogene status [24], [25] have also been used in etiological approaches as a proxy for potential modifiers of the associations with risk factors.

To date, several genetic alterations have been described in neuroblastoma, which concern gene amplification, polymorphisms and chromosome alterations with loss or gain of chromosome material.

The amplification of the oncogene *MYCN*, at chromosome 2p24, is the most frequently reported tumor genetic alteration. The *MYCN* gene provides instructions for making a protein

that plays an important role in the formation of tissues and organs during the embryonic development. Amplification of the *MYCN* oncogene is observed in 20-25% of all neuroblastoma. It is more frequent among children with neuroblastoma diagnosed later and harbors a particularly poor prognosis (reviewed in [26]).The *MYCN* amplification can occur in a context of co-activation with other genes. Owing to their similar locations on 2p, *ALK* and *MYCN* can be co-amplified. Somatic mutations in *ALK* are reported in approximately 14% of high-risk neuroblastoma[27].

Segmental chromosomal alterations (SCAs) are characterised by losses of chromosomes (1p, 3p, 4p and 11p) and/or gains of chromosomes (1q, 2p and/or 17q)(reviewed in[28]). They are associated with poor prognosis in most cases.

Finally, *LIN28B* polymorphisms have been shown to be associated with high-risk neuroblastoma. *LIN28B* is known to play a crucial role throughout embryonic development, controlling cell growth during neural crest cell lineage differentiation. In neuroblastoma cells, misexpression of *LIN28B* leads to high levels of N-MYC, the protein encoded by *MYCN* [29].

1.3 Natural history: the hypothesis of a perinatal origin

During the early seventies, Knudson[30] proposed the hypothesis that a “two hit” model may explain familial neuroblastoma development, like in retinoblastoma. The first hit being a germline mutation and the second hit an acquired somatic mutation. Although the majority of neuroblastoma are sporadic cases, the multiple-hit model is still supported by considerable evidence suggesting that neuroblastoma is initiated in utero during sympathoadrenal development[31] (Figure 1).

During the early embryonic development (three to five weeks after conception), the neural crest develops from the neural tube. The neural crest cells will then migrate towards the dorsal aorta and differentiate into sympathoadrenal progenitor cells, from which develop the cells of the peripheral nervous system. The migration process requires high expression levels of N-MYC and bone morphogenetic proteins (BMPs). However, once this process has been completed, N-MYC proteins levels should gradually reduce to allow sympathoadrenal maturation and differentiation into sympathetic neurones. The prenatal origin hypothesis suggests that the earliest origin for neuroblastoma may be a neural crest cell that has not received or has not responded to cues that determine cell differentiation. This would represent the first hit towards malignant differentiation. During normal embryonic development, these aberrant undifferentiated cells are detected and the excess of neural precursors undergoes

apoptotic cell death. A second hit would provide to these cells the resistance to apoptotic signals and give raise to the postnatal survival of neuroblast precancer cells. A third hit during the postnatal period may induce precancer cell transformation, and result in neuroblastoma in early childhood[31].

Paternal periconceptional exposures may also be involved. It has been shown that some exposures like tobacco smoke may have germ-cell mutagen effects[32]. Furthermore, epigenetic changes in human sperm may impair normal embryo development, particularly cell death and apoptosis[33].

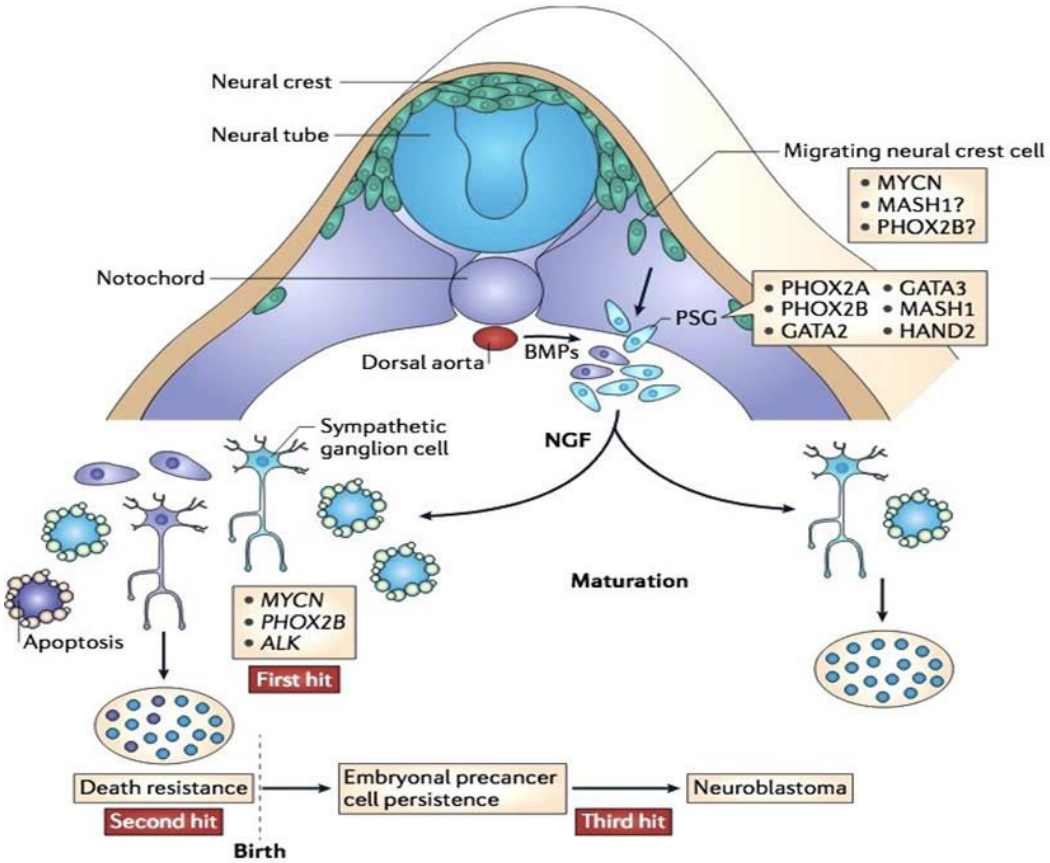


Figure 1: Neural crest development and neuroblastoma. Extracted from Marshall et al "The prenatal origins of cancer".

1.4 Etiology of neuroblastoma

1.4.1 Genetic aspects of neuroblastoma

The possibility of neuroblastoma genetic predisposition has long been suggested after the observation of familial cases. Neuroblastoma has been described in the context of disorders related to abnormal development of the neural crest derived tissues like central congenital

hypoventilation syndrome and Hirschsprung disease. In addition, the associations, while rare, between neuroblastoma and the familial tumor syndrome neurofibromatosis type 1 (NF1), Noonan or Costello syndromes, underlay the potential importance of the *RAS-MAPK* pathway in neuroblastoma development.

Familial neuroblastoma *per se* is rare. A family history of the disease is found in about 1-2% of newly diagnosed cases.

The missense mutations of *PHOX2B* gene on chromosome 4p were the first germ line mutations to be identified in neuroblastoma predisposition[34]. The *PHOX2B* gene provides instructions for making a protein, very active in the neural crest, that acts early in development to help promote the formation and differentiation of neurons. The germline gain-of-function in the anaplastic lymphoma kinase gene (*ALK*) on chromosome 2p23 has been identified in more than half of familial cases[27], [35]. The *ALK* gene provides instructions for making a protein called ALK receptor tyrosine kinase, which is thought to act early in development to help regulate the proliferation of nerve cells. Taken together, the presence of constitutional *PHOX2B* and *ALK* mutations constitute key events in neuroblastoma oncogenesis in affected individuals.

As shown by genome-wide association studies (GWAS) (reviewed in [28]), some constitutional genomic variants correlate with either high-risk or low-risk neuroblastoma and indicate that favorable and unfavorable forms of neuroblastoma may represent distinct entities in terms of genetic events that initiate tumorigenesis.

1.4.2 Birth-related characteristics

The embryonic nature of the tumor cells and the early age at neuroblastoma onset suggest that preconceptional and perinatal events may be involved in its etiology.

1.4.2.1 Birth-weight, gestational age and fetal growth

Previous studies have shown that in-utero events may influence later susceptibility to certain diseases. Fetal growth abnormalities are associated with increased risk of stillbirth, increased neonatal morbidity and mortality. Both fetal growth restriction (when the fetus does not achieve its growth potential) and macrosomia (or fetal over-growth) have been associated with long-term risks to health, such as type 2 diabetes and cardiovascular pathology[36]. In addition, fetal growth abnormalities have been associated with increased risk of some cancers in childhood. A positive association has been consistently reported between fetal over-growth

and childhood acute lymphoblastic leukemia (LLA)[37] and Wilms' tumor[38], [39], while for other childhood cancers, results are still heterogeneous.

The insulin-like growth factors (IGFs) are thought be involved in the biological mechanisms underlying such associations[40]. IGFs are mitogenic and anabolic proteins that are important in the regulation of cell proliferation during normal embryogenesis. However, they are also believed to play a role in carcinogenesis.

Two different approaches have been advocated for the study of fetal growth, both adjusting birth-weight for gestational age. One is based on the premise that fetal growth is mainly influenced by genetics factors and uses growth charts customized for specific phenotypic traits like maternal ethnicity, height and weight[41]. The other is based on the theory that growth potential is similar within populations and uses optimal fetal growth standards at a population level. This second approach has mainly been used in epidemiological studies that have used the combined analysis of birth-weight and gestational age as a proxy for fetal growth. For each gestational age, birth weight below the 10th and above the 90th percentiles of the expected distribution of birth weight in the population are commonly used cut-offs to define the fetus at risk that may benefit from higher surveillance of fetal well-being. Some other studies have used birth-weight *per se* for the characterization of the exposure, with low birth-weight defined by "less than 2500 g" and high birth-weight defined by "more than 4000 g".

Gestational age has also been analyzed in order to assess its potential association with childhood cancer as an independent risk factor.

With regards to neuroblastoma, several case-control studies have analyzed the relationship between gestational age, birth-weight and neuroblastoma, some of them based on maternal interview at the time of diagnosis[23], [38], [42], [43] and some using information from birth certificates[44]–[49]. They are summarized in Table 3.

Overall, no association has been shown between neuroblastoma and gestational age. By contrast, a slight positive relationship was observed with high birth-weight with a pooled OR of 1.2 [95% CI 1.0-1.4] for birth-weight >4000g, compared to those ≤4000 g, in a meta-analysis[50]. Results were heterogeneous among the five studies[38], [46]–[49] that considered birth-weight by gestational age.

Table 3: Literature review - association between neuroblastoma and birth related characteristics

Study		Exposure	Prevalence	Reference group	OR [95% CI]
Interview-based case-control studies					
US-Canada,1992-1994		<32 weeks	2%	37-42 weeks	1.9 [0.7-4.4]
504cases<19 years[42]		33-36 weeks	6%		0.5 [0.3-1.0]
		> 42 weeks	1%		0.9 [0.3-3.0]
		<1500 g	1%	2501-4000 g	2.6 [0.7-10.3]
		1500-2500 g	6%		1.1 [0.6-2.0]
		4000-4499 g	11%		1.1 [0.7-1.7]
		≥ 4500 g	1%		1.4 [0.6-3.2]
Germany, 1988-1994,		<37 weeks	4%	37-42 weeks	2.5 [1.3-4.6]
183 cases< 15 years[23]		< 2500 g	3%	2500-4000 g	2.4 [1.2-4.7]
		> 4000 g	11%		1.3 [0.8-2.2]
Germany, 1992-1994,		SGA	11%	AGA	1.2 [0.7-2.1]
160 cases <14 years[38]		LGA	8%		1.6 [0.9-2.7]
Italy, 1998-2001,		≤37 weeks	15%	38-42 weeks	0.8 [0.5-1.3]
207 cases < 15 years[43]		>42 weeks	5%		0.8 [0.3-2.2]
		< 2500 g	6%	2500-4000 g	0.6 [0.2-1.6]
		> 4000 g	8%		1.1 [0.6-2.0]
Record linkage studies					
Norway, 1967-2004,	≤ 18 months	<37 weeks	N/S	40-41 weeks	0.6 [0.2-2.0]
178 cases < 15 years[44]		> 42 weeks	N/S		1.2 [0.7-2.0]
		< 2500 g	N/S	3000-3499 g	1.1 [0.3-3.7]
		≥ 4000g	N/S		1.8 [1.0-3.1]
	> 18 months	<37 weeks	N/S	40-41 weeks	0.7 [0.2-2.8]
		> 42 weeks	N/S		1.6 [0.8-3.0]
		≥ 4000g	N/S	3000-3499 g	0.9 [0.4-1.8]
US New-York state, 1976-1987		<37 weeks	11%	37-42 weeks	0.4 [0.1-0.9]
155 cases <6 years[45]		>42 weeks	12%		0.3 [0.1-0.7]
		< 2500 g	7%	3000-3499	0.9 [0.4-2.2]
		> 4000 g	12%		1.2 [0.6-2.2]

US Minnesota state, 1976-2004, 155 cases <14 years[46]	<37 weeks	9%	≥37 weeks	1.0 [0.6-1.8]
	< 2500 g	7%	2500-4000 g	1.2 [0.6-2.3]
	> 4000 g	15%		1.1 [0.7-1.7]
	SGA	7%	AGA	2.1 [1.1-4.0]
	LGA	24%	AGA	1.0 [0.7-1.5]
US California state,1988-1997, 508 cases<5 years[47]	<37 weeks	12%	37-41 weeks	0.8 [0.6-1.2]
	>42 weeks	11%		1.1 [0.8-1.5]
	< 2500 g	5%	2500-3999 g	1.0 [0.6-1.6]
	≥ 4000g	12%		1.2 [0.9-1.7]
	term and < 2500 g	2%	Term and /2500-3999 g	1.4 [0.6-3.0]
	Term and ≥ 4000g	10%		1.2 [0.9-1.8]
US Washington state, 1980-2004, 240 cases <15 years[48]	<37 weeks	8%	37-42 weeks	0.6 [0.3-1.1]
	>42 weeks	4%		1.2 [0.9-1.8]
	< 2500 g	5%	2500-3999 g	0.7 [0.4-1.5]
	> 4000 g	13%		1.4 [0.7-2.6]
	SGA	9%	AGA	0.9 [0.6-1.5]
	LGA	10%		1.3 [0.8-1.9]
US New-York state, 1983-2001, 529 cases <15 years[49]	<38 weeks	14%	38-40 weeks	0.9 [0.7-1.2]
	< 2500 g		2500-4500 g	1.5 [1.0-2.1]
	> 4500 g			1.4 [0.7-2.5]
	SGA		AGA	1.0 [0.5-1.9]
	LGA			1.1 [0.8-1.6]

%E: proportion of exposed; SGA: small for gestational age; LGA: large for gestational age; AGA: appropriate for gestational age; N/S: not specified

1.4.2.2 Congenital malformations

Congenital malformations, also known as birth defects or congenital anomalies, are structural or functional anomalies, which may be diagnosed prenatally, at birth or later in life. The term encompasses a heterogeneous group of pathologies that can be caused by single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens and micronutrient deficiencies or excesses[51].

Congenital malformations have been long described to be associated with different types of childhood cancer, especially leukemia and brain tumors[52]–[54]. They may suggest the existence of genetic factors or materno-fetal exposures that increase cancer risk. For example, the reported association between Wilms' tumor and WAGR syndrome (Wilms' tumor, aniridia, genitourinary malformations and mental retardation) led to the identification of the *WT1* gene[55], [56]. Evidence about the increased risk of cervical and vaginal clear cell adenocarcinoma and reproductive tract abnormalities among women exposed to diethylstilbestrol (DES) raised the hypothesis that biological processes, corresponding to non-optimal development, might then induce possible carcinogenicity processes.

Nine studies which investigated the link between congenital malformations and neuroblastoma reported positive associations (Table 4). Both congenital malformations and neuroblastoma are rare, which has limited the ability to do detailed analyses. The association with any malformation has been consistently reported by both interview- [43], [57] and birth certificate-based studies[44], [47], [48], [53], [58]–[60]

1.4.2.3 Maternal folic acid intake

Folic acid is a synthetic form of folate, which is water soluble B vitamin most commonly found in green vegetables. Periconceptional folic acid supplementation has been shown to reduce the risk of neural tube defects by almost three-quarters[61]. Neural tube defects occur when the neural tube, which originates on the neural crest, fails to close early during the embryonic development (after 28 days after conception). This can result in significant morbidity and mortality because of damage of the exposed neural tissue. In 1991, the Centers for Disease Control and Prevention (CDC) recommended that women with a previous history of neural tube defects in offspring should consume 400 µg of folic acid daily starting at the time they begin planning a pregnancy. Subsequently, in 1992, the U.S. Public Health Service extended this recommendation to all women of childbearing age[62]. Because these recommendations may be difficult to achieve for a large part of the general population,

mandatory fortification programs have been implemented in many countries. Up to 2010, the CDC reported that 53 countries had regulations for mandatory fortification of wheat flour with folic acid[63]. However, in France, no such mandatory fortification program has been implemented. Health promotion campaigns encouraging prenatal folic acid supplementation began in 2004, after the ESCALE study period.

Because neural tube defects arise from the same embryonic structures as neuroblastoma, it has been hypothesized that the risk of neuroblastoma could also be reduced by maternal folic acid supplementation before conception and in the first trimester of pregnancy. Biological plausibility of this association is supported by evidence suggesting that low folate levels during the periconceptional period may be associated with increased DNA methylation, thereby modifying the expression of genes, such as *TFAP2A,* a critical gene for neural crest development or *STX11*, a gene implicated in acute myeloid leukemia[64].

Four studies have investigated the association between vitamin/folic acid supplementation during pregnancy and neuroblastoma[65]–[68] (Table 5), three of which reported an inverse association with supplementation during pregnancy[65]–[67], based on maternal interview. In addition, an interventional time series analysis found that the incidence of neuroblastoma significantly declined after the implementation of a mandatory food fortification program in Canada[65].

1.4.2.4 Breastfeeding

Breastfeeding is known to be protective against many childhood diseases because its nutritional, immune modulating and growth-promoting benefits. Inverse associations have been reported with childhood leukemia[69]. Biological plausibility for this association is supported by the fact that breastfeeding provides an influx of growth factors that promote cell differentiation[70] influences microbiota composition[71] and strengthens the infant immune system[72].

Studies on neuroblastoma and breastfeeding are summarized in Table 6. Breastfeeding was reported to reduce risk of neuroblastoma in a large study performed in US and Canada (OR 0.6 [95% CI 0.5-0.9])[73], while two other studies[74], [75] based in less than 50 cases did not report significant associations.

Table 4: Literature review - association between congenital malformations and risk of neuroblastoma

Study		Exposure (Classification System)	%E	RR [95% CI]
Interview-based case-control studies				
US and Canada, 1992-94,		Any malformation (ICD-10)	5.0%	2.5 [1.6-4.2]
538 cases <19 years [57]		Major malformation	1.0%	7.5 [2.2-25.5]
Italy, 1998-2001,		N/S	1.3%	4.9 [1.8-13.6]
207 cases <15 years[43]				
Record linkage studies				
England, Scotland and Wales, 1971-1986,		Spina bifida (ICD-10)	N/S	0.7 (ns)
1208 cases < 15 years[58]		Cardiac septal defects (ICD-10)	N/S	1.1 (ns)
		Tetralogy of Fallot (ICD-10)	N/S	8.3 (ns)
		Genitourinary (ICD-10)	N/S	1.5 (ns)
		Spine malformations (ICD-10)	N/S	1.7 (ns)
Canada, 1977-1993		Any malformation (ICD-9)	4%	1.9 ($p < 0.03$)
141 cases < 15 years [59]				
Norway, 1978-1997,	< 18 months	Any malformation (N/S)	N/S	3.7 [1.7-8.0]
178 cases<15 years[44]			N/S	0.7 [0.1-4.7]
	≥ 18 months			
Australia, 1984-1993,		Any malformation (ICD-9, British	2.5%	7.9 [3.3 – 18.8]
52 cases < 15 years [53]		Pediatric Association modification)		
US, Washington state, 1980-2004,		Any malformation (N/S)	4.8%	2.1 [1.3-3.4]
240 cases < 20 years[48]		Major malformations	0.6%	6.9 [2.9-16.1]
US, California state, 1988-1997	< 1-4 years	Any malformation (N/S)	N/S	1.0 [0.4-2.7]
508 cases < 5 years[47]	< 1 year			1.6 [0.8-3.3]
US Washington state, 1984-2013				
327 cases <20 years[60]		Non-chromosomal malformations	5%	1.9 [1.3-2.8]
		(ICD-9)		

%E: proportion of exposed; CI: confidence interval; N/S: not specified; RR: relative risk estimate (odds ratio or standardized incidence ratio).

Table 5: Literature review - association between neuroblastoma and folic acid/vitamins supplementation in the periconceptional period

Study	Exposure	Exposure period	%E	RR [95% CI]
Interview-based case-control studies				
US-Canada, 1992-1994, 538 cases <15 years[67]	Vitamins use	12 to 2 months before pregnancy	17%	0.7 [0.4-1.1]
		1 month before pregnancy	19%	0.7 [0.7-1.1]
		1st trimester	52%	0.7 [0.5-1.0]
		2nd trimester	80%	0.6 [0.4-0.9]
		3rd trimester	78%	0.6 [0.4-0.9]
	Vitamin, folic acid ≥ 0.4 mg	1st trimester	N/S	0.7 [0.5-0.9]
		2nd and 3rd trimester	N/S	0.6 [0.5-0.9]
New York state, 1976-1987 183 cases < 15 years [66]	Vitamin use	During pregnancy	57%	0.5 [0.3-0.7]
Incidence studies				
Ontario 1985-2000, < 17 years [65]	Trend before/after population food fortification program with folic acid			0.4 [0.2-0.6]
Norway, 1999-2010, 72 cases <15 years [68]	Vitamins only	Before and/or during pregnancy	8%	1.0 [0.3-2.8]
	Folic acid only		17%	1.1 [0.5-2.1]
	Both		19%	1.0 [0.5-2.1]

%E: proportion of exposed; CI: confidence interval; N/S: not specified; RR: relative risk estimate (odds ratio, Incidence rate, or hazard ratio)

Table 6: Literature review - association between neuroblastoma and breastfeeding

Study	Exposure	Reference	%E	RR [95% CI]
Interview-based case-control studies				
Russia,1986-1988,	As reported by interviewed mothers	> 12 months	N/S	7.5 [0.7-97.3]
42 cases < 14years [74]	< 1 months			
	1-2 months		N/S	1.1 [0.2-8.1]
	3-4 months		N/S	2.1 [0.5-9.3]
	5-6 months		N/S	1.9 [0.4-10.9]
	7-12 months		N/S	2.1 [0.5-9.6]
US-Canada, 1992-1994	Breast and bottle	Never	13%	0.7 [0.5-1.2]
393 cases > 6 months-19 years[73]	Breast only		56%	0.6 [0.5-0.9]
	0-3 months		16%	0.7 [0.4-1.0]
	4-6 months		13%	0.7 [0.5-1.2]
	7-9 months		9%	0.6 [0.4-1.1]
	9-12 months		7%	0.6 [0.3-1.1]
	≥ 13 months		10%	0.5 [0.3-0.9]
				p-trend < 0.01
Record linkage studies				
Sweden,1988-1991,	1-6 months	<1 month	ns	0.6 [0.1-2.8]
34 cases <14 years[75]	≥ 6 months		ns	0.5 [0.1-2.6]

%E: proportion of exposed; CI: confidence interval; N/S: not specified; RR: relative risk estimate (odds ratio or standardized incidence rate)

1.4.3 Environmental risk factors

1.4.3.1 *Pesticides*

Pesticides are defined as any substance, molecule or product aimed to eliminate pests[76]. The term covers a large, heterogeneous group of chemicals. Pesticides are used in a large number of settings including the rural sector, public spaces and around the home. France is the second leading European country with regards to agricultural pesticides purchase. This is mainly related to its agricultural surface (the biggest in Europe) and the prevalence of high pesticide use (grapevine, wheat and canola).

The general population is exposed to multiple sources of pesticides, which contribution to the overall individual exposure is yet to be determined. The main sources of pesticide exposure in general population are food, contamination from outdoor and indoor air, soil or indoor dust, and the use of pesticides in gardens and on domestic animals.

As well as playing a role in enhancing food production and contributing to the control of diseases like malaria, pesticides have also been associated with adverse health events such as childhood leukemia[77]. The International Agency for Research on Cancer (IARC) has classified seven pesticides as "probable or possible" human carcinogens and many more as carcinogenic to laboratory animals[78][79]. Previous studies have shown that pesticides can reach the fetus after maternal exposure and, in many cases, induce genotoxic damage (reviewed in[80]).

Exposures to pesticides have been the most investigated environmental exposures with regards to neuroblastoma and the published papers are summarized in Table 7. This thesis focused on maternal use of household pesticides and maternal occupational exposure during pregnancy. Increased risk of neuroblastoma was associated to self-reported use of household pesticides before[25] or after birth[23] in two previous case-control studies.

Maternal occupational exposures were addressed by five case-control studies[23], [81]–[84] and three of them[23], [81], [83] reported increased risk of neuroblastoma with farming or pesticides use. Two cohorts[85], [86] and seven case-control studies [23], [25], [81]–[84] investigated paternal exposure with inconsistent results, as summarized by a meta-analysis showing no association with neuroblastoma[87].

1.4.3.2 Tobacco smoking and alcohol

Tobacco smoke and alcohol consumption are of interest as potential risk factors of neuroblastoma. The IARC has classed tobacco smoke and its metabolites as proven carcinogens to humans (Group 1)[88]. Tobacco smoke compounds, either directly or after metabolism, react with specific sites in DNA to form covalent binding products called addition products (or adducts) that can cause mistakes during DNA replication. Several adducts have been found in placental and umbilical cord DNA, showing that tobacco smoke compounds can cross the placenta leading to fetal exposure and DNA damage [89]. It has also been shown that paternal smoking affects both genomic and epigenomic components of the sperm and could be related with developmental defects in the offspring[90].

Teratogen and carcinogenic effects have also been shown with regards to alcohol consumption. The underlying biological mechanisms are not clear, but explanatory hypotheses highlight the potential of ethanol and its metabolites to generate oxidative stress, induction of mitogen-activated protein kinases (MAPK) and folate and DNA methylation, among others [91].

Overall, literature suggests that maternal tobacco smoking during pregnancy is associated with a slight increase in risk of neuroblastoma, based on 8 studies[23], [24], [43], [45], [46], [48], [92], [93] estimated by a pooled OR of 1.2 [95% CI 1.0-1.4] in a recent meta-analysis[94]. Fewer studies report data on paternal smoking and no consistent pattern has been observed to date. Findings on maternal alcohol drinking during pregnancy are also heterogeneous[23], [24], [45], [46], [92]. The literature on tobacco smoking and alcohol are described in more detail in the Results section (3.4.2.3 Meta-analysis).

1.4.3.3 Other environmental factors

Associations with parental occupational exposures to magnetic fields, hydrocarbons or other chemicals [82]–[84], [95] or residential exposure to air pollutants [96], [97] have been investigated reported and no consistent pattern has emerged to date.

Table 7: Literature review - association between parental pesticide exposure and neuroblastoma

Study	Exposure	Parent	Period	%E (controls)	RR [95% CI]
Parental occupational exposures - cohorts					
Norway, 1952-1991, 27 cases [85]	Field vegetable farming (agricultural census)	Any parent	Any time	N/S	2.5 [1.0;6.1]
US, 1993-1997, 3 cases[86]	Farming	Any parent	Any time	N/S	1.3 [0.4;3.9]
Parental occupational exposures – case-control studies					
US Greater Philadelphia area, 1970-1979, 104 cases[81]	Farming	Any parent	Pregnancy	N/S	0.7 [0.1;5.8]
			Preconception	N/S	3.5 [0.7-35]
US and Canada, 1992-1996, 504 cases[82]	Farming	Maternal	Any time	N/S	2.2 [0.6;8.8]
		Paternal	Any time	N/S	0.9 [0.4;1.8]
US New-York state, 1976-1987, 183 cases[83]	Field vegetable farming	Maternal	Pregnancy	N/S	0.8 [0.2;3.2]
		Paternal	Pregnancy	N/S	1.0 [0.2;3.9]
	Insecticides use	Maternal	Pregnancy	N/S	2.3 [1.4;3.7]
		Paternal	Pregnancy	N/S	1.7 [1.0;2.7]
Germany, 1988-1994, 183 cases[23]	Farming	Maternal	After birth	3%	1.2 [0.4;3.7]
	Any occupational use	Maternal	Any time	1%	5.1 [1.1;23.4]
		Paternal	Any time	4%	1.8 [0.8;3.7]
Great Britain, 1962-1999, 2920 cases[84]	Agriculture	Paternal	Pregnancy	2%	0.9 [0.6;1.3]
	Agrochemical industry	Paternal	Pregnancy	3%	1.0 [0.7;1.4]
Parental non-occupational exposures - case-control studies					
US and Canada, 1992-1994, 538 cases[25]	Household use	Both parents	Ever	31%	1.6 [1.0;2.3]
			Preconception-pregnancy	18%	1.3 [0.8;3.3]
			After birth	18%	1.4 [0.9-2.2]
	Garden	Both parents	Ever	22%	1.7 [0.9;2.1]
			Preconception-pregnancy	13%	1.3 [0.8;2.0]
			After birth	12%	1.8 [1.0-3.1]
Germany, 1988-1993, 183 cases[23]	Household insecticides use		After birth	6%	1.8 [0.9;3.4]
	Garden pesticides		After birth	10%	0.9 [0.5;1.6]

RR [95%CI]: RR estimated (Standardized incidence ratio or odds ratio) and its 95% confidence interval; N/S: not stated

1.5 Aims of the work

The present work aimed at further understanding the perinatal and environmental risk factors of childhood neuroblastoma, with the period around pregnancy as a critical window of exposure.

With regards to perinatal factors, we investigated the following factors:

- Birth-related characteristics (gestational age, birth-weight and fetal growth), with the hypothesis that increased fetal growth could be related to neuroblastoma.
- The presence of congenital malformations, with the hypothesis that developmental genes are responsible of both congenital malformations and embryonic tumors, particularly neuroblastoma.
- Maternal intake of folic acid before or during pregnancy, with the hypothesis that low folate levels during the periconceptional period may be associated with increased risk of neuroblastoma because of abnormal gene expression during neural crest development.
- Breastfeeding, which is suggested to reduce the risk of many childhood cancers.

With regards to environmental exposures related to parental habits, we focused on the following exposures around pregnancy, which are suspected risk factors:

- Maternal use of household pesticides during pregnancy, and maternal occupational exposure to pesticides.
- Maternal smoking during pregnancy and paternal smoking in the year before pregnancy
- Maternal alcohol consumption during pregnancy

Methods

2 Population and methods

These analyses used data from the ESCALE and ESTELLE studies, two nationwide case-controls studies based on the French national registry of childhood cancers. The ESCALE study (Etude Sur les Cancers et les Leucémies de l'Enfant) was performed in 2003-2004 and included all cases of neuroblastoma, leukemia, lymphoma and malignant brain tumor. The ESTELLE study (Etude Sur les Tumeurs Embryonnaires, Leucémies et Lymphomes de l'Enfant) was performed in 2010-2011 and included cases of neuroblastoma, leukemia, lymphoma, malignant brain tumor, Wilms' tumor and hepatoblastoma.

2.1 Study population

2.1.1 Case and control ascertainment

2.1.1.1 The French National Registry of Childhood cancers (RNCE)

As a Health Registry, the RNCE is a continuous and exhaustive collection of individual data for public health and research purposes, on childhood cancers and some borderline tumors diagnosed in France (http://rnce.inserm.fr).

The RNCE is composed by two entities:

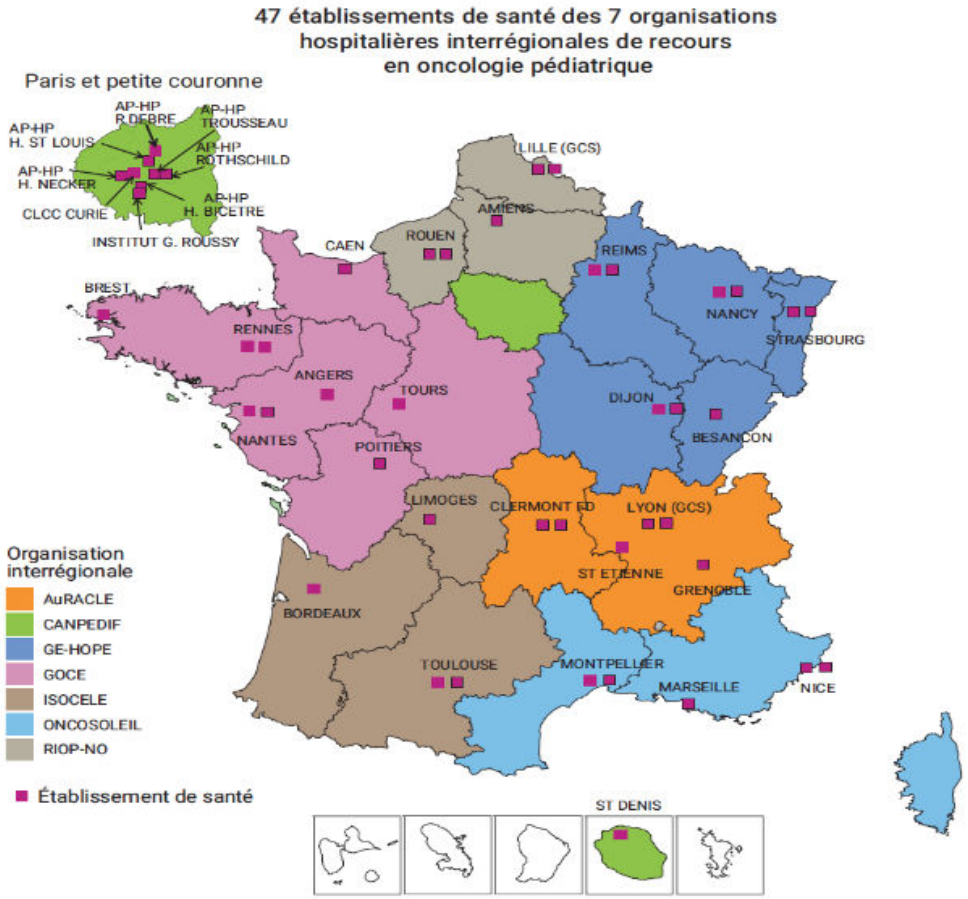
- The registry of childhood hematological malignancies (RNHE), starting in 1990
- The registry of childhood solid tumors (RNTSE), starting in 2000

The RNCE was created with three main goals:

1. To estimate and monitor childhood cancer incidence and survival rates in France by histologic/cytological/molecular subtypes, as well as to detect geographical and temporal variations of the rates.
2. To support etiological research on the potential risk factors of childhood cancers.
3. To provide assistance for the assessment of health providers and quality of care.

The RNCE currently includes all children younger than 18 years old and living in mainland France or in the overseas at the time of diagnosis. Up to 2011, the RNCE included cases under 15 years of age and living in France mainland only.

Complete information about each patient is collected by active searching in the forty-seven Hospital Centres that provide care for children with a cancer or borderline tumor (Figure 2) and in additional services where cases are detected using medico-administrative databases. Trained clinical research associates transmit the information to the RNCE coordinators, responsible for data monitoring and validation, and for diagnoses coding and staging.



CLCC Centre de lutte contre le cancer ; GCS Groupement de coopération sanitaire ; AP-HP Assistance publique-Hôpitaux de Paris. Source et traitement : INCa 2014

Figure 2: Location of oncology centres in France and overseas

2.1.1.2 Inclusion in the ESCALE and ESTELLE studies

The parents of the eligible cases were contacted at hospital by the network of the RNCE clinical research assistants. The eligible cases were children diagnosed with a neuroblastoma during the study period, younger than 15 years old and living in mainland France at the moment of diagnosis (Figure 3). Their biological mother had to be eligible for interview, which led to exclusion of adopted children, children whose biological mother had died, did not speak French, or had a serious psychiatric disorder (n=34). Children who had died and those in palliative care (n=22) were not eligible because of ethical reasons.

2.1.1.3 Control selection (Figure 4)

Eligible controls were children from general population that had not been diagnosed with cancer. Like the cases, the children who had been adopted, or whose biological mother had died or did not speak French were not eligible as controls (Figure 4).

The procedure for control sampling was slightly different for the two studies. In the ESCALE study, a base of 60,000 telephone numbers was randomly extracted from the national telephone directory. The set was representative of the population in terms of the administrative regions and urbanization. By incrementing each number by 1, a new set of 60,000 numbers was generated. The new set included unlisted numbers and had geographic and demographic distribution similar to those of the initial set (same first six digits, which indicate the location of the line). In the ESTELLE study, the population controls were children free from cancer selected in France. Forty successive sets of 5,000–25,000 allocatable telephone numbers were randomly generated and dialed over the 2 years of the subject recruitment period.

In both studies, the telephone numbers were generated by sequential sets so as to refresh the base every 3 months. The obtained numbers had to be dialed up to six times at different schedules before being abandoned.

Quotas of age and gender were used to make the age and gender distribution of the controls similar to the case distribution of all the cancers. The expected numbers were based on RNCE data from previous years. Overall, there was at least one control by case in all the age and gender strata, for each type of cancer. Controls younger than one year were overrepresented to increase power in that category.

The quotas also ensured that the control group had the same distribution as the overall French population for the number of children aged <15 years living in the household, conditional on age, based on data from the National statistics office (INSEE).

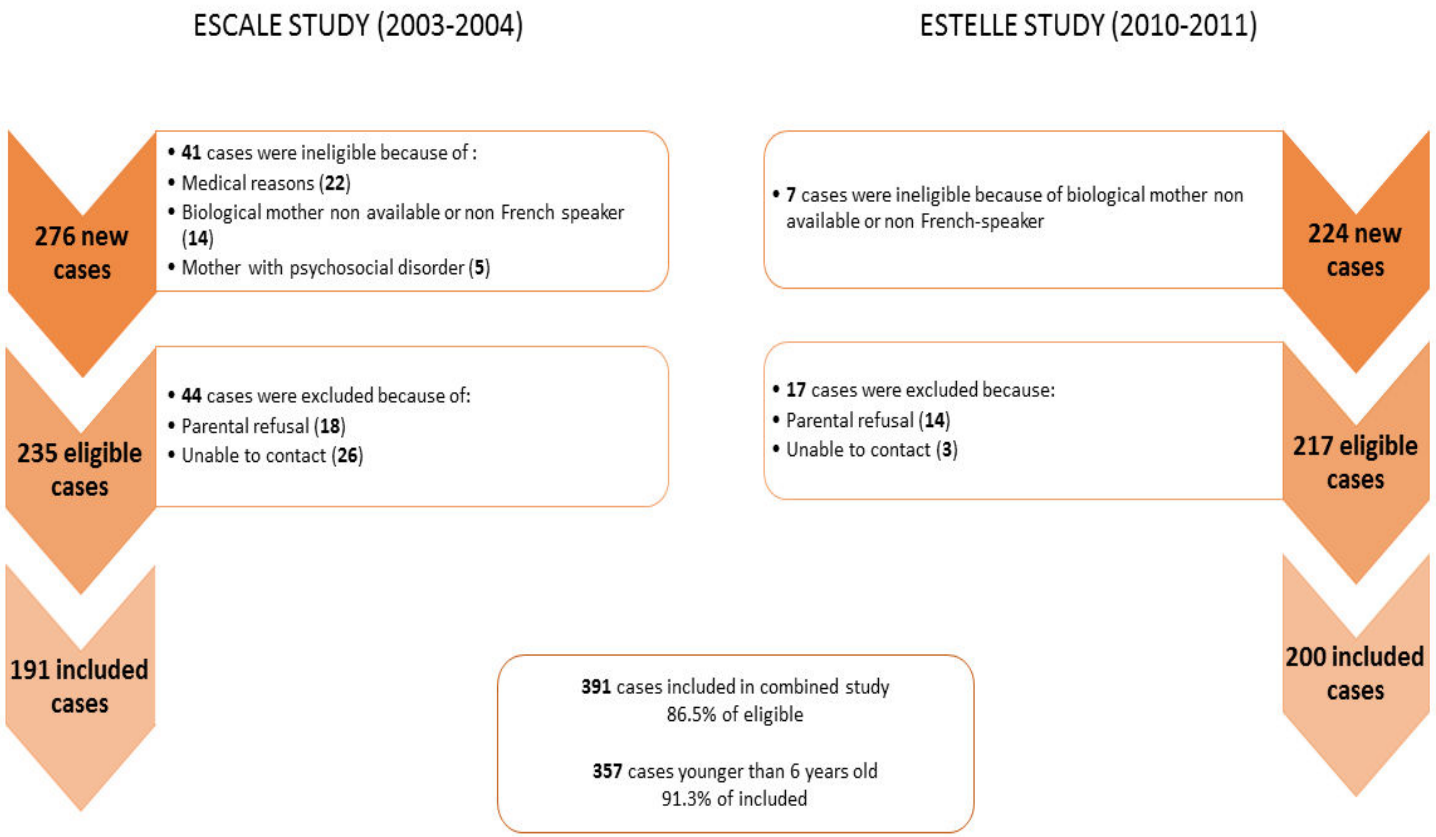


Figure 3: Case recruitment by contact to parents in the hospital departments

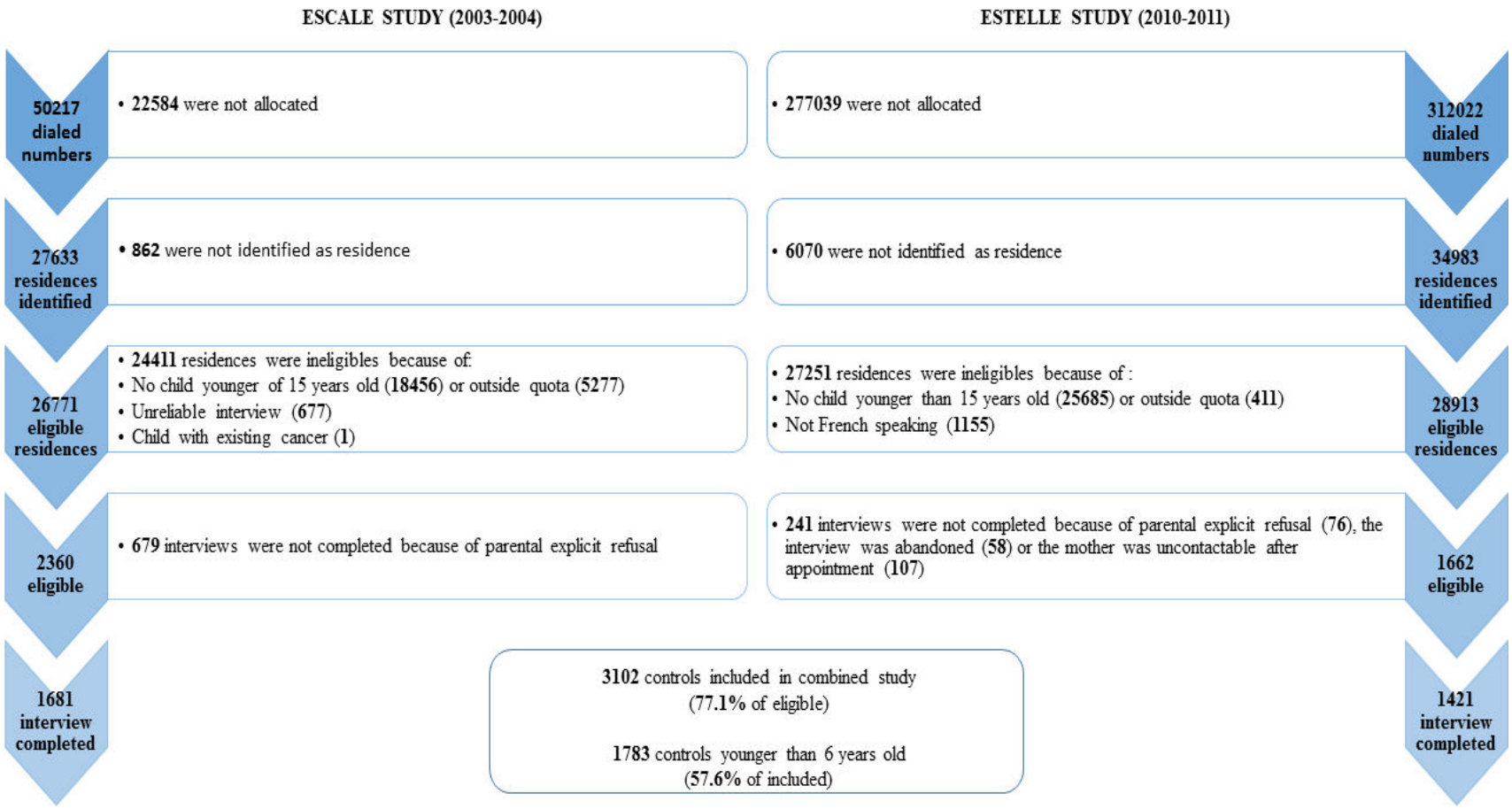


Figure 4: Flowchart of the phone calls dialled for the recruitment of controls

2.2 Data collection and data management

The same trained interviewers carried out the interviews with the mothers of case and control children using structured questionnaires with computer-assisted telephone interviewing. The questionnaire elicited information on demographic and socioeconomic characteristics, childhood environment and lifestyle, familial and personal medical history, and history of pregnancy.

2.2.1 Perinatal characteristics

2.2.1.1 Birth-weight and gestational age

The mothers were asked about the child's birth-weight and gestational age. For this question, the mothers were advised to consult the relevant pages of the child's personal heath record, which were available for over 95% of cases and 98% of controls.

Birth-weight was classified with cut-offs in grams (< 2,500, 2,500–2,999, 3,000–3,499, 3,500–3,999, 4,000) comparable with those used by the French national perinatal surveys. Gestational age was classified as: pre-term (< 37 complete weeks of pregnancy), post-term (42 weeks or more) and two at-term categories (37–39 and 40–41 weeks). Using these data, we estimated fetal growth using the birth-weight Z-score based on gender-specific percentiles for birth-weight by gestational age in weeks from a large French cohort by the Association des Utilisateurs de Dossiers Informatisés en Pédiatrie, Obstétrique et Gynécologie (AUDIPOG)[98]. Using the standard definitions, fetal growth was classified as small (SGA, < 10th percentile), appropriate (AGA, 10th through the 90th percentile), or large for gestational age (LGA, > 90th percentile)

2.2.1.2 Congenital malformations

Mothers were asked if the child was diagnosed at birth with congenital malformations. If so, details of the type and site of malformations were collected.

The congenital malformations were coded using the International Classification of Diseases, 10th Revision (ICD-10) [99] by two independent researchers (Paula Rios and Helen Bailey). The coding process was reviewed by a third researcher (Jacqueline Clavel) when doubts or disagreement. All the coders were blind to case-control status. Then, the children with only minor or unspecified malformations were excluded in accordance with the European Surveillance of Congenital Anomalies (EUROCAT) recommendations [100].

2.2.1.3 Maternal history and folic acid supplementation

The mothers were asked if they took any vitamin, mineral or folic acid supplements three months before pregnancy or in the 1st, 2nd or 3rd trimester of pregnancy. If yes, they were asked to name the specific product.

The use of vitamin, mineral or folic acid supplements was considered as a binary (ever/never), and by trimester from three months before pregnancy to birth, taking as reference category the absence of supplementation in any of those periods. We also used a more specific classification, defining "substantiated" folic acid supplementation as supplementation reported for the relevant period with a valid product name, and "unsubstantiated" supplementation as supplementation reported for the relevant time period but with no valid name given.

The mothers were asked about their age at the index child's birth, the parity of that pregnancy and if they had difficulty becoming pregnant. Difficulty becoming pregnant was defined as taking more than one year to conceive the index child and/or the need to consult a doctor and/or the need for the mother or father to undergo fertility treatment, which could influence intake of folic acid. In the latter case, the mother was asked to specify the type of treatment.

2.2.1.4 Breastfeeding

Mothers were asked if they breastfed the index child and if yes, for how long. Breastfeeding was defined as having breastfed for at least three days after birth. Ever breastfed children were categorized by breastfeeding duration (<3 months, 3-5 months and 6 months or more). In order to properly address breastfeeding in relation to the development of neuroblastoma, analyses were limited to children aged six months or older at diagnosis or at the reference date for controls (269 case and 1589 control children), so that the cases and controls would have had the opportunity of being breastfed for up to six months.

2.2.2 Environmental exposures related to parental habits

2.2.2.1 Pesticides

The mother was asked if she used herbicides (''weed killers''), fungicides, or insecticides (and whether they were used indoors, for gardening or outdoors, or on pets). She was also asked if she was exposed to any type of pesticides in the workplace. The ESTELLE study included additional questions about maternal pesticide use in the three months prior to conception and after birth, and whether there had been any professional pest control treatments of the home.

Pesticide exposure was categorized into the following groups: None, any pesticide (declined in any insecticide, any herbicide or any fungicide) and categories based on whether the mothers reported that they had been used alone (only insecticides, only herbicides and only fungicides) or in combination with other pesticides (insecticides + other pesticides, only herbicides and fungicides). The reported use of insecticides was categorized based on the place of application declared by the mothers (indoor use, gardening and outdoor, for pets). The maternal professional exposure was considered as a dichotomous variable (yes/no) based on the maternal report of being exposed to pesticides at the workplace.

2.2.2.2 Tobacco smoking and alcohol

The mothers were asked whether they had smoked cigarettes during the pregnancy with the index child, and if yes, their average daily consumption. The ESTELLE study included additional questions about their smoking habits in the three months before the pregnancy and in each trimester of pregnancy, which was used for analyses by time window.

Mothers were also asked if the father of the index child smoked during the year before to her pregnancy. To validate the maternal responses about paternal exposures, a small convenience subset of ESTELLE fathers also did a telephone interview about their exposures. The fathers of 134 childhood cancer cases and 174 controls were interviewed about their exposures, which included the same questions about their smoking habits as had been asked in the maternal interview.

Maternal and paternal tobacco smoking were analyzed as dichotomous variables (ever/never) and as quantitative variables depending on the reported cigarettes per day (CPD). Maternal and paternal average daily consumptions were grouped in three categories based on the tertiles among smoking control parents. The cut-offs were: 0; 1-4; 5-9 and ≥10 CPD for mothers and 0; 1-9; 10-15 and > 15 CPD for fathers. The joint effect of maternal and paternal smoking was also analyzed (neither parent, only mother, only father, both).

Mothers were asked about alcohol consumption (wine, beer/cider, and spirits) during pregnancy and to quantify their consumption if applicable. In the Estelle study, they were also asked specifically about consumption in the first trimester. Alcohol consumption was categorized three ways: a dichotomous variable (ever/never), a quantitative variable (cut-offs defined *a priori* as following: nil, <1, 1-2 or >2 glasses per week), and by type (wine, beer or cider, spirits).

2.3 Statistical methods

2.3.1 Case-control analyses

We first analyzed study-specific ORs and 95% confidence intervals (CIs) using unconditional logistic regression methods. The analyses on perinatal risk factors of neuroblastoma were performed using STATA software (STATA version 11, StatCorp LP, College Station TX), while the analysis on environmental exposures were performed using SAS software (SAS version 9; SAS Institute Inc., Cary, NC, USA).

In addition to exploring differences by case–control status, we explored the differences of each of the key exposure variables by study among the controls to see if there had been changes in parental characteristics and behavior between the study time periods. In pooled analyses of the ESCALE and ESTELLE studies, between-study heterogeneity was systematically tested by fitting an interaction term between the study and the exposure of interest.

All the models included the study matching factors child's age and gender and, for the pooled analyses, the indicator of the study of origin. Because of the particular distribution of neuroblastoma cases, only cases and controls younger than six years old were included in the pooled analysis of the ESCALE and ESTELLE studies (91.3% of included cases). To avoid possible residual confounding by age, the first year of age was split into quarters (< 3 months, 3–5 months, 6–8 months, 9–11 months), the second year into semesters (12–17 months, 18–23 months), and the other ages were kept as single years (2, 3, 4 and 5 years).

Sociodemographic variables paternal and maternal age at the child's birth, maternal education, birth order and degree of urbanization of the place of residence were assumed *a priori* to be potential confounders because of their possible relationship with unmeasured confounders and their possible influence on parents' participation in the studies. They were tested to determine whether they met the empirical definition of confounding (i.e. independent association with both the exposure of interest and with neuroblastoma). All the models included birth order, maternal age and urban status of the area of residence in addition to matching variables (child's age and sex) and the study of origin (ESCALE or ESTELLE).

For the analyses of maternal household use of pesticides during pregnancy, the type of housing was also retained as a potential confounder. The analyses on maternal and paternal smoking were mutually adjusted for these factors.

Tests for linear trend were computed for quantitative variables (e.g. breastfeeding duration,

number of cigarettes per day, number of glasses of alcohol per week). First, the deviation from linearity was tested by a likelihood ratio test, comparing the model with the newly generated quantitative variable, where subjects in each class of the categorical variables were assigned the median value of that class, to the full model with the categorical variable. If linearity was not rejected, the *p* value of the trend was determined by testing the slope of the quantitative variable using a Wald-test.

We performed additional analyses by age at diagnosis (<18 months/≥18 months) and by *MYCN* status (amplified/non-amplified). For the study of the maternal use of household pesticides during pregnancy, we also performed stratified analysis on urban/rural status of the area of residence, and maternal level of education (less than baccalaureate/baccalaureate or higher).

2.3.2 Meta-analysis on maternal smoking and alcohol consumption during pregnancy

A systematic review on maternal smoking and alcohol consumption during pregnancy was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[101] (see Supplementary material).

The main author (Paula Rios) undertook the study selection, data abstraction and assessment of study quality of the systematic review process. In case of doubts about study inclusions or estimates extraction, these were discussed with co-authors (Jaqueline Clavel and Helen Bailey).

The Medline and Embase databases were searched from inception to October 2018.

Studies were included if they were published in English, French or Spanish. In addition, all references cited in original studies and reviews were manually searched.

To be included in the meta-analysis, each study was required to:

1. Be an original report.
2. Be a cohort or case–control study that presented ORs and corresponding 95% CIs for the association between maternal smoking and alcohol consumption during pregnancy and risk of childhood neuroblastoma (or provide data that allowed these to be calculated).
3. Include pediatric neuroblastoma cases (less than 19 years old)

Exclusion criteria for studies were:

1. Studies reporting “parental” consumption without specifying whether this was maternal or paternal exposure.

2. Studies exclusively reporting maternal smoking or alcohol consumption but not related to around the pregnancy time window.
3. Studies reporting estimates on "All childhood cancers" but not specifying numbers or estimates for neuroblastoma.

The extracted study characteristics for meta-analysis encompassed: publication year, study time frame, study design, study size, recruitment source, and source of exposure information, age at diagnosis, matching variables, effect measures and confounders.

We used random effects, precision-based weighting to calculate the summary OR with our results. Statistical heterogeneity between studies was assessed using the Cochrane Q test. Publication bias was assessed via inspection of the funnel plot and formal testing for funnel plot asymmetry using Egger's test.

In the assessment of the quality of included studies, and as to evaluate comparability of cases and controls on the basis of design, age at diagnosis was set *a priori* as the most important matching or adjusting factor in the Newcastle-Ottawa Quality scale[102].

In the assessment of the possibility of bias in study designs, conduct and analyses, we used the Joanna Briggs checklists for case-control studies[103].

Sensitivity analysis were performed by stratifying our analysis by study type (studies based on record linkage/interview) and by excluding studies with lower quality scores (Newcastle-Ottawa Quality scale <7).

2.3.3 Statistical power

Power calculations were done for the 351 cases included in the pooled analysis of the ESCALE and ESTELLE studies. The calculations assumed a 5% level of significance and the estimate for the prevalence for each exposure was based on that reported by the National Perinatal Survey (ENP) performed during the same time periods and EUROCAT for the prevalence of congenital malformations. Power calculations were not possible with regards to household use of pesticides as no information about the exposure on the general population was available for the relevant time period. Table 8 shows the relative risk that this study had the power to detect at the >80% level.

Table 8: Statistical power

Prevalence of exposure	Examples of activity, time period, person with this level of prevalence in the control series	Odds ratio	
		Minimum	Maximum
0.4	Folic acid supplementation during pregnancy	0.7	
0.2	Smoking, alcohol consumption during pregnancy		1.5
	Folic acid supplementation before pregnancy	0.6	
0.07	Preterm (< 37 weeks) LBW (< 2500 g) HBW (> 4000 g)	1.8	
0.03	Congenital malformations	2.2	

LBW: low birth-weight; HBW: high birth-weight

2.3.4 Sensitivity analysis

Since controls were recruited from a sample of landline telephone numbers, sensitivity analyses were performed excluding case mothers with no landline at home (restricted to ESTELLE, the only study for which these data were collected). Landline subscribers might be different from mobile phone only owners in terms of age and other unknown characteristics.

With regards to birth-related characteristics, analyses were performed after exclusion of multiple births (related to lower birth weight and length) and children born following in vitro fertilization (whose mother was more likely to have received folic acid supplementation). Finally, children with genetic syndromes (one case and 3 controls) were excluded in sensitivity analyses on congenital malformations.

Results

3 Results

3.1 Case-control comparability

The study included 357 cases (174 in ESCALE, 183 in ESTELLE) and 1783 (949 in ESCALE and 834 in ESTELLE) controls aged less than six years. The *MYCN* oncogene was amplified in 64 cases (17.9 %), not amplified in 270 cases (75.6%) and non-informative (NI) in 23 cases (6.4%). The amplification of *MYCN* was more frequent among older children (11% of the cases under 18 months of age versus 25% of the older cases). Overall case participation rates were 81.2% for ESCALE and 92.2% for ESTELLE.

The ESCALE and ESTELLE studies included cases of leukemia, lymphoma, brain tumors, neuroblastoma, Wilms' tumor and hepatoblastoma. In both studies the cases and the controls were generally similar on sociodemographic characteristics (Table 9), although the cases tended to be younger, with younger mothers and in more urban areas than the controls. The control sampling was performed so that the control group would have the same age distribution as the complete group of childhood cancer cases in the ESCALE and ESTELLE studies, and not specifically neuroblastoma. However, there were at least two controls for each case in each age stratum. The participation rates were 71.2% for ESCALE and 85.5% for the ESTELLE study.

3.2 Between-study heterogeneity

The prevalence of some of the exposures of interest differed between the studies. Control mothers in the ESTELLE study lived more often in less populated areas and were more highly educated than those in the ESCALE study. They were also more likely to have used pesticides at home (39.6% for ESTELLE and 32.2% for ESCALE; p value for heterogeneity < 0.01).

Fewer control mothers reported drinking alcohol (24.5 vs. 36.6%, p value <0.001) during the index pregnancy in the ESTELLE study than in the ESCALE study, while there were no differences in the reported prevalence of maternal smoking (19.3% in ESCALE and 20.4% in ESTELLE).

Table 9: Characteristics of the cases and controls of the ESCALE and ESTELLE studies

	ESCALE (2003-2004)				ESTELLE (2010-2011)				POOLED			
	Cases (n=174)		Controls (n=949)		Cases (n=183)		Controls (n=834)		Cases (n=357)		Controls (n=1783)	
	N	%	N	%	N	%	N	%	N	%	N	%
***MYCN* status**												
Non-amplified	131	*75.3*			139	*76.0*			270	75.6		
Amplified	34	*19.5*			30	*16.4*			64	17.9		
Missing	9	*5.2*			14	*7.6*			23	6.4		
Age (years)												
< 1	74	*42.5*	187	*19.7*	70	*38.2*	188	*22.5*	144	*40.3*	375	*21*
1	36	*20.7*	182	*19.2*	37	*20.2*	123	*14.7*	73	*20.4*	305	*17*
2	30	*17.2*	153	*16.1*	35	*19.1*	148	*17.7*	65	*18.2*	301	*16.9*
3	13	*7.5*	166	*17.5*	21	*11.5*	139	*16.7*	34	*9.5*	305	*17.1*
4	11	*6.3*	145	*15.3*	12	*6.6*	131	*15.7*	23	*6.4*	276	*15.5*
5	10	*5.7*	116	*12.2*	8	*4.4*	105	*12.6*	18	*5*	221	*12.4*
<18 months	93	*53.4*	283	*29.8*	95	*51.9*	261	*31.3*	188	*52.7*	544	30.5
≥18 months	81	*46.5*	666	*70.2*	88	*48.1*	573	*68.7*	169	*47.3*	1239	*69.5*
Birth order												
First born	86	*49.4*	520	*54.7*	100	*54.6*	446	*53.5*	186	*52.1*	966	*54.2*
Second or more	88	*50.6*	429	*45.2*	83	*45.3*	388	*46.5*	171	*47.9*	817	*45.8*
Maternal age at child's birth (years)												
< 25	31	*17.8*	80	*8.4*	25	*13.7*	86	*10.3*	56	*15.7*	166	*9.3*
25-29	61	*35.1*	314	*33.1*	70	*38.2*	250	*30*	131	*36.7*	564	*31.6*
30-34	54	*31.0*	360	*37.9*	50	*27.3*	293	*35.1*	104	*29.1*	653	*36.6*
≥ 35	28	*16.1*	195	*20.5*	38	*20.8*	205	*24.6*	66	*18.5*	400	*22.4*
Maternal education												
< Baccalaureate	55	*31.6*	319	*33.6*	50	*27.3*	203	*24.3*	105	*29.4*	522	*29.3*
Baccalaureate	32	*18.4*	195	*20.5*	44	*24*	192	*23*	76	*21.3*	387	*21.7*
> Baccalaureate	87	*50.0*	435	*45.8*	88	*48.1*	439	*52.6*	175	*49.0*	874	*49.0*
Missing	0	*0*	0	*0*	1	*0.5*	0	*0*	1	*0.3*	0	*0*
Size of urban unit of residence (inhabitants)												
< 5000	57	*32.8*	360	*37.9*	59	*32.2*	351	*42.1*	116	*32.5*	711	*39.9*
5000-99,999	36	*20.1*	211	*22.2*	43	*23.5*	174	*20.9*	79	*22.1*	385	*21.6*
100,000-1,999,999	41	*23.6*	233	*24.5*	50	*27.3*	158	*18.9*	91	*25.5*	391	*21.9*
Paris unit	37	*21.3*	145	*15.3*	30	*16.4*	149	*17.9*	67	*18.8*	294	*16.5*
Missing	3	*1.7*			1	*0.5*	2	*0.2*	4	*1.1*	2	*0.1*

3.3 Perinatal characteristics

3.3.1 Gestational age, birth-weight and congenital malformations

There was no significant association between gestational age or birth-weight and neuroblastoma. SGA and LGA were both associated with neuroblastoma (ORs 1.4 95% [CI 1.0–2.0] and 1.5 95% CI [1.1–2.2], respectively) (Table 10). The associations were specially marked for children younger than 18 months (ORs 1.7 [95% CI 1.0–3.1] and 2.0 [95% CI 1.2–3.3], respectively).

Overall, congenital malformations were reported slightly more often for cases (4%) than for controls (3%), and the association tended to be more marked, although based on small numbers, when the child had two or more malformations (OR 6.3 [95% CI 1.3–29]). The most frequently reported sites of malformations were the skeletal and genitourinary systems. Lastly, the association between malformations and neuroblastoma was only observed in children aged <18 months (OR 3.6 [95% CI 1.3–8.9]). Results were similar when the analysis by subgroups defined by case *MYCN* amplification status was performed.

3.3.2 Maternal intake of folic acid, vitamins or minerals during the periconceptional period

There was an inverse association with maternal use of any supplement containing folic acid, vitamins or minerals in the three months before conception (OR 0.5 [95% CI 0.3–0.9]) (Table 11). There was little change when the analysis was restricted to substantiated supplements containing folic acid (OR 0.4 95% CI 0.2–1.1). There was no association with the use of supplements during the other trimesters of pregnancy. There was no association between neuroblastoma and difficulty becoming pregnant or the use of fertility treatments for the index pregnancy, although based on small numbers (Table 12).

3.3.3 Breastfeeding

The breastfeeding analysis was restricted to the 1,589 controls and 269 cases who were aged 6 months or older (Table 13). The cases were breastfed less often than the controls (OR 0.7 [95% CI 0.5–1.0]) but there was no evidence of a decrease in risk with increasing breastfeeding duration. The inverse trend was significant when the association was restricted to the cases with *MYCN* amplification (p values for trend< 0.01)

Table 10: Birth related characteristics, congenital malformations and risk of neuroblastoma. Pooled analysis of the ESCALE and ESTELLE studies.

	All neuroblastomas						Age <18 months						Age ≥18 months					
	Controls		**Cases**		**OR**	**95%CI**	**Controls**		**Cases**		**OR**	**95%CI**	**Controls**		**Cases**		**OR**	**95%CI**
	n	%	n	%			n	%	n	%			n	%	n	%		
Gestational age (weeks)																		
<37	126	*7.1*	32	*9.0*	1.2	[0.8-1.9]	39	*7.2*	17	*9.0*	1.0	[0.5-1.9]	87	*7.0*	15	*8.9*	1.4	[0.7-2.5]
37-39	652	*36.6*	136	*38.1*	1.0	Ref	217	*39.9*	79	*42.0*	1.0	Ref	435	*35.1*	57	*33.7*	1.0	Ref
40-41	942	*52.8*	176	*49.3*	0.9	[0.7-1.2]	270	*49.6*	89	*47.3*	0.8	[0.6-1.2]	672	*54.2*	87	*51.5*	1.1	[0.7-1.5]
≥ 42	35	*2.0*	3	*1.0*	*		12	*2.2*	1	*0.5*	*		23	*1.8*	2	*1.2*	*	
Missing	28	*1.6*	10	*2.8*	-	-	6	*1.1*	2	*1.1*	-	-	22	*1.7*	8	*4.7*	-	-
Birth-weight (grams)																		
<2500	105	*5.6*	25	*7.0*	1.2	[0.9-1.7]	31	*5.7*	11	*5.8*	1.3	[0.6-2.9]	74	*5.9*	14	*8.2*	1.4	[0.7-2.6]
2500-2999	342	*19.2*	73	*20.4*	1.3	[0.8-2.2]	102	*18.7*	45	*23.9*	1.6	[1.0-2.6]	240	*19.4*	28	*16.6*	0.9	[0.6-1.5]
3000-3499	683	*38.3*	123	*34.4*	1.0	Ref	210	*38.6*	59	*31.4*	1.0	Ref	473	*38.2*	64	*37.9*	1.0	Ref
3500-3999	494	*27.7*	95	*26.6*	1.1	[0.8-1.5]	143	*26.3*	46	*24.4*	1.3	[0.8-2.1]	351	*28.3*	49	*29.0*	1.0	[0.6-1.5]
≥ 4000	159	*8.9*	40	*11.2*	1.4	[0.9-2.2]	58	*10.6*	26	*13.8*	1.8	[1.0-3.2]	101	*8.1*	14	*8.3*	1.1	[0.6-2.1]
Missing			1	0.3	-				1	0.5								
Fetal growth																		
SGA	200	*11.2*	46	*12.9*	1.4	[1.0-2.0]	47	*8.6*	25	*13.3*	1.7	[1.0-3.1]	153	*12.3*	21	*12.4*	1.2	[0.7-2.0]
AGA	1366	*76.6*	249	*69.7*	1.0	Ref	430	*79.0*	128	*68.1*	1.0	Ref	936	*75.5*	121	*71.6*	1.0	Ref
LGA	189	*10.6*	51	*14.3*	**1.5**	**[1.1-2.2]**	61	*11.2*	32	*17.0*	**2.0**	**[1.2-3.3]**	128	*10.3*	19	*11.3*	1.1	[0.7-2.0]
Missing	28	*1.6*	11	*3.1*	-	-	6	*1.1*	3	*1.6*	-	-	22	*1.8*	8	*4.7*	-	-
Malformations																		
No	1736	*97.5*	344	*96.3*	1.0	Ref	535	*98.3*	179	*95.2*	1.0	Ref	1201	*96.9*	165	*97.6*	1.0	Ref
Any	47	*2.5*	13	*3.6*	1.6	[0.8-3.0]	9	*1.6*	9	*4.8*	**3.6**	**[1.3-8.9]**	38	*3.1*	4	*2.4*	0.8	[0.3-2.3]
1	43	*2.4*	10	*2.8*	1.3	[0.6-2.6]	9	*1.6*	8	*4.2*	**2.9**	**[1.1-7.9]**	34	*3.1*	2	*1.0*	*	
≥ 2	4	*0.2*	3	*0.8*	*				1	*0.5*	*		4	*0.3*	2	*1.0*	*	
Cardiovascular	13	*0.7*	1	*0.3*	*													
Digestive	1	*0.1*	1	*0.3*	*													
Genitourinary	15	*0.8*	6	*1.7*	2.2	[0.8-6.0]												
Skeleton	14	*0.8*	3	*0.9*	*													
CNS	0	-	1	*0.3*	*													
Head and neck	4	*0.2*	1	*0.3*	*													

Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by logistic regression models adjusted for age and sex, birth-order, maternal age, urban status of the area of residence and study, after prior testing of heterogeneity between the ESCALE and ESTELLE studies.

* Too few cases to fit model

Table 11: Maternal supplementation of folic acid, vitamins or minerals around the index pregnancy and risk of neuroblastoma (ESTELLE study only)

	Preconception period				First trimester				Second trimester				Third trimester				At any time			
	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI
Never	496	107	1.0	Ref	496	107	1.0	Ref	496	107	1.0	Ref	496	107	1.0	Ref	496	107	1.0	Ref
Vitamin supplementation	107	16	**0.5**	**[0.3-0.9]**	206	53	0.9	[0.6-1.4]	201	43	0.8	[0.5-1.2]	176	40	0.8	[0.6-1.3]	338	76	0.8	[0.6-1.2]
Folic acid substantiated[1]	45	7	0.4	[0.2-1.1]	86	24	0.9	[0.5-1.5]	71	19	0.9	[0.5-1.6]	63	18	0.9	[0.5-1.7]	118	30	0.8	[0.5-1.4]
Folic acid unsubstantiated[2]	48	8	0.6	[0.3-1.4]	95	25	1.0	[0.6-1.7]	79	18	0.9	[0.5-1.7]	78	17	0.9	[0.5-1.7]	150	35	0.9	[0.6-1.5]
Without folic acid	14	1			25	4			51	6			38	5			70	11		
Only in another period	231	60			132	23			137	33			162	36						

Ca: cases; Co: controls; Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by logistic regression models adjusted for age and sex, birth-order, maternal age, urban status of the area of residence and study

[1] supplementation reported for the relevant period with a valid product name

[2] supplementation reported for the relevant time period but with no valid name given

Table 12: Maternal reproductive history and risk of neuroblastoma. Pooled analysis of the ESCALE and ESTELLE studies.

	All neuroblastomas						Age <18 months						Age ≥18 months					
	Controls n=1783		Cases n=357		OR[1]	95%CI	Controls n=544		Cases n=188		OR[1]	95%CI	Controls n=1239		Cases n=169		OR[1]	95%CI
	n	%	n	%			n	%	n	%			n	%	n	%		
Difficulty to get pregnant																		
No	1498	*84.0*	299	*83.7*	1.0	Ref	446	*82.0*	158	*84.0*	1.0	Ref	1052	*84.9*	141	*83.4*	1.0	Ref
Yes	285	*16.0*	58	*16.3*	1.0	[0.6-1.3]	98	*18.0*	30	*16.0*	0.8	[0.5-1.3]	187	*15.1*	28	*16.6*	1.1	[0.7-1.7]
Use of fertility treatment for the index child																		
No	1669	*93.6*	335	*93.8*	1.0	Ref	512	*94.1*	175	*93.1*	1.0	Ref	1157	*93.4*	160	*94.7*	1.0	Ref
Yes	114	*6.4*	22	*6.2*	1.0	[0.6-1.6]	32	*5.9*	13	*6.9*	1.2	[0.6-2.4]	82	*6.6*	9	*5.3*	0.8	[0.4-1.3]
Type of fertility treatment																		
No	1669	*93.6*	335	*93.8*	1.0	Ref	512	*94.1*	175	*93.1*	1.0	Ref	1157	*93.4*	160	*94.7*	1.0	Ref
Stimulation only	47	*2.6*	12	*3.4*	1.2	[0.6-2.3]	13	*2.4*	8	*4.2*	1.7	[0.7-4.4]	34	*2.7*	4	*2.4*	0.8	
In vitro fertilization	33	*1.8*	4	*1.1*	*		11	*2.0*	2	*1.1*	*		22	*1.8*	2	*1.2*	*	
Artificial insemination	15	*0.8*	1	*0.3*	*		2	*0.4*	1	*0.5*	*		13	*1*	-	-	-	-
Another technique	16	*0.9*	5	*1.4*	1.2	[0.4-3.7]	5	*0.9*	2	*1.1*	1.2	[0.3-6.9]	11	*0.9*	3	*1.8*	*	
Missing	3	*0.2*					1	*0.2*					2	0.2				

[1]Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by logistic regression models adjusted for age and sex, birth-order, maternal age, urban status of the area of residence and study, after prior testing of heterogeneity between the ESCALE and ESTELLE studies. * Too few cases to fit model

Table 13: Breastfeeding and risk of neuroblastoma. Pooled analysis of the ESCALE and ESTELLE studies (children older than 6 months)

	All neuroblastomas				MYCN -			MYCN +			< 18 months				≥ 18 months			
	Co[1]	Ca[1]	OR[2]	95% CI	Ca	OR[3]	95% CI	Ca	OR[3]	95% CI	Co	Ca	OR[2]	95% CI	Co	Ca	OR[2]	95% CI
Breastfeeding																		
Never	642	118	1.0	Ref	78	1.0	Ref	31	1.0	Ref	118	40	1.0	Ref	524	78	1.0	Ref
Ever	947	151	0.7	[0.5-1.0]	109	0.8	[0.6-1.1]	30	0.6	[0.3-1.0]	232	60	0.6	[0.4-1.0]	715	91	0.8	[0.6-1.1]
Breastfeeding duration																		
Never breastfed	642	118	1.0	Ref	78	1.0	Ref	31	1.0	Ref	118	40	1.0	Ref	524	78	1.0	Ref
< 3 months	348	64	0.8	[0.6-1.1]	40	0.7	[0.5-1.1]	17	0.9	[0.5-1.6]	88	23	0.5	[0.3-1.0]	260	41	1.0	[0.6-1.5]
3-5 months	293	42	0.6	[0.4-1.0]	31	0.7	[0.4-1.1]	8	0.5	[0.2-1.2]	82	21	0.5	[0.3-1.1]	211	21	0.7	[0.4-1.1]
≥ 6 months	287	42	0.7	[0.5-1.1]	35	0.9	[0.6-1.4]	5	0.3	[0.1-0.9]	51	13	0.7	[0.3-1.5]	236	29	0.7	[0.4-1.2]
Missing	19	3			3						11	3			8			
			p-trend=0.06					**p-trend <0.01**									p-trend=0.1	

Ca: cases; Co: controls;

[1]N=269 case and 1589 control children aged less than six years.

[2]Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by logistic regression models adjusted for age and sex, birth-order, maternal age, urban status of the area of residence and study, after prior testing of heterogeneity between the ESCALE and ESTELLE studies.

[3]Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by polytomous logistic regression models adjusted for age, sex and birth-order, maternal age, urban status of the area of residence and study

3.4 Environmental exposures related to parental habits

3.4.1 Exposure to pesticides

3.4.1.1 Household use of pesticides

Overall, maternal use pesticide during pregnancy was reported for 43.7% of the cases and 35.7% of the controls (Table 14). Insecticides were the most commonly used (40.6% of cases and 33.9% of the controls) and they were mostly used alone. Their use was mainly reported indoors (80.0% of insecticides use in cases and 81.0% in controls). Mothers rarely used herbicides or fungicides and they most often also used insecticides.

The maternal use of any type of pesticide during pregnancy was associated with the risk of neuroblastoma (OR 1.5 [95% CI 1.2–1.9]). There was a positive association with the use of insecticides alone (OR 1.4 [95% CI 1.1–1.9]) or insecticides with other pesticides (OR 2.0 [95% CI 1.1–3.4]). There was no between-study heterogeneity except for herbicide use (pooled OR 2.0 [95% CI 1.1–3.7]); ESCALE (OR 3.8 [95% CI 1.8–8.0]); ESTELLE (OR 1.1 [95% CI 0.4–3.2]); p value for interaction = 0.07).

Although based on less than 10 exposed cases, the maternal use of any fungicide during pregnancy was associated with increased risk of neuroblastoma among cases with MYCN amplification (OR 4.3 [95% CI 1.6-11.5] (Table 14).

The use of any herbicide or fungicide was associated with neuroblastoma in children older than 18 months, but the associations cannot be dissociated from that with insecticide and either herbicide or fungicide use.

Among the controls, the prevalence of pesticide use varied by urban/rural status, but not by maternal level of education, and the results did not change when stratification for either of these factors was used instead of adjustment.

Maternal use of pesticides before and after pregnancy and the use of professional pest control treatments at home were not collected in the ESCALE study. In the ESTELLE study, more than a quarter of the mothers reported pesticide use during all three time periods (29.0% of the cases and 27.6% of the controls) and very few mothers reported the use during pregnancy only (6.0% of cases and 3.5% of controls). This precluded specific analyses by time window.

3.4.1.2 Maternal occupational exposure and pest control treatments at home

Maternal occupational pesticide exposure during pregnancy was associated with the risk of neuroblastoma (OR 2.0 [95% CI 1.0–4.0), although the frequency of exposure was low (3.6% of cases and 1.8% of controls).

No association was found with professional pest control treatments at home during pregnancy (OR 1.2 [95% CI 0.5–2.8])

Table 14: Maternal use of household pesticides during pregnancy and risk of neuroblastoma. Pooled analysis of the ESCALE and ESTELLE studies.

	Controls		All neuroblastoma				MYCN –				MYCN +			
	n	%	n	%	OR[1]	95% CI	n	%	OR[2]	95% CI	n	%	OR[2]	95% CI
Maternal use of pesticides														
None	1112	*62.4*	198	*55.5*	1.0	Ref	155	*57.4*	1.0	Ref	32	*50.0*	1.0	Ref
Any pesticide	636	*35.7*	156	*43.7*	**1.5**	**[1.2-1.9]**	113	*41.8*	**1.4**	**[1.1-1.9]**	31	*48.4*	1.7	[1.0-2.9]
Any insecticide	604	*33.9*	145	*40.6*	**1.5**	**[1.1-1.9]**	105	*38.9*	**1.4**	**[1.1-1.9]**	28	*43.7*	1.6	[0.9-2.7]
Any herbicide	61	*3.4*	20	*5.6*	**2.0**	**[1.1-3.7]**	14	*5.2*	1.9	[0.9-3.6]	3	*4.7*	*	
Any fungicide	50	*2.8*	14	*3.9*	1.6	[0.8-3.1]	7	*2.6*	1.1	[0.5-2.6]	6	*9.4*	**4.3**	**[1.6-11.5]**
Only insecticides	537	*30.1*	125	*35*	**1.4**	**[1.1-1.9]**	93	*34.4*	1.4	[1.0-1.9]	23	*35.9*	1.5	[0.8-2.6]
Only herbicides	13	*0.7*	5	*1.4*	1.5	[0.4-4.8]	5	*1.8*	1.9	[0.6-6.2]	0	*0*	*	
Only fungicides	19	*1.1*	6	*1.7*	1.7	[0.7-4.6]	3	*1.1*	*		3	*4.7*	*	
Insecticides + other pesticides	67	*3.8*	20	*5.6*	**2.0**	**[1.1-3.4]**	12	*4.4*	1.6	[0.8-3.1]	5	*7.8*	2.4	[0.9-6.8]
Only herbicides and fungicides	0		0											
Use of insecticides														
Indoor use	489	*27.4*	116	*32.5*	**1.5**	**[1.1-2.0]**	81	*30.0*	1.4	[1.0-1.9]	25	*39.1*	1.8	[1.0-3.1]
Gardening and outdoor use	57	*3.2*	13	*3.6*	1.3	[0.7-2.5]	7	*2.6*	0.9	[0.4-2.1]	3	*4.7*	*	
For pets	224	*12.6*	49	*13.7*	1.3	[0.9-1.9]	37	*13.7*	1.3	[0.9-2.0]	10	*15.6*	1.2	[0.5-2.8]
Missing	35	*1.9*	3	*0.8*			2	*0.7*			1	*1.6*		

[1]Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by logistic regression models adjusted for age and sex, birth-order, maternal age, urban status of the area of residence and study, after prior testing of heterogeneity between the ESCALE and ESTELLE studies.

[2]Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by polytomous logistic regression models adjusted for age, sex and birth-order, maternal age, urban status of the area of residence and study after prior testing of heterogeneity between the ESCALE and ESTELLE studies.
* Too few cases to fit model

Table 15: Maternal use of household pesticides and risk of neuroblastoma by age at diagnosis. Pooled analysis of the ESCALE and ESTELLE studies.

	Age < 18 months						Age ≥ 18 months					
	Cases		Controls		OR[1]	95% CI	Cases		Controls		OR[1]	95% CI
	n=188	%	n=544	%			n=169	%	n=1239	%		
Maternal use of pesticides												
None	114	*60.6*	369	*67.8*	1.0	Reference	84	*49.7*	743	*59.9*	1.0	Reference
Any pesticide	73	*38.8*	166	*30.5*	1.5	[1.0-2.1]	83	*49.1*	470	*37.9*	**1.6**	**[1.1-2.2]**
Any insecticide	65	*34.6*	155	*28.5*	1.4	[0.9-2.0]	80	*47.3*	449	*36.2*	**1.6**	**[1.1-2.3]**
Any herbicide	7	*3.7*	22	*4.0*	1.1	[0.4-3.1]	13	*7.7*	39	*3.1*	**3.4**	**[1.6-7.1]**
Any fungicide	5	*2.7*	15	*2.8*	1.0	[0.3-3.3]	9	*5.3*	35	*2.8*	2.3	[1.0-5.1]
Only insecticides	62	*32.9*	134	*24.6*	1.5	[1.0-2.2]	63	*37.3*	403	*32.5*	1.4	[1.0-2.0]
Only herbicides[2]	4	*2.1*	7	*1.3*			1	*0.6*	6	*0.5*	*	
Only fungicides[2]	4	*2.1*	4	*0.7*			2	*1.2*	15	*1.2*	*	
Insecticides + other pesticides	3	*1.6*	21	*3.9*	*		17	*10.0*	46	*3.7*	**3.4**	**[1.8-6.5]**
Only herbicides and fungicides	0		0				0		0			
Use of insecticide												
Indoor use	50	*26.6*	121	*22.2*	1.5	[1.0-2.2]	66	*39.0*	368	*29.7*	**1.6**	**[1.1-2.3]**
Gardening and outdoor use	8	*4.3*	23	*4.2*	1.5	[0.6-3.6]	5	*2.9*	34	*2.7*	1.2	[0.4-3.2]
For pets	23	*12.2*	58	*10.7*	1.3	[0.7-2.3]	26	*15.4*	166	*13.4*	1.3	[0.8-2.2]
Missing	1	*0.5*	9	*1.6*			2	*1.2*	26	*2.1*		

[1]Odds ratios (OR) and 95% confident intervals (CI) estimated by unconditional logistic regression models adjusted for children age and sex, study, maternal age, birth order, size of the urban unit of residence and type of housing during pregnancy, after prior testing of heterogeneity between the ESCALE and ESTELLE studies. *Too few cases to fit model

3.4.2 Parental smoking and maternal alcohol consumption during pregnancy

3.4.2.1 Parental smoking during pregnancy

Maternal smoking during pregnancy was slightly more often reported for the cases (24.1%) than for the controls (19.7%), with an OR of 1.3 [95% CI 0.9 -1.7] (Table 16). There was no trend with the average number of cigarettes smoked per day (OR for 5 CPD increase was 1.1 [95% CI 1.0–1.3]). The prevalence of smoking among mothers was similar between the ESCALE and ESTELLE studies. In the ESTELLE study, most of the mothers that reported having smoked during pregnancy started before conception and smoked during the whole pregnancy. Maternal smoking before and during pregnancy were highly correlated which precluded specific analysis by time window (Spearman's rho =0.69).

Paternal tobacco consumption during pregnancy was not associated with the risk of neuroblastoma as an independent exposure (OR 1.1 [95% CI 0.9-1.4], but having both parents reported as having smoked during pregnancy was associated with neuroblastoma (OR 1.5 [95% CI 1.1-2.1]) (Table 16). The maternal average daily consumption of cigarettes did not differ significantly according to whether only the mother or both parents were reported smokers (mean 5.7 CPD versus 6.1 CPD, respectively). However, the percentage of mothers who smoked was higher when the fathers also smoked (34.4% versus 10%, results not tabulated). The associations seemed to be only present among children younger than 18 months (OR 1.4 [95% CI 0.9–2.2]) vs. (OR 1.1 [95% CI 0.7–1.7]) among older children, but interaction with age was not significant (p-value for interaction 0.4) (Table 16). Although based on small numbers, the analyses did not reveal differences by MYCN status (results not tabulated).

3.4.2.2 Maternal alcohol consumption during pregnancy

Maternal alcohol consumption during pregnancy was not associated with the risk of neuroblastoma (OR 1.0 [95 % CI 0.8–1.4] (Table 17). There was no interaction between maternal smoking and alcohol consumption (p-value=0.4). The results were similar with regards to different types of beverages and there was no increasing risk with increasing alcohol consumption or differences by age at diagnosis or MYCN status (results not tabulated).

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Table 16: Association between neuroblastoma and parental smoking for the whole sample and according to age group. Pooled analysis of the ESCALE and ESTELLE studies.

	Total				< 18 months				≥ 18 months			
	Co (%)	Ca (%)	OR[1]	[95% CI]	Co(%)	Ca (%)	OR[1]	[95% CI]	Co (%)	Ca (%)	OR[1]	[95% CI]
Maternal smoking during pregnancy [2]												
None	80.3	75.9	1.0	Ref	75	82.2	1.0	Ref	76.9	79.4	1.0	Ref
Any	19.7	24.1	1.3	[0.9-1.7]	25	17.8	1.4	[0.9-2.2]	23.1	20.5	1.1	[0.7-1.7]
< 5 cigarettes/day	7.2	9	1.2	[0.7-1.8]	10.1	7.5	1.3	[0.7-2.4]	7.7	7	1.1	[0.6-2.1]
5-9 cigarettes/day	6	7.3	1.3	[0.8-2.2]	7.4	4.6	1.8	[0.9-3.8]	7.1	6.6	1.1	[0.6-2.0]
≥10 cigarettes/day	4.8	5.3	1.3	[0.7-2.2]	5.3	4	1.4	[0.6-3.1]	5.3	5.2	1.1	[0.5-2.3]
Per 5 cigarettes/day			1.10	[1.0-1.3]			1.03	[0.98-1.09]			1.01	[0.96-1.07]
Paternal smoking in the year before birth [2]												
None	57.9	53.5	1.0	Ref	52.7	62.3	1.0	Ref	54.4	55.9	1.0	Ref
Any	40.8	44.5	1.1	[0.9-1.4]	45.7	37.1	1.2	[0.8-1.8]	43.2	42.4	1.0	[0.7-1.4]
< 10 cigarettes/day	9	12.3	1.3	[0.9-2.0]	13.3	10.5	1.4	[0.8-2.4]	11.2	8.1	1.4	[0.8-2.4]
10-15 cigarettes/day	16.1	14.8	0.9	[0.6-1.3]	16	15.6	1.2	[0.7-1.9]	13.6	16.4	0.8	[0.5-1.3]
> 15 cigarettes/day	16.3	18.5	1.3	[0.9-1.8]	17.5	11.2	1.8	[1.0-2.9]	19.5	18.5	1.1	[0.7-1.7]
Per 10 cigarettes/day			1.00	[0.99-1.02]			1.01	[0.98-1.03]			0.99	[0.97-1.01]
Maternal smoking during pregnancy and paternal smoking in the year before birth[2]												
Neither parent	52.6	48.7	1.0	Ref	47.9	56.4	1.0	Ref	49.7	50	1.0	Ref
Only mother	6	4.8	0.9	[0.5-1.6]	4.8	5.9	1.0	[0.5-2.3]	4.7	6	0.8	[0.4-1.7]
Only father	27.8	25.8	1.0	[0.8-1.4]	26.1	25.4	1.2	[0.8-1.8]	25.4	28.3	0.9	[0.6-1.3]
Both parents	13.5	18.8	**1.5**	**[1.1-2.1]**	19.7	11.8	**1.9**	**[1.2-3.1]**	17.7	14	1.2	[0.8-1.9]

[1] Odd ratio (OR) and 95 confident intervals (95% CI) estimated by unconditional logistic regression adjusted for age, sex and birth-order, maternal age, urban status of the area of residence and study of origin, after prior testing of heterogeneity between the ESCALE and ESTELLE studies.
[2] ORs on maternal and paternal smoking were mutually adjusted

Table 17: Association between neuroblastoma and alcohol consumption during pregnancy. Pooled analysis of the ESCALE and ESTELLE studies.

	Controls		Cases		OR	[95% CI]
	N	%	N	%		
Maternal alcohol drinking during pregnancy						
Never	1293	*72.5*	268	*75.1*	1.0	Ref
Ever	490	*27.2*	88	*24.6*	1.0	[0.8-1.4]
Missing	*0*		1	0.3		
Glasses per week						
None	1293	*72.5*	268	*75.1*	1.0	Ref
<1	225	*12.6*	50	*14*	1.1	[0.8-1.6]
1-2	110	*6.2*	12	*3.4*	0.6	[0.3-1.2]
>2	143	*8.0*	22	*6.2*	1.0	[0.6-1.6]
Missing	*12*	*0.7*	4	*1.1*		
Types of alcohol						
None	1293	*72.5*	268	*75.1*	1.0	Ref
Wine	330	*18.5*	54	*15.1*	0.9	[0.6-1.3]
Beer or cider	167	*9.4*	34	*9.5*	1.2	[0.8-1.9]
Spirits	189	*10.6*	31	*8.7*	0.8	[0.5-1.2]

[1]Odd ratio (OR) and 95 confident intervals (95% CI) estimated by unconditional logistic regression adjusted for age, sex and birth order, maternal age, urban status of the area of residence and study

3.4.2.3 Meta-analyses on maternal smoking and alcohol consumption

The results of the search strategy are summarized in Figure 5. Of the 973 articles identified by the algorithm-based search, 929 were deemed irrelevant using their title or abstract. The full texts of the potentially eligible 41 remaining publications were obtained and assessed using the eligibility criteria, leading to the exclusion of 28 studies for various reasons (see Supplementary material).

We identified thirteen studies (5 record linkage and 8 case-control studies) that provided data on maternal smoking during pregnancy and seven of them also presented data on maternal alcohol consumption. Their details and main findings are summarized in Table 18.

Ratings according to the Newcastle-Ottawa Quality scale spanned between 6 and 9. All the included studies ensured comparability of cases and controls on the basis of study design. Despite the variability of exposure ascertainment among included studies, the same data collection method was used for cases and controls within individual studies; no study, however, had validated alcohol consumption records or used structured interviews blinded as to case/control status. With regards to risk of bias assessment, five studies presented insufficient data regarding the comparability of cases and controls. Funnel plots did not provide any evidence of publication bias and Egger test was not significant (p-value= 0.2).

The summary OR for maternal smoking during pregnancy was 1.1 [95% CI 1.0-1.3] (Figure 6). Between-study heterogeneity was low (I^2= 17.3 %). The funnel plot did not provide any evidence of publication bias and Egger test was not significant (p-value= 0.2).

The analyses did not suggest any association between maternal alcohol consumption during pregnancy and neuroblastoma, with a summary OR of 1.0 [95% CI 0.9–1.2] (Figure 7). Funnel plot were performed but there were insufficient studies.

Slightly lower estimates were observed on meta-analysis of maternal smoking and neuroblastoma when only record linkage studies were included, compared with interview based studies. However, results may be interpreted with caution because based on only five studies (See supplementary material). Results did not change when studies with less than 7 in the Newcastle-Ottawa Quality scale were excluded.

3.4.3 Sensitivity analysis

All the results remained unchanged after exclusion of the ESTELLE cases with no telephone landline. No changes were observed after exclusion of multiple births, children born following in vitro fertilization and children with genetic syndromes.

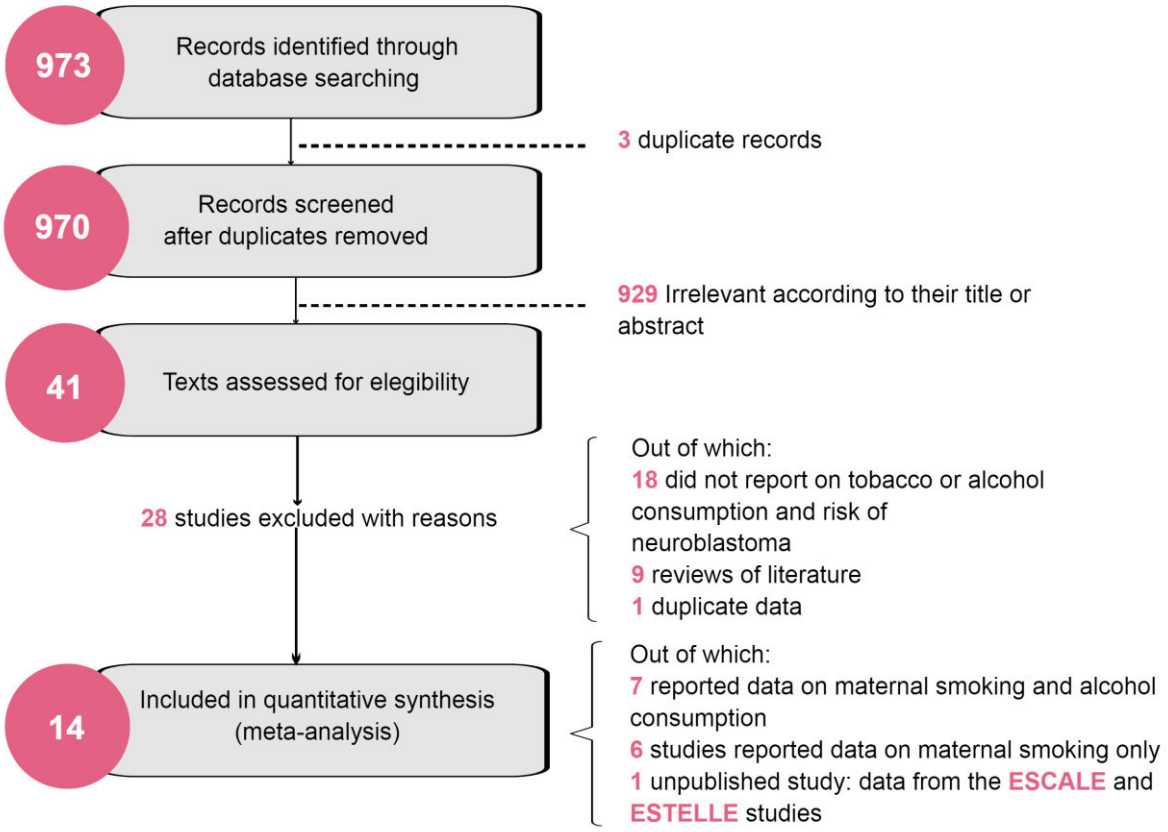

Figure 5: Search strategy on meta-analyses of maternal smoking, alcohol consumption and risk of neuroblastoma

Table 18: Included studies on the meta-analysis about maternal smoking, alcohol consumption and neuroblastoma.

Study	Cases and controls selection				Maternal consumption during pregnancy (ever/never)				
Author, country, year of case accrual	Cases		Controls		Smoking		Alcohol drinking		Matched factors/Adjustments
	Source	n	Source	n	Crude OR [95% CI][a]	Adjusted OR [95% CI]	Crude OR [95% CI][a]	Adjusted OR [95% CI]	
Data obtained by record linkage									
Johnson et al, 2008 US (1976-2004)[46]	Cancer Registry Minnesota state	155	Birth Registry	8752	1.4 [0.9-2.2]	1.4 [0.9-2.3]	1.1 [0.4-3.5]	-	year of birth, sex
Chow et al, 2003 US (1980-1992)[48]	Cancer Registry Washington state	240	Birth Registry	2400	0.8 [0.6-1.2]	0.8 [0.6-1.2]	-	-	year of birth, sex/ gestational age, birth-weight, parental age, ethnicity, maternal residence
McLaughlin et al, 2009 US (1985-2001)[49]	Cancer Registry New York state	529	Birth Records	12010	-	1.0 [0.7-1.3]	-	1.2 [0.5-2.6]	date of birth, sex
Stavrou et al, 2009 Australia, (1994-2005)[104]	Cancer Registry New South Wales	122	Midwives data collection	1045966	0.8 [0.5-1.3]	1.0 [0.6-1.7]	-	-	children age and sex, maternal age, birth-weight, gestational age, socioeconomics, maternal hypertension, gestational diabetes, preeclampsia
Heck et al, 2016 USA (2007-2013)[105]	Cancer Registry California state	238	Birth certificates	40356	1.1 [0.5-2.4]	1.2 [0.5-2.5]	-	-	year of birth/ maternal ethnicity, maternal education
Data obtained by Interview									
Kramer et al, 1987 US (1970-1979)[92]	Cancer Registry Great Delaware valley	93	General population	93	1.3 [0.8-2.1]		1.4 [0.9-2.2]		date of birth, race, area code,
Buck et al, 2001 US (1976-1987)[45]	Cancer Registry New York state	155	Birth Registry	310	1.3 [0.8-2.1]	1.4 [0.9-2.1]	1.2 [0.8-1.9]	1.2 [0.8-1.9]	year of birth, parity, maternal age, smoking and alcohol consumption
Sorahan et al, 1994 UK (1977-1981)[106]	Cancer Registry	93	Birth Registry	93	-	1.0 [0.8-1.3]	-	-	date of birth, sex
Schwartzbaum et al, 1992 US (1979-1986)[93]	Hospital Cancer Registry	101	Hospital Cancer Registry	690	-	1.9 [1.1-3.2]	-	0.7 [0.4-1.1]	age, race, maternal age, social class, exposure to x-ray, miscarriage, others (not

									specified)
Schuz et al, 2001 Germany (1988-1993)[23]	Cancer Registry	183	Residents database	1785	1.4 [1.0-1.9]	-	0.9 [0.6-1.3]	-	age, sex, year of birth /SES, degree of urbanization
Pang et al, 2003 UK (1992-1994)[107]	Cancer Registry	188	Family Health Services database	6987	-	0.9 [0.6-1.3]	-	-	age, sex, parental age, deprivation score
Yang et al, 2000 US (1992-1994)[24]	Oncology Group	538	General population	538	1.2 [0.9-1.6]	1.1 [0.8-1.4]	1.1 [0.9-1.4]	1.1 [0.8-1.4]	date of birth /sex, race, maternal education, household income in the birth year
Parodi et al, 2014 Italy (1998-2001)[43]	Oncology Group	153	National health service database	1044	1.4 [0.9-2.2]	1.2 [0.7-2.1]	-	-	Gender, date of birth, area of residence/ maternal age and maternal education
France (2003-2004 and 2010-2011)	Cancer Registry	357	General population	1783	1.3 [1.0-1.7]	1.3 [0.9-1.7]	0.9 [0.7-1.1]	1.0 [0.8-1.4]	age, sex, maternal age, study of origin

[a] Calculated using raw numbers stated in the published paper.

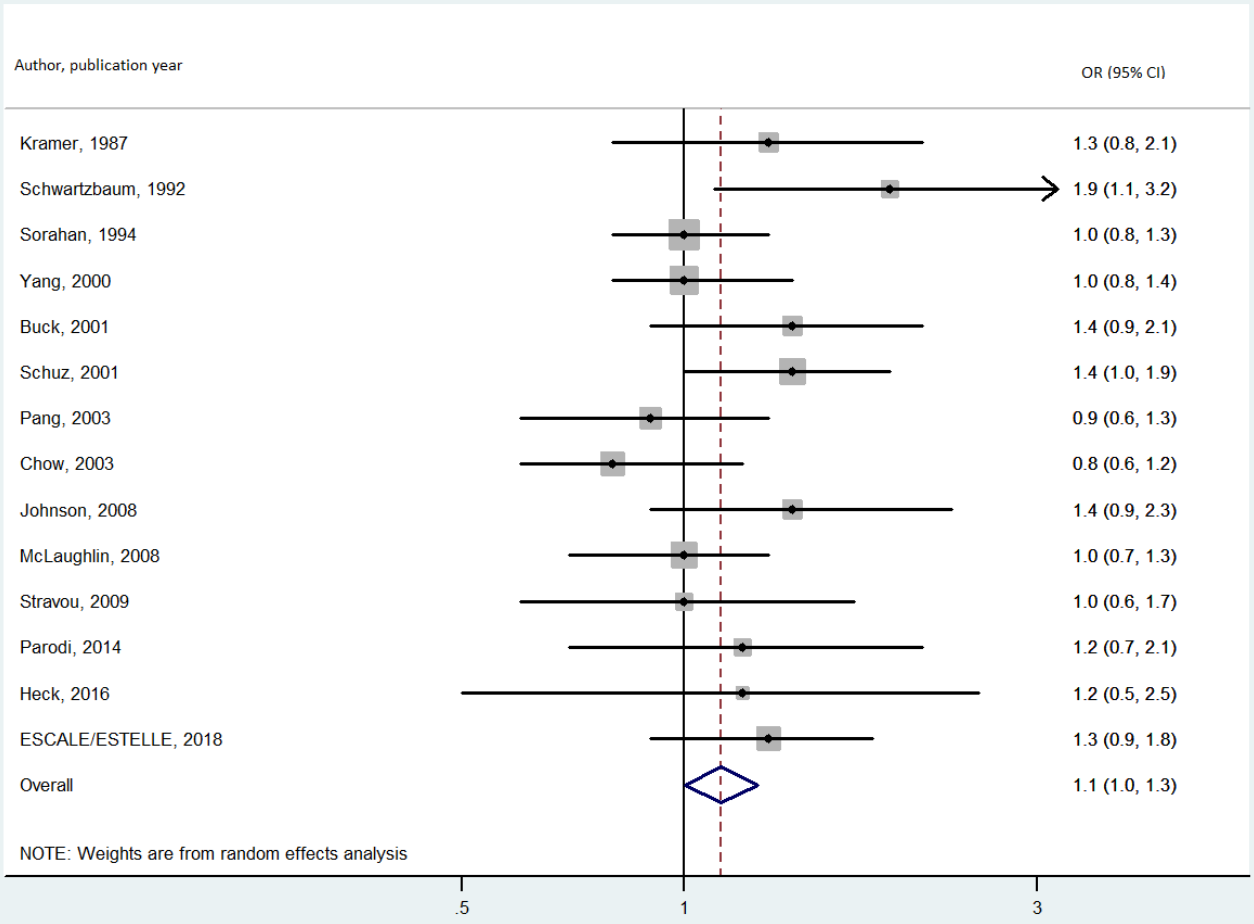

Figure 6: Forest plot. Studies on maternal smoking and risk of neuroblastoma

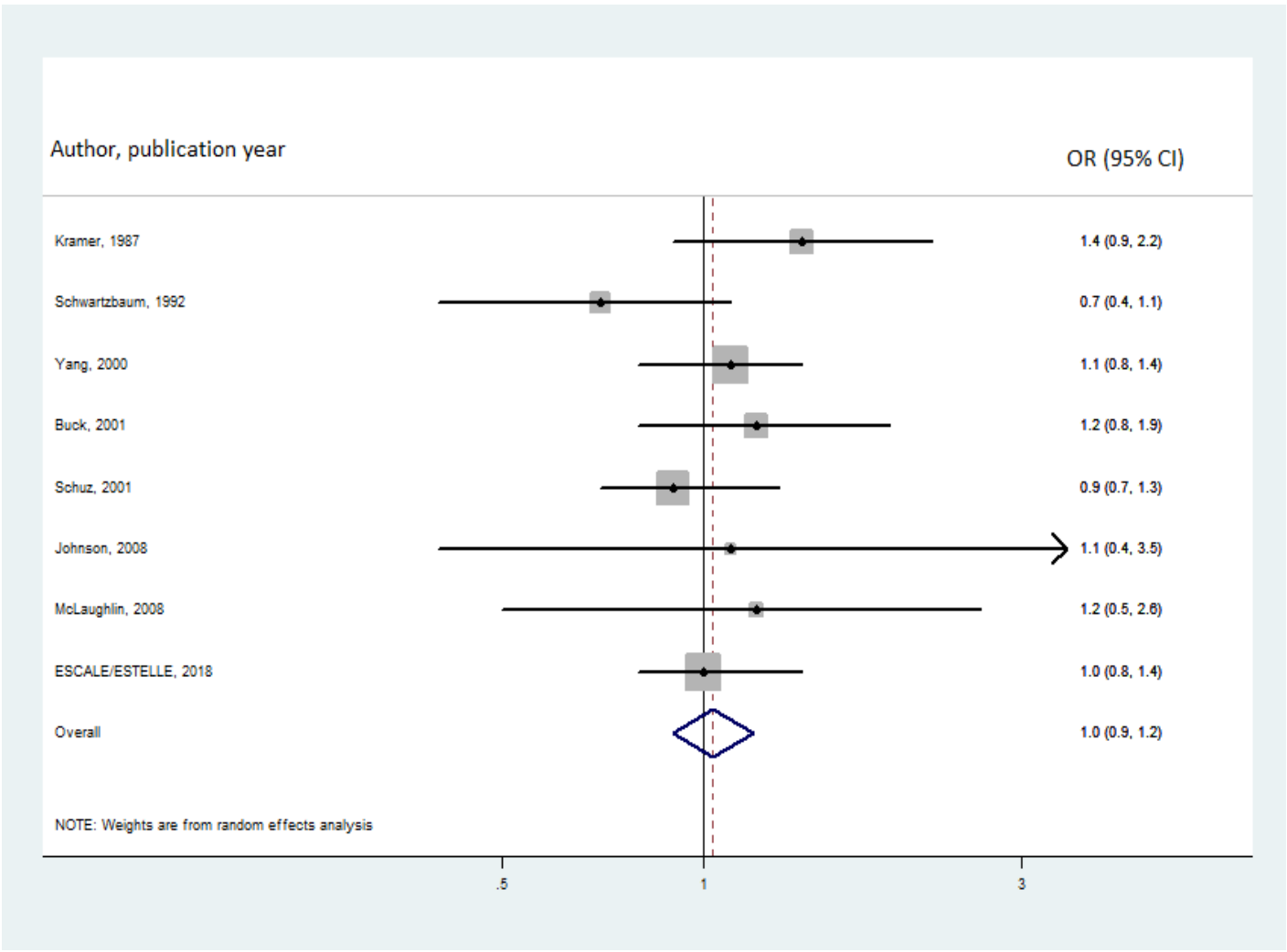

Figure 7: Forest plot. Maternal alcohol consumption during pregnancy and risk of neuroblastoma.

Discussion

4 Discussion

4.1 Summary of main findings

The aim of this study was to investigate whether specific birth-related characteristics and environmental exposures around pregnancy were related with the risk of childhood neuroblastoma. Because the embryonic characteristics of neuroblastoma and the early age at onset suggests that exposures before birth may play a role in disease development, the periconceptional period was the assessed as the key time period. Most of the studied exposures were investigated during pregnancy as a proxy for fetal exposures, while the year before conception and after birth were used to assess parental and childhood exposures, respectively. The exposures of interest in this study were chosen on the basis of previous research that suggested a biologically plausible association with neuroblastoma in the offspring.

Our main findings on perinatal factors were the positive associations with being born either small or large for gestational age and with having a congenital malformation, and the inverse associations with breastfeeding and maternal use of supplements containing folic acid, vitamins or minerals in the preconceptional period. Our findings did not support the association between neuroblastoma and gestational age, difficulty becoming pregnant or undergoing fertility treatment.

Our main findings on environmental exposures related to parental lifestyle were the increased risk with the maternal use of pesticides and maternal smoking during pregnancy. No associations were observed with paternal smoking in the year before pregnancy or maternal alcohol consumption during pregnancy.

4.2 Internal validity

Like all case-control interview-based studies, our study is exposed to a risk of selection and recall bias, measurement error and potential confounding.

4.2.1 Selection bias

The ESCALE and ESTELLE studies were designed to minimize bias in case and control selection. The cases were ascertained by a nationwide cancer registry, which has a high degree of completeness and all the diagnosis were verified before inclusion of cases. Since case children were excluded if they had died or were in palliative care, a bias may have

occurred if the exposures of interest were associated with the children's prognosis. For example, some major malformations may have increased the risk of death. Then the true association should be stronger than the observed association in our study. As expected, the proportion of cases ineligible because this reason was smaller under 18 months of age than for those aged 18 months or more as these children have better survival. A bias may have occurred if the exposures would be expected to be stronger after 18 months. There is no known evidence of such effects, and the age-stratified analyses did not provide evidence of substantial differences in the associations between neuroblastoma and maternal exposures.

The controls were randomly selected from the same population as cases. Controls were recruited from landline subscribers, who may differ in age and socioeconomic status from non-subscribers. The extent of possible bias was limited in France where telephone coverage was high during the years of recruitment[108]. The two studies had been designed to include landline owners who request to be unlisted ("liste rouge"), because they often belong to the highest socioeconomic categories. In the ESTELLE study, 24% of case families were not landline subscribers and the proportion is likely to have been lower for the ESCALE study. In the ESTELLE study, we were able to restrict the analyses to the case families that, like the control families, were landline subscribers, which did not change the findings.

Non-response or refusal is an important source of participation bias. In our study, the proportion of participants was higher for eligible cases (86.5%) than for eligible controls (77.1 %). Because no demographic information was available about non-participants, we do not know if these children were different from the study sample.

It is known that subjects who refuse to participate in case-control studies may have a different exposure distribution from those who do participate[109]. In that case, if the factor of interest is under-represented in the control population, it may result in a spurious positive association due to selection bias and non-response bias. Similarly, if the exposure of interest is over represented among controls, the estimates may tend to the null. In our study, the control mothers were generally older than mothers reported in the National Perinatal Surveys (ENP)[110] conducted during the same time periods as the ESCALE and ESTELLE studies (i.e. 20% mothers were older than 35 years old in ESCALE and only 16% in the ENP performed in 2003; 25% in ESTELLE versus 19% in the ENP 2010) (Table 19). Control mothers were also older compared with case mothers. It has been shown in previous studies[111] that participation of younger mothers tend to be low among control families despite adequate sampling frame and satisfactory response rate among controls. Although

maternal age is not thought to be related to neuroblastoma, all the analyses were adjusted on maternal age in an effort to account for this participation bias. The prevalence of several other reported characteristics were comparable to those reported by the ENP, namely the distribution of gestational age and high weight children (> 4000 g at birth), maternal education level, and the prevalence of use of fertility treatments. The prevalence of reported folic acid intake during pregnancy during pregnancy or preconception in the ESTELLE study was similar to the 2010 ENP.

Similarly, the prevalence of congenital malformations among controls was comparable with the prevalence of congenital malformations in the general population reported by the European Surveillance of Congenital Anomalies Register (EUROCAT)[112]. Information was not collected for children who had died or were in palliative care, which may have biased the study of association with congenital malformations.

Table 19: Comparison between reported prevalence of exposures in the ESCALE-ESTELLE studies and National Surveys

	Year			
	ENP 2003	**ESCALE**	**ENP 2010**	**ESTELLE**
Maternal education	*%*	*%*	*%*	*%*
Less than secondary	*36*	*34*	*28*	*24*
Secondary	*21*	*20*	*20*	*23*
More than secondary	*43*	*46*	*52*	*53*
Maternal age (years)				
< 25	19	18	17	10
25-29	*33*	*35*	*33*	*30*
30-34	*32*	*31*	*31*	*35*
≥ 35	*16*	*20*	*19*	*25*
Folic acid intake				
Before/during pregnancy	*Not stated*	-	*40*	*41*
Before pregnancy	*Not stated*	-	*15*[*]	*13*
Fertility treatment for the index pregnancy				
In-vitro fertilization	*1.7*	*1.8*	*2.3*	*2.0*
Artificial insemination	*0.6*	*0.8*	*1.0*	*0.9*
Hormonal stimulation	*2.4*	*2.5*	*2.0*	*3.0*
Maternal smoking during pregnancy				
	20	*19*	*18*	*20*
Maternal alcohol intake during pregnancy				
	Not stated	-	*20*	*20*
Breastfeeding	*62*	*55*	*68*	*65*
Gestational age				
< 37 weeks	*7*	*7*	*7*	*7*
37-39 weeks	*45*	*37*	*47*	*39*
40-41 weeks	*46*	*54*	*44*	*53*
> 42	*2*	*1*	*2*	*0.3*
Birth-weight				
< 2500 g	*6*	*8*	*5*	*7*
2500-3999 g	*85*	*85*	*86*	*86*
≥4000 g	*8*	*7*	*9*	*7*

[*] From Tort et al[113]

4.2.2 Misclassification bias

Non-differential misclassification

Errors in the measurement of variables are unavoidable in studies where exposures are self-reported, particularly when the information is obtained retrospectively. In the present context, the delay between exposure and interview was relatively short, which may have reduced recall difficulties.

Since most of the mothers had the child's personal health records (over 95% of cases and 98% of controls) in their hands, gestational age and birth-weight were unlikely to be misclassified. On the other hand, the interviews could not elicit all the characteristics of specific exposures and, given their complexity, it is likely that mothers may not have been able to determine the agent of true interest. For example, mothers might not be aware that supplementation products for pregnancy contained folic acid, or even that the supplementation product contained a product other than folic acid.

A woman's total plasma folate level is determined by multiple factors. Dietary folate might be high among mothers that did not report folic acid intake around pregnancy. Dietary folate is absorbed differently from supplemental folic acid and as well as naturally occurring folate. In this study, we were not able to assess the dietary folate intake. In the same line, exposure opportunity and the intensity of pesticide exposure depends on many factors (nature, frequency and amount of pesticides used, methods of application, time spent in the exposed place, etc.) that could not be collected in the interviews. These types of exposure misclassification are likely to be randomly distributed among cases and it may tend to minimize the strength of an association, if present.

With regards to congenital malformations, interview-based studies are subjects to non-differential misclassification bias as they use unconfirmed information on birth defects. In our study, two independent reviewers with medical background assessed the maternal responses about congenital malformations. The reviewers were blinded to the case-controls status of children.

Paternal smoking was assessed through the maternal report of the father's tobacco consumption. This could introduce a bias if the provided information was not accurate. In this study, the extent of the bias is limited since in the subset used for validation, agreement between maternal and paternal responses was high with regards to both ever smoking and number of cigarettes smoked per day[114]. Furthermore, control parents were also similar to the source population in the same age group in terms of tobacco smoking, when compared to estimates from the 2005 and 2010 French Health Barometer surveys[115]. For example,

42.5% and 44.3% of fathers reported regular smoking during the ESCALE and ESTELLE studies, respectively. This is similar to 42.6% and 47.7% of men of similar age (26-34 years) that reported regular smoking in 2005 and 2010, respectively.

Recall bias

The main concern about interview-based case-control studies is that the mothers of cases may recall previous exposures differently than the mothers of controls. The ESCALE and ESTELLE studies were designed to reduce the risk of recall bias by the use of computer-assisted standardized interviews conducted by the same trained interviewers, contemporaneously and in identical conditions for cases and controls, although not blind to the case-control status. For example, over-declaration of malformations among neuroblastoma cases in which case mothers are more likely to remember minor defects than control mothers, may introduce differential classification bias in case-control studies. In order to limit this potential bias, we have excluded minor anomalies that are not always truly congenital in origin, sometimes associated with immaturity at birth, and have lesser medical or functional consequences. The criteria for exclusion were based on EUROCAT group recommendations[100], as the group's experience showed that the definition, diagnosis and reporting of minor malformations vary considerably, while major malformations are less liable to differential recall bias. Despite the fact that recall bias cannot be completely ruled out, other studies based on birth records provide support for the validity of our findings[44], [47], [48], [53], [59], [60]. Another concern was the fact that medical diagnosis of some types of malformation may be more frequent among cases. For example, since most of neuroblastoma tumors are located in the abdominal cavity, the use of tomography scan for risk stratification may also lead to the diagnosis of an asymptomatic malformation, such as renal agenesis. Although we cannot exclude this possibility, a specific question was included in the ESTELLE study and none of the cases declared that the malformation had been diagnosed in the context of another pathology.

Pesticide exposure and maternal smoking can also be subject to recall bias, and the direction of the bias is impossible to foresee. Mothers of cases may have been thinking more deeply and have less under-reported pesticide exposures than control mothers. The opposite scenario may also be possible and a true association may be underestimated. Deleterious effects of maternal smoking during pregnancy are well known and potential carcinogenic effects of pesticides raise concern in society. As social desirability is known to influence self-report of

substance abuse[116] case mothers may under-report these exposures if they try to deny any responsibility for the disease.

4.2.3 Confounding

The detailed questionnaires of the ESCALE and ESTELLE studies allowed for the consideration of multiple potential confounding. After reviewing the literature about potential risk factors of neuroblastoma, potential confounders were selected and tested if they met the empiric criteria of confounder (that is being associated with both the exposure of interest and the occurrence of the disease). All the analyses were adjusted on children age and sex, which were the matching variables. All the models of the pooled analysis accounted for the study effect to take into consideration the variations between the ESCALE and ESTELLE studies.

As discussed above, control mothers were older than cases mothers and older than the general population of women giving birth. This could reflect the underrepresentation in our studies of young mothers without landline using mobile phone only. However, a high proportion of French homes had landlines at the time of the study[108], and in the ESTELLE study, exclusion of the case mothers with mobile phone only did not compensate the age difference between cases and controls. All our analyses were adjusted for maternal age at birth of the index child. Maternal education and degree of urbanization of the area of residence were also included as they may have been associated to control selection.

Despite adjustment for maternal age and other factors in the final models, there is the possibility that some residual confounding remained or that there was confounding by unmeasured factors.

4.2.4 Statistical power of this analysis

Since the ESCALE and ESTELLE studies were designed as to be pooled, with similar defined exposures, this is one of the largest studies of neuroblastoma at present. Therefore, the study had overall power for detecting the association between neuroblastoma and the studied exposures, except for the pre-conceptional intake of folic acid supplements since the prevalence of the exposure was still low during the ESTELLE study period and no data was available during the ESCALE study period.

On the contrary, our study did not have enough power with regards to stratified analysis by age at diagnosis, or by sub-groups of *MYCN* status of cases. Therefore, findings based on

small sub-groups of cases, such as the inverse trend of neuroblastoma risk with increased breastfeeding duration among *MYCN* amplified cases, may be due to chance.

It is worth noting that despite the fact that multiple tests have been performed, our analyses were based on *a priori* hypotheses suggested by the current literature and supported by biological plausibility.

4.3 Comparation with literature

4.3.1 Birth-weight, gestational age and fetal growth

Our findings support the hypothesis that link neuroblastoma with fetal growth disorders. In this study, the odds ratios were slightly higher when gestational age was considered, suggesting that the risk of cancer may be principally related to fetal growth, rather that birth-weight *per se*. Harder et al [50] conducted a meta-analysis of 11 studies, conducted between 1987 and 2016, and reported that a birth-weight > 4,000 g was consistently associated with an increased risk of neuroblastoma across studies. In addition, five of the included studies [38], [46]–[49] also analyzed fetal growth, with three studies reporting associations with fetal growth anomalies, which is in line with our findings. The association with high birth-weight was also reported by a large meta-analysis of studies from the UK and USA[117]. The biological hypothesis regarding the mechanisms underlying associations between fetal growth anomalies and neuroblastoma risk remains speculative. The association with fetal overgrowth may be related to the observation that embryonic tumors such as neuroblastoma are more frequent among children with overgrowth disorders, such as Beckwith-Wiedemann syndrome[118]. The syndrome is caused by overexpression of the gene for insulin-like growth factor IGF2, which is suspected to play a role in the development of several childhood malignancies[40]. However, the overexpression of IGF2 would not explain the association with low birth-weight that we and Johnson et al.[46] have observed. In the meta-analysis, Harder et al[50] suggested that the observed association with low birth-weight might be related to recall bias, since studies using interview data reported stronger associations than those using registries as the data source. A child born with low birth-weight may be subject to more medical care than a child with normal or high birth-weight.

4.3.2 Congenital malformations

We found a positive association between neuroblastoma and congenital malformations. This finding is consistent with previous publications[43], [44], [47], [48], [53], [57], [60] and

strengthens the hypothesis that developmental factors could have an etiological role in neuroblastoma.

A previous Swedish study cohort[44] found that the association with congenital malformations was only present among younger children (<18 months at diagnosis), which is consistent with our findings. The underlying explanation is not clear. As it has been shown that some genetic alterations are different among younger and older neuroblastoma cases (i.e *MYCN* amplification is more frequent among children >18 months), it may reflect different genetic conditions involved in both congenital malformations and childhood neuroblastoma. However, it could also arise from unmeasured confounding or differential recall bias between the mothers of younger and older children.

Since our study included only one case and three control children with genetic syndromes and results did not change after their exclusion in sensitivity analysis, our findings support the associations between neuroblastoma and non-chromosomal congenital malformations. The associations between chromosomal congenital malformations and childhood cancer are consistently reported in literature. Some of these associations are well documented, especially those between childhood cancer and chromosomal syndromes (i.e. leukemia and Down syndrome[119] or Wilm's tumor and Beckwith-Weidemann syndrome[120]). Two recent record linkage studies[60], [121] analyzed the association between neuroblastoma and non-chromosomal malformations using data from high-quality registers. Their results are consistent with ours. The US case-control study[60] found that non-chromosomal malformations were associated to a moderate increase of neuroblastoma risk OR 1.9 [95% CI 1.3-2.8]. Finally, an Australian study cohort[121] found a positive association between childhood cancer among children younger than four years old and congenital malformations that were not known to be related to a cancer (OR 1.7 [95% CI 1.3-2.4]. They reported a positive association with neuroblastoma, but confident intervals were wide as based only in five exposed cases (OR 1.4 [95% CI 0.6-3.5]).

The biological plausibility of relationships between birth defects and childhood cancer is supported by recent studies (reviewed in[31]) suggesting that disruption of normal developmental processes may be linked with oncogenesis.

Further investigation is needed. However, since the associations between childhood cancer and congenital malformations have long been consistently reported, new research on the topic may identify new approaches to clarify the underlying mechanisms of the observed associations as well as replicate previous findings. Wellesley et al[122] highlighted the fact

that current research on congenital malformations etiology, like our study, is based on the ICD classification system, which uses body systems for classification categories which does not take account of new knowledge in clinical genetics. The authors suggest that, for etiological research, new classification methods should be developed based on presumed etiological commonality. This could allow a better understanding of the etiology of congenital malformations and further investigation of those cases where a yet unknown environmental element could be important. Furthermore, it is hoped that the study of the association of childhood cancer and congenital anomalies will lead to identification of new genes that are involved in development and cancer.

4.3.3 Maternal intake of folic acid, vitamins or minerals

Our study suggests that maternal folic acid; vitamin or mineral supplementation in the preconception period may reduce the incidence of neuroblastoma. In line with these findings, a significant reduction in neuroblastoma incidence was observed in Canada after mandatory flour fortification with folic acid was instituted[65]. Moreover, two US case-control studies[66], [67], also reviewed in Goh et al.[123] concluded that periconceptional folic acid supplementation, three months before pregnancy and early in pregnancy, should be recommended. By contrast, a recent study performed by Mortensen et al [68] found no association between supplemental folic acid before and/or pregnancy and neuroblastoma[68]. Although this large nation-wide study included almost 800 children with cancer, results on neuroblastoma were based on only 71 cases and lack of power cannot be completely ruled out.

As discussed in Chapter 1, the periconceptional folic acid supplementation has been shown to reduce the risk of neural tube defects by almost three-quarters [61]. Neural tube defects arise from the same embryonic structures as neuroblastoma. The biological plausibility of the associations derives from evidence that folic acid derivatives are essential for the synthesis of nucleic acids and amino acids, cell division, tissue growth, and DNA methylation. In our study, the potential protective effect was only observed when supplementation started before pregnancy. One explanation for this finding could be that the inception, migration, divergence and maturation of neural crest progenitors occurs early in pregnancy (between 3rd and 5th week of gestation) a critical period of time when most women may still not know they are pregnant. The association may also result from confounding since the women who began supplementation prior to pregnancy may differ in terms of profile and lifestyle from those

who did not use supplements or started them after discovering they were pregnant. A study by Tort et al, based on the French 2010 National Perinatal Survey[113] showed that women who took folic acid supplements before pregnancy were more likely to be: older; married or cohabiting; European; non-smokers; with a body mass index less than 25. Folic acid use during preconception was also higher in low-parity highly educated women. However, the cases and controls in our study were similar in terms of maternal education and parity, and the estimates were adjusted for maternal age. Since health promotion campaigns on folic acid intake during pregnancy started in France after the ESCALE study period, the low frequency of supplementation in both cases and controls in that study (only two cases and three controls reported the use of folic acid or vitamin/mineral supplements in the pre-conception period) prevented supplementation being addressed in that study.

Only half of the mothers who reported folic acid supplementation during the preconception period could name a valid folic acid product. However, the imprecision was similar for case and control mothers, and there was little change in the estimates when only those mothers were analysed. Although various constituents of the multivitamin supplements may have been responsible for the protective effects, only 2% of controls and 1% of cases reported using a supplement not containing folic acid during the preconception period.

4.3.4 Maternal reproductive history before the index pregnancy

This study did not find any association between fertility treatments and neuroblastoma and as such is consistent with the results of a large British cohort study[124]. In our study the estimates were very close to unity and do not suggest any increased risk of neuroblastoma. However, the findings were based on small numbers of exposed subjects and cannot be interpreted as meaning there is no risk. The literature remains discordant. A Danish cohort study suggested that maternal fertility treatment with progesterone before childbirth might increase the risk of sympathetic nervous system tumors[125]. Additionally, a meta-analysis by the same author, based on five studies of neuroblastoma, found an increased risk of neuroblastoma among children born after fertility treatment (OR 4.0 [95% CI 1.24–13.18])[126].

4.3.5 Breastfeeding

Our finding of an inverse relationship with breastfeeding is consistent with a large US study, which reported an association of the same order of magnitude (OR 0.63 [95% CI 0.41–

0.96])[57]. The association between breastfeeding and neuroblastoma has been less documented than the association with leukemia or brain tumors. A meta-analysis published in 2005[127], which reported a 41% reduction in the risk of neuroblastoma, relied on only three studies[73]–[75], two of which[74], [75] involved <45 cases each.

The hypotheses with respect to underlying biological mechanisms are unclear at present. Greaves' hypothesis [128] regarding childhood leukemia suggests that breast milk may play an important role in the prevention of childhood leukemia by actively stimulating or modulating the immune system and promoting its development in early life. However, an infectious etiology and a role of immunological modifiers in neuroblastoma development have not been prominent hypotheses. Epigenetic mechanisms have also been suggested based on the contribution of some human milk compounds to metabolic and differentiation processes, and to the development of the infant's immune system[129].

The proportion of control mothers who reported breastfeeding during the ESCALE study (55%) was lower than the reported by the ENP (62%), while the proportion was similar between ESTELLE study (65%) and the 2010 ENP report (68%).

The differences between our studies and the relevant ENPs could be related to different definitions, which was breast-feeding for at least three days in our studies, while in the ENP, it was the breast-feeding status in the maternity unit. In our studies, it is plausible that women who breast-fed for a short duration did not report it. The increased breastfeeding prevalence between the ESCALE and ESTELLE studies was similar to the observed trend between 2003 and 2010

The increased breastfeeding prevalence between the ESCALE and ESTELLE studies was similar to the observed trend between 2003 and 2010. However, we cannot dismiss the possibility that our results might be influenced by residual confounding by socioeconomic status or other unmeasured characteristics associated with participation among controls.

4.3.6 Maternal use of household pesticides

Our findings suggest that the maternal use of household pesticides during pregnancy may be associated with an increased risk of neuroblastoma. Only two previous studies have assessed the relationship between household use of pesticides and neuroblastoma. In a large US case–control study, Daniels et al.[25] also reported modest associations (OR around 1.5) with the use of pesticides at home or in the garden during the preconception-pregnancy period or childhood. Consistent with our findings, this study showed similar estimates irrespective of

the *MYCN* status, which does not support the potential for pesticides to act through different pathways in the two subtypes. In that study, the estimates were stronger among older children. Since the use of pesticides at home may be associated with similar patterns over the life span, the stronger associations observed in older children may reflect the effect of longer period of exposures to pesticides. Previous studies have also suggested that different etiologic factors may be specific to age at neuroblastoma diagnosis[23], [47], [130]. In our study, there was a suggestion of difference by age with the use of herbicides and fungicides. However, this was based on small numbers and the confidence intervals in the two groups overlapped substantially. Another study conducted in Germany by Schuz et al[23] found an association between neuroblastoma and the use of household pesticides after the child's birth, but did not investigate pesticide exposure during pregnancy.

Despite the homogeneity of exposure definition among these previous studies and ours, differences were observed with regards to the assessed time-period. In the US study, positive associations were observed when both parents reported pesticide use between the month before pregnancy and diagnosis date (or equivalent date for controls), but not when only the preconception-pregnancy period was assessed. Up to 70% of parents reported ever use of pesticides in the US study, which was consistent with the reported prevalence in the ESTELLE study (60% for any use of pesticides before or during pregnancy, or after birth). Because the use of pesticides was correlated between time periods (preconception, pregnancy, and childhood), we cannot conclude that exposure specifically during pregnancy was associated with neuroblastoma. It is possible that patterns of exposure are related to lifetime habits, which are consistent throughout the periconceptional period and later in childhood. The German study only assessed household pesticide use after birth. The reported prevalence of household pesticides use was much lower (10%). In the ESTELLE study, 10% of control mothers reported having use household pesticides only after pregnancy, but as discussed above, pesticide use was strongly correlated between time periods.

This study had limited ability to investigate associations with pesticides other than insecticides since the majority of mothers who reported any pesticide use (94%) reported using insecticides, either alone or combined with herbicides or fungicides. A French cohort study[131] performed between 2002-2006 found pesticides in up to 84% of urine samples from pregnant women living in the Bretagne region. Organophosphate insecticides were the most frequently observed pesticides among the 3 pesticides families that were investigated (triazine herbicides, organophosphate and carbamate insecticides).

We could not identify the active ingredients in the products used as in our study we only asked women about the category of pesticide as we thought this would be recalled with greater accuracy than the actual product name. Commercial household pesticides often contain multiple active ingredients, all which may have different properties including potentially carcinogenic actions. In our study, we mainly focused on maternal use of pesticides at home. However, the mothers may be exposed to other direct or indirect sources of pesticides, which we did not account for, like the paternal use of household pesticides or other sources of environmental exposure.

Previous studies have found consistency between self-reported pesticide treatments and pesticides concentrations in dust[132] and that agreement about pesticides exposure between parents did not differ by case–control status[25], suggesting no differential recall based on motivation of case parents.

It is biologically plausible that maternal pesticide exposure during pregnancy could be associated with the risk of neuroblastoma. It has been shown that maternal exposure during pregnancy can lead to fetal exposure since these compounds or their residues pass through the placenta and can be found in cord blood, infant hair, and meconium[133], [134].The potential underlying mechanisms are still unknown. Some individual pesticides have been classed as ‘‘probable or possible carcinogens’’ by the IARC[78], [79]. In addition, because of the similarity of brain biochemistry, some insecticides that target the nervous system of insects may also be neurotoxic to humans[135].

Despite the biological plausibility of an association and the consistency with the literature, our findings raise concern about how to interpret the lack of variation by broad type of pesticide, by geographical area and by time-period.

Pesticides are a heterogeneous group of substances with diverse biological targets and modes of action. Therefore, a common biological effect such as carcinogenicity seems unlikely. However, interpretations may also consider the fact that childhood cancers are complex and multi-step diseases and a unique biological pathway is also unlikely. Carcinogenicity may be the consequence of different mutagenic or immunotoxic properties of pesticides that could impair different biological processes. As observed in our study, participants might be exposed to multiple types of pesticides, with unknown cumulative effects and between which there may be interactions.

The fact that pesticide use patterns was too similar in each of the three time periods (preconception-pregnancy-after birth) might explain the homogeneity of results across the three studies despite the fact that they assessed different time periods.

The lack of variation across different countries and study periods is also worth noting. The US and the German studies covered the late eighties and early nineties, while the ESCALE and ESTELLE studies where performed at least ten year later. The active ingredients of pesticides and practices of use may vary over time and by geographical area. However, household use of insecticides may be subject to less variation than agricultural use of pesticides. Unless not identical, the molecules are likely to be similar. For example, insecticides where the also the most frequent household pesticide reported by the Northern California Childhood Leukemia Study, and identification of chemical classes showed that up to 77% were pyrethroids[136]. Finally, we cannot rule out recall bias as all the studies on household pesticide exposure and neuroblastoma relied on self-report. Since literature on household use of pesticides and neuroblastoma is scarce, further studies with a better exposure assessment are needed.

4.3.7 Professional pesticides exposure

Consistent with our findings, associations between neuroblastoma and maternal occupational exposure to pesticides were reported in two case–control studies in the US[23], [82]. In a cohort study in Norway, an increased risk of neuroblastoma was observed in offspring of parents having worked with field vegetables[85]. However, these findings should be interpreted with caution since they were based on small numbers. In two of these studies[82], [85] like in ours, estimates were based on less than ten exposed cases and could represent a chance finding. Literature on paternal occupational exposure to pesticides is not supportive of an association with neuroblastoma as shown in a meta-analysis conducted by Moore et al.[87]. A large Texan case–control study estimated residential exposures to pesticides due to neighboring agricultural activities and found no association[137]. However, estimates were inconsistent between low and high level of exposure and were based on less than 15 exposed cases. A meta-analysis performed by Vinson et al[138] reported an OR 1.7 [95% CI 1.1-2.5] with parental occupational exposure to pesticides. However, results on neuroblastoma where not clearly stated and did not distinguish between paternal and maternal exposure.

4.3.8 Parental smoking and alcohol consumption

With regards to parental smoking, the main finding of the ESCALE and ESTELLE studies was the slight positive association between maternal smoking during pregnancy and the risk of neuroblastoma. By contrast, no association with maternal alcohol consumption or paternal smoking around pregnancy was observed.

Our meta-analysis also suggested a slight but significant association between neuroblastoma and maternal smoking during pregnancy. This is consistent with the two previous meta-analyses by Chu et al.[139] (OR = 1.3 [95% CI 1.0–1.6]) and Müller-Schulte et al.[94] (OR = 1.2 [95% CI 1.0–1.4], which included almost the same studies. Our meta-analysis added nearly 500 cases from an Australian study[104] and the ESCALE and ESTELLE studies.

Yang et al.[24], whose findings were consistent with ours, also accounted for *MYCN* status. Their findings do not support the potential for maternal smoking to act through different pathways in the two subtypes.

Paternal tobacco smoking has been shown to be associated with increases in DNA damage, aneuploidies, and mutations in sperm and may act as a human germ cell mutagen[140]. Despite this, our study did not support the link between paternal smoking around pregnancy and the risk of neuroblastoma as did two previous studies[24], [107].

The meaning of the apparent stronger association between maternal smoking and neuroblastoma when both parents smoked is still unclear. We first hypothesized that when both parents smoked the mothers may have been heavier smokers, but there was no difference with regards to the quantity of cigarettes smoked per day. However, the prevalence of tobacco consumption was higher among mothers when the fathers also smoked. Yang et al.[24] also found a slightly stronger association when both parents smoked (OR 1.3 [95% CI 0.9–2.0]) compared to only mothers (OR 1.1 [95% CI 0.8–1.4]. Consistent with our findings, previous studies did not support the link between maternal alcohol intake during pregnancy and neuroblastoma[23], [24], [46], [49], [93].

4.4 Public health implications

This thesis adds weights to the existing evidence that some perinatal exposures may play a role in NB development. This has implications for parents, health care professionals and politicians.

Since it is recommended that women take folic acid supplements prior to conception and continue in early pregnancy, timing is one of the biggest challenges with regards to folic acid supplementation. In France, recommendations on the preconceptional intake of folic acid should be strengthened among women of childbearing age. The 2016 ENP reported a significant increase of the prevalence of preconceptional folic acid supplementation among women compared to 2010[141]. Fifteen per cent of mothers reported folic acid intake before pregnancy in 2010 and 23% in 2016 ($p < 0.001$). However, the challenging is still enormous

since overall 80% of women do not benefits of this effective preventive measure nowadays, far below the level reported in other high-income countries, as shown by an Australian study which reported 51% of women had taken folic acid supplements during the preconception period as far back as 2009[142]. The National Institute for Prevention and Health Education (INPES) advocates that health professionals should prescribe a daily intake of folic acid to women from the time they start to planning to get pregnant and until the 12th week of amenorrhea[143]. Nevertheless, opportunity for this action seems limited as only 35% of women declared a preconceptional medical consultation and 28% an early consultation once they knew they were pregnant[141]. Furthermore, this measure does not take account unplanned pregnancies.

Additional strategies should be considered to reach a bigger proportion of women. As previously discussed, folates are naturally present in some foods. However, to achieve the equivalent of 400 µg folic acid through dietary food folate intake may be difficult and most of the time requires behavioural change. Governments should evaluate the feasibility and efficacy of a mandatory food fortification program in their specific context. If successful, this measure also has the advantage of reach hard to reach groups [144] since it has been shown that women who take folic acid during the periconceptional period tend to be older, better educated and from high socioeconomic categories[113].

Women should be advised to avoid pesticide exposure around pregnancy and there is a need to limit the use of pesticides in homes as well as public and private spaces where women and children might be exposed, such as parks, child care centres and schools. In 2005, the National Institute for Agronomic research (INRA) in collaboration with Cemagref released an expert's report on demand of the Ministry for Ecology and Sustainable Development (MEDD)[145]. The report provides a detailed analysis of existing data for evaluating the magnitude of pesticides use in France, as well as to estimate population-level exposure. It also proposes several strategies to reduce the use of pesticides in France, as well as its environmental impact. The report drew on previous international experiences such as the US Integrated Pest Management program[146] in order to propose better strategies for the French context.

National efforts are being implemented to achieve these goals. However, the implementation of changes is taking longer than planned. The European Union has adopted a common framework in line with sustainable development recommendations. In France, the Ecophyto program aimed to halve the agricultural use of pesticides. In 2015, Ecophyto II program extended the achievement of this goal to 2025.

Further efforts should be made to limit tobacco consumption among prospective parents and supporting smoking cessation during pregnancy. After a significant reduction of maternal smoking during pregnancy over the period 2003-2010[107] the 2016 ENP[141] reported an stagnation of the prevalence of maternal tobacco consumption during pregnancy (overall 17%). It is worthy to note that the report states that less than half of pregnant women who declared tobacco consumption declared to have benefit from medical advice or professional support for smoking cessation. Finally, current practices should continue to support breastfeeding, which is reported nowadays by almost 65% of women.

4.5 Implications for etiological research

This thesis adds to the existing knowledge about risk factors for neuroblastoma. Specifically, it supports the hypothesis that malignant tumors may be initiated during fetal development. However, there are still many unanswered questions.

The rarity of neuroblastoma and the lack of precision on measurement of some exposures are the main limitations to overcome. It is hoped that several ongoing projects will help to improve our understanding of neuroblastoma etiology.

For instance, international consortiums such as the CLIC+, which expands the Childhood Leukemia International Consortium, aim to achieve better statistical power by pooling data from good-quality studies conducted by different countries. Some of these, like the ESCALE and ESTELLE studies, have collected biological samples that will allow to study genetic predisposition to neuroblastoma and interactions between genetic and environmental factors

Finally, recent studies[147], [148] and governmental programs[149] have been undertaken to improve the quality of pesticide exposures assessment. However, the challenges are still great in regards to childhood cancer research, since cohort studies are not feasible and the characterization of pesticides exposure relies on retrospective data collection.

5 Conclusion

Our findings support the hypothesis that fetal growth anomalies and congenital malformations could increase the risk of neuroblastoma, which may suggest defective embryogenesis. They also add to the evidence that neuroblastoma is related to maternal use of household pesticides and to maternal smoking, which are additional reasons for advising and helping pregnant women to limit these exposures in this period. Further investigations are needed to clarify the role of folic acid supplementation and breastfeeding, given their potential importance in neuroblastoma prevention.

6 References

[1] B. Lacour, A. Guyot-Goubin, S. Guissou, S. Bellec, E. Désandes, and J. Clavel, "Incidence of childhood cancer in France: National Children Cancer Registries, 2000–2004," *Eur. J. Cancer Prev.*, vol. 19, no. 3, pp. 173–181, May 2010.

[2] P. A. Trott, "International Classification of Diseases for Oncology," *Journal of Clinical Pathology*, 2007. [Online]. Available: http://codes.iarc.fr/. [Accessed: 14-Feb-2019].

[3] E. Steliarova-Foucher, C. Stiller, B. Lacour, and P. Kaatsch, "International classification of childhood cancer, third edition," *Cancer*, vol. 103, no. 7, pp. 1457–1467, Feb. 2005.

[4] C. Spix, G. Pastore, R. Sankila, C. A. Stiller, and E. Steliarova-Foucher, "Neuroblastoma incidence and survival in European children (1978-1997): Report from the Automated Childhood Cancer Information System project," *Eur. J. Cancer*, vol. 42, no. 13, pp. 2081–2091, Sep. 2006.

[5] K. K. Matthay, L. S. Baskin, M. H. Hsieh, M. V Meng, and T. J. Walsh, "Increasing Incidence of Neuroblastoma and Potentially Higher Associated Mortality of Children From Nonmetropolitan Areas: Analysis of the Surveillance, Epidemiology, and End Results Database," *J. Pediatr. Hematol. Oncol.*, vol. 31, no. 12, pp. 942–946, Dec. 2009.

[6] F. Berthold, C. Spix, P. Kaatsch, and F. Lampert, "Incidence, Survival, and Treatment of Localized and Metastatic Neuroblastoma in Germany 1979–2015," *Pediatr. Drugs*, vol. 19, no. 6, pp. 577–593, 2017.

[7] J. Kamihara, C. Ma, S. L. Fuentes Alabi, C. Garrido, A. L. Frazier, C. Rodriguez-Galindo, and M. A. Orjuela, "Socioeconomic status and global variations in the incidence of neuroblastoma: call for support of population-based cancer registries in low-middle–income countries," *Pediatr. Blood Cancer*, vol. 64, no. 2, pp. 321–323, 2017.

[8] E. Steliarova-Foucher, M. Colombet, L. A. G. Ries, F. Moreno, A. Dolya, F. Bray, P. Hesseling, H. Y. Shin, and C. A. Stiller, "International incidence of childhood cancer, 2001–10: a population-based registry study," *Lancet Oncol.*, vol. 18, no. 6, pp. 719–731, 2017.

[9] M. K. Georgakis, R. Gheorghiu, E. Bouka, E. Hatzipantelis, A. Tragiannidis, E. Stiakaki, M. Kourti, A. Demetriou, L. Antunes, M. Sekerija, D. Coza, E. T. Petridou, N. Dessypris, H. Dana, S. Polychronopoulou, E. Steliarova-Foucher, A. Ryzhov, P. Panagopoulou, M. Moschovi, A. Zborovskaya, V. Papadakis, M. Trojanowski, D. Agius, J. Bastos, T. Zagar, S. Eser, and M. Baka, "Neuroblastoma among children in Southern and Eastern European cancer registries: Variations in incidence and temporal trends compared to US," *Int. J. Cancer*, vol. 142, no. 10, pp. 1977–1985, 2017.

[10] S. Barrette, M. L. Bernstein, J. M. Leclerc, M. A. Champagne, Y. Samson, J. Brossurad, and W. G. Woods, "Treatment complications in children diagnosed with neuroblastoma during a screening program," *J. Clin. Oncol.*, vol. 24, no. 10, pp. 1542–1545, Apr. 2006.

[11] K. Yamamoto, S. Ohta, E. Ito, Y. Hayashi, T. Asami, O. Mabuchi, M. Higashigawa, and M. Tanimura, "Marginal decrease in mortality and marked increase in incidence as a result of neuroblastoma screening at 6 months of age: cohort study in seven prefectures in Japan.," *J. Clin. Oncol.*, vol. 20, no. 5, pp. 1209–14, Mar. 2002.

[12] F. H. Schilling, C. Spix, F. Berthold, R. Erttmann, N. Fehse, B. Hero, G. Klein, J. Treuner, J. Sander, U. Zorn, K. Schwarz, and J. Michaelis, "Neuroblastoma Screening at One Year of Age," *N. Engl. J. Med.*, vol. 346, no. 14, pp. 1047–1053, 2002.
[13] J. M. Maris, M. D. Hogarty, R. Bagatell, and S. L. Cohn, "Neuroblastoma," *Lancet*, 2007.
[14] R. Haupt, A. Garaventa, C. Gambini, S. Parodi, G. Cangemi, F. Casale, E. Viscardi, M. Bianchi, A. Prete, A. Jenkner, R. Luksch, A. Di Cataldo, C. Favre, P. D'Angelo, G. A. Zanazzo, G. Arcamone, G. C. Izzi, A. R. Gigliotti, G. Pastore, and B. De Bernardi, "Improved survival of children with neuroblastoma between 1979 and 2005: A report of the Italian neuroblastoma registry," *J. Clin. Oncol.*, vol. 28, no. 14, pp. 2331–2338, May 2010.
[15] V. Moroz, D. Machin, A. Faldum, B. Hero, T. Iehara, V. Mosseri, R. Ladenstein, B. De Bernardi, H. Rubie, F. Berthold, K. K. Matthay, T. Monclair, P. F. Ambros, A. D. J. Pearson, S. L. Cohn, and W. B. London, "Changes over three decades in outcome and the prognostic influence of age-at-diagnosis in young patients with neuroblastoma: A report from the International Neuroblastoma Risk Group Project," *Eur. J. Cancer*, vol. 47, no. 4, pp. 561–571, Mar. 2011.
[16] G. Gatta, L. Botta, S. Rossi, T. Aareleid, M. Bielska-Lasota, J. Clavel, N. Dimitrova, Z. Jakab, P. Kaatsch, B. Lacour, S. Mallone, R. Marcos-Gragera, P. Minicozzi, M. J. Sánchez-Pérez, M. Sant, M. Santaquilani, C. Stiller, A. Tavilla, A. Trama, O. Visser, and R. Peris-Bonet, "Childhood cancer survival in Europe 1999-2007: Results of EUROCARE-5-a population-based study," *Lancet Oncol.*, vol. 15, no. 1, pp. 35–47, Jan. 2014.
[17] D. Coughlan, M. Gianferante, C. F. Lynch, J. L. Stevens, and L. C. Harlan, "Treatment and survival of childhood neuroblastoma: Evidence from a population-based study in the United States," *Pediatr. Hematol. Oncol.*, vol. 34, no. 5, pp. 320–330, 2017.
[18] P. Panagopoulou, E. Bouka, M. K. Georgakis, A. Zborovskaya, J. Bastos, D. Coza, D. Agius, E. Stiakaki, S. Eser, S. Polychronopoulou, A. Ryzhov, T. Žagar, H. Dana, A. Tragiannidis, M. Baka, N. Dessypris, M. Moschovi, D. Morgenstern, R. Gheorghiu, L. Antunes, M. Kourti, A. Demetriou, M. Šekerija, E. Hatzipantelis, V. Papadakis, M. Trojanowski, and E. T. Petridou, "Persisting inequalities in survival patterns of childhood neuroblastoma in Southern and Eastern Europe and the effect of socio-economic development compared with those of the US," *Eur. J. Cancer*, vol. 96, pp. 44–53, Jun. 2018.
[19] N. O. Basta, G. C. Halliday, G. Makin, J. Birch, R. Feltbower, N. Bown, M. Elliott, L. Moreno, G. Barone, A. D. Pearson, P. W. James, D. A. Tweddle, and R. J. Q. McNally, "Factors associated with recurrence and survival length following relapse in patients with neuroblastoma," *Br. J. Cancer*, vol. 115, no. 9, pp. 1048–1057, 2016.
[20] C. Laverdiére, Q. Liu, Y. Yasui, P. C. Nathan, J. G. Gurney, M. Stovall, L. R. Diller, N. K. Cheung, S. Wolden, L. L. Robison, and C. A. Sklar, "Long-term outcomes in survivors of neuroblastoma: A report from the childhood cancer survivor study," *J. Natl. Cancer Inst.*, vol. 101, no. 16, pp. 1131–1140, 2009.
[21] E. Sokol and A. Desai, "The Evolution of Risk Classification for Neuroblastoma," *Children*, vol. 6, no. 2, p. 27, 2019.
[22] S. L. Cohn, A. D. J. Pearson, W. B. London, T. Monclair, P. F. Ambros, G. M. Brodeur, A. Faldum, B. Hero, T. Iehara, D. Machin, V. Mosseri, T. Simon, A. Garaventa, V. Castel, and K. K. Matthay, "The International Neuroblastoma Risk Group (INRG) classification system: An INRG task force report," *J. Clin. Oncol.*, vol. 27, no. 2, pp. 289–297, Jan. 2009.
[23] J. Schüz, U. Kaletsch, R. Meinert, P. Kaatsch, C. Spix, and J. Michaelis, "Risk factors

for neuroblastoma at different stages of disease. Results from a population-based case-control study in Germany," *J. Clin. Epidemiol.*, vol. 54, no. 7, pp. 702–709, 2001.
[24] Q. Yang, A. F. Olshan, M. L. Bondy, N. R. Shah, B. H. Pollock, R. C. Seeger, A. T. Look, and S. L. Cohn, "Parental smoking and alcohol consumption and risk of neuroblastoma," *Cancer Epidemiol. Biomarkers Prev.*, vol. 9, no. 9, pp. 967–972, Sep. 2000.
[25] J. L. Daniels, A. F. Olshan, K. Teschke, I. Hertz-Picciotto, D. A. Savitz, J. Blatt, M. L. Bondy, J. P. Neglia, B. H. Pollock, S. L. Cohn, A. T. Look, R. C. Seeger, and R. P. Castleberry, "Residential pesticide exposure and neuroblastoma," *Epidemiology*, vol. 12, no. 1, pp. 20–27, Jan. 2001.
[26] M. Huang and W. A. Weiss, "Neuroblastoma and MYCN," *Cold Spring Harb. Perspect. Med.*, vol. 3, no. 10, 2013.
[27] I. Janoueix-Lerosey, D. Lequin, L. Brugières, A. Ribeiro, L. De Pontual, V. Combaret, V. Raynal, A. Puisieux, G. Schleiermacher, G. Pierron, D. Valteau-Couanet, T. Frebourg, J. Michon, S. Lyonnet, J. Amiel, and O. Delattre, "Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma," *Nature*, vol. 455, no. 7215, pp. 967–970, 2008.
[28] G. Schleiermacher, I. Janoueix-Lerosey, and O. Delattre, "Recent insights into the biology of neuroblastoma," *International Journal of Cancer*, vol. 135, no. 10. pp. 2249–2261, 2014.
[29] J. J. Molenaar, R. Domingo-Fernández, M. E. Ebus, S. Lindner, J. Koster, K. Drabek, P. Mestdagh, P. Van Sluis, L. J. Valentijn, J. Van Nes, M. Broekmans, F. Haneveld, R. Volckmann, I. Bray, L. Heukamp, A. Sprüssel, T. Thor, K. Kieckbusch, L. Klein-Hitpass, M. Fischer, J. Vandesompele, A. Schramm, M. M. Van Noesel, L. Varesio, F. Speleman, A. Eggert, R. L. Stallings, H. N. Caron, R. Versteeg, and J. H. Schulte, "LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression," *Nat. Genet.*, vol. 44, no. 11, pp. 1199–1206, 2012.
[30] A. G. Knudson and L. C. Strong', "Mutation and Cancer: Neuroblastoma and Pheochromocytoma," *Amer J Hum Genet*, vol. 24, pp. 514–532, 1972.
[31] G. M. Marshall, D. R. Carter, T. Liu, W. A. Weiss, B. B. Cheung, M. K. Mateos, and J. G. Meyerowitz, "The prenatal origins of cancer," *Nat. Rev. Cancer*, vol. 14, no. 4, pp. 277–289, 2014.
[32] D. M. DeMarini, "Declaring the existence of human germ-cell mutagens," *Environ. Mol. Mutagen.*, vol. 148, no. April 2014, pp. 166–172, 2015.
[33] E. L. Marczylo, A. A. Amoako, J. C. Konje, T. W. Gant, and T. H. Marczylo, "Smoking induces differential miRNA expression in human spermatozoa: A potential transgenerational epigenetic concern?," *Epigenetics*, vol. 7, no. 5, pp. 432–439, 2012.
[34] C. Coz, J. Amiel, A. Ribeiro, O. Delattre, A. Deville, J.-F. Michiels, D. Trochet, S. Lyonnet, I. Janoueix-Lerosey, and F. Bourdeaut, "Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma," 2005.
[35] Y. P. Mossë, M. Laudenslager, L. Longo, K. A. Cole, A. Wood, E. F. Attiyeh, M. J. Laquaglia, R. Sennett, J. E. Lynch, P. Perri, G. Laureys, F. Speleman, H. Hakonarson, A. Torkamani, N. J. Schork, G. M. Brodeur, G. P. Tonini, E. Rappaport, M. Devoto, and J. M. Maris, "Identification of ALK as the Major Familial Neuroblastoma Predisposition Gene," *Nature*, vol. 455, no. 7215, pp. 930–935, 2008.
[36] P. D. Gluckman, M. A. Hanson, D. Phil, C. Cooper, and K. L. Thornburg, "Effect of In Utero and Early-Life Conditions on Adult Health and Disease," 2008.
[37] L. L. Hjalgrim, T. Westergaard, K. Rostgaard, K. Schmiegelow, M. Melbye, H. Hjalgrim, and E. A. Engels, "Birth weight as a risk factor for childhood leukemia: a meta-analysis of 18 epidemiologic studies.," *Am. J. Epidemiol.*, vol. 158, no. 8, pp.

724–35, Oct. 2003.
[38] J. Schüz and M. R. Forman, “Birthweight by gestational age and childhood cancer,” *Cancer Causes Control*, vol. 18, no. 6, pp. 655–663, Jun. 2007.
[39] J. Schüz, L. S. Schmidt, P. Kogner, P. M. Lähteenmäki, N. Pal, T. Stokland, and K. Schmiegelow, “Birth characteristics and Wilms tumors in children in the Nordic countries: a register-based case-control study.,” *Int. J. cancer*, vol. 128, no. 9, pp. 2166–73, May 2011.
[40] A. C. Callan and E. Milne, “Involvement of the IGF system in fetal growth and childhood cancer: An overview of potential mechanisms,” *Cancer Causes and Control*, vol. 20, no. 10. pp. 1783–1798, 2009.
[41] J. Gardosi, A. Chang, B. Kalyan, D. Sahota, and E. M. Symonds, “Customised antenatal growth charts.,” *Lancet*, vol. 339, no. 8788, pp. 283–7, Feb. 1992.
[42] S. E. G. Hamrick, A. F. Olshan, J. P. Neglia, and B. H. Pollock, “Association of pregnancy history and birth characteristics with neuroblastoma: A report from the Children’s Cancer Group and the Pediatric Oncology Group,” *Paediatr. Perinat. Epidemiol.*, vol. 15, no. 4, pp. 328–337, Jul. 2001.
[43] S. Parodi, D. F. Merlo, A. Ranucci, L. Miligi, A. Benvenuti, R. Rondelli, C. Magnani, R. Haupt, D. Andreuccetti, L. Anglesio, M. Bertolotti, P. Bevitori, R. Biancotto, A. Biggeri, S. Bucci, R. Calisti, P. Comba, P. Crosignani, G. D’Amore, E. Duglio, M. Erna, D. Ferrante, L. Gelli, M. Gilardetti, P. Guidotti, M. Lombardi, D. Loomis, M. Magnoni, F. Merletti, G. Miceli, D. Monetti, P. Mozzo, M. Nardi, S. Panico, A. Poggi, O. Pons, A. Rasulo, S. Roletti, M. Rosa, O. Ru, G. Russo, G. Sgorbati, L. Simonato, D. Sivo, B. Stievano, S. Tofani, F. Troti, R. Tumino, M. Valle, P. Vecchia, G. Erminio, and B. Galleni, “Risk of neuroblastoma, maternal characteristics and perinatal exposures: The SETIL study,” *Cancer Epidemiol.*, vol. 38, no. 6, pp. 686–694, 2014.
[44] T. Bjørge, A. Engeland, S. Tretli, and I. Heuch, “Birth and parental characteristics and risk of neuroblastoma in a population-based Norwegian cohort study,” *Br. J. Cancer*, vol. 99, no. 7, pp. 1165–1169, Oct. 2008.
[45] B. M. Buck, M. Michalek, C. Chen, N. Nasca, and B. Baptiste, “Perinatal factors and risk of neuroblastoma,” *Paediatr. Perinat. Epidemiol.*, vol. 15, no. 1, pp. 47–53, Jan. 2001.
[46] K. J. Johnson, S. E. Puumala, J. T. Soler, and L. G. Spector, “Perinatal characteristics and risk of neuroblastoma,” *Int. J. Cancer*, vol. 123, no. 5, pp. 1166–1172, Sep. 2008.
[47] K. Y. Urayama, J. Von Behren, and P. Reynolds, “Birth characteristics and risk of neuroblastoma in young children,” *Am. J. Epidemiol.*, vol. 165, no. 5, pp. 486–495, Mar. 2007.
[48] E. J. Chow, D. L. Friedman, and B. A. Mueller, “Maternal and perinatal characteristics in relation to neuroblastoma,” *Cancer*, vol. 109, no. 5, pp. 983–992, 2007.
[49] C. C. McLaughlin, M. S. Baptiste, M. J. Schymura, M. S. Zdeb, and P. C. Nasca, “Perinatal risk factors for neuroblastoma,” *Cancer Causes Control*, vol. 20, no. 3, pp. 289–301, Apr. 2009.
[50] T. Harder, A. Plagemann, and A. Harder, “Birth weight and risk of neuroblastoma: A meta-analysis,” *Int. J. Epidemiol.*, vol. 39, no. 3, pp. 746–756, Jun. 2010.
[51] WHO, “AtlasCongenitalAnomalies.WHO2014,” 2014.
[52] M. Nishi, H. Miyake, T. Takeda, and Y. Hatae, “Congenital malformations and childhood cancer,” *Med. Pediatr. Oncol.*, vol. 34, no. 4, pp. 250–254, 2000.
[53] A. E. Altmann, J. L. Halliday, and G. G. Giles, “Associations between congenital malformations and childhood cancer. A register-based case-control study,” *Br. J. Cancer*, vol. 78, no. 9, pp. 1244–1249, 1998.
[54] F. Mili, M. J. Khoury, W. D. Flanders, and R. S. Greenberg, “Risk of childhood cancer

for infants with birth defects: I. A record-linkage study, atlanta, georgia, 1968-1988," *Am. J. Epidemiol.*, vol. 137, no. 6, pp. 629–638, Mar. 1993.
[55] F. H. Epstein, M. J. Coppes, D. A. Haber, and P. E. Grundy, "Genetic Events in the Development of Wilms' Tumor," *N. Engl. J. Med.*, vol. 331, no. 9, pp. 586–590, 2002.
[56] M. J. Coppes, V. Huff, and J. Pelletier, "Denys-Drash syndrome: Relating a clinical disorder to genetic alterations in the tumor suppressor gene WT1," *J. Pediatr.*, vol. 123, no. 5, pp. 673–678, Nov. 1993.
[57] F. Menegaux, A. F. Olshan, P. J. Reitnauer, J. Blatt, and S. L. Cohn, "Positive association between congenital anomalies and risk of neuroblastoma," *Pediatr. Blood Cancer*, vol. 45, no. 5, pp. 649–655, Oct. 2005.
[58] S. A. Narod, M. M. Hawkins, C. M. Robertson, and C. A. Stiller, "Congenital anomalies and childhood cancer in Great Britain," *Am.J.Hum.Genet.*, vol. 60, no. 3, pp. 474–485, 1997.
[59] W. D. Foulkes, P. N. Buu, D. Filiatrault, J. M. Leclerc, and S. A. Narod, "Excess of congenital abnormalities in French-Canadian children with neuroblastoma: A case series study from montreal," *Med. Pediatr. Oncol.*, vol. 29, no. 4, pp. 272–279, Oct. 1997.
[60] M. S. Norwood, P. J. Lupo, E. J. Chow, M. E. Scheurer, S. E. Plon, H. E. Danysh, L. G. Spector, S. E. Carozza, D. R. Doody, and B. A. Mueller, "Childhood cancer risk in those with chromosomal and non-chromosomal congenital anomalies in Washington State: 1984-2013," *PLoS One*, vol. 12, no. 6, 2017.
[61] L. M. De-Regil, J. P. Peña-Rosas, A. C. Fernández-Gaxiola, and P. Rayco-Solon, "Effects and safety of periconceptional oral folate supplementation for preventing birth defects," Dec. 2015.
[62] K. S. Crider, L. B. Bailey, and R. J. Berry, "Folic acid food fortification-its history, effect, concerns, and future directions," *Nutrients*, vol. 3, no. 3. pp. 370–384, 2011.
[63] Centers for Disease Control, "Public Health Grand Rounds Presents: 'Global Prevention of Neural Tube Defects,'" 2017.
[64] S. Gonseth, R. Roy, E. A. Houseman, A. J. de Smith, M. Zhou, S. T. Lee, S. Nusslé, A. W. Singer, M. R. Wrensch, C. Metayer, and J. L. Wiemels, "Periconceptional folate consumption is associated with neonatal DNA methylation modifications in neural crest regulatory and cancer development genes," *Epigenetics*, vol. 10, no. 12, pp. 1166–1176, 2015.
[65] A. E. French, R. Grant, S. Weitzman, J. G. Ray, M. J. Vermeulen, L. Sung, M. Greenberg, and G. Koren, "Folic acid food fortification is associated with a decline in neuroblastoma," *Clin. Pharmacol. Ther.*, vol. 74, no. 3, pp. 288–294, 2003.
[66] A. M. Michalek, A. N. Freedman, G. M. Buck, M. C. Mahoney, M. S. Baptiste, and P. C. Nasca, "Gravid Health Status, Medication Use, and Risk of Neuroblastoma," *Am. J. Epidemiol.*, vol. 143, no. 10, pp. 996–1001, 2012.
[67] A. F. Olshan, J. C. Smith, M. L. Bondy, J. P. Neglia, and B. H. Pollock, "Maternal vitamin use and reduced risk of neuroblastoma," *Epidemiology*, vol. 13, no. 5, pp. 575–580, Sep. 2002.
[68] J. H. S. Mortensen, N. Øyen, T. Fomina, S. Tretli, M. Melbye, S. E. Vollset, and T. Bjørge, "Supplemental folic acid in pregnancy and childhood cancer risk," *Br. J. Cancer*, vol. 114, no. 1, pp. 71–75, 2016.
[69] C. Infante-Rivard, I. Fortier, and E. Olson, "Markers of infection, breast-feeding and childhood acute lymphoblastic leukaemia," *Br. J. Cancer*, vol. 83, no. 11, pp. 1559–1564, Dec. 2000.
[70] G. E. Gaull, C. E. Wright, and C. E. Isaacs, "Significance of growth modulators in human milk.," *Pediatrics*, vol. 75, no. 1 Pt 2, pp. 142–5, Jan. 1985.

[71] A. J. Macpherson, M. Gomez de Agüero, and S. C. Ganal-Vonarburg, “How nutrition and the maternal microbiota shape the neonatal immune system,” 2017.
[72] A. S. Goldman, “The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties.,” *Pediatr. Infect. Dis. J.*, vol. 12, no. 8, pp. 664–71, Aug. 1993.
[73] J. L. Daniels, A. F. Olshan, B. H. Pollock, N. R. Shah, and D. O. Stram, “Breast-feeding and neuroblastoma, USA and Canada,” *Cancer Causes Control*, vol. 13, no. 5, pp. 401–405, 2002.
[74] V. B. Smulevich, L. G. Solionova, and S. V. Belyakova, “Parental occupation and other factors and cancer risk in children: I. Study methodology and non-occupational factors,” *Int. J. Cancer*, vol. 83, no. 6, pp. 712–717, 1999.
[75] L. Hardell and A. C. Dreifaldt, “Breast-feeding duration and the risk of malignant diseases in childhood in Sweden,” *Eur. J. Clin. Nutr.*, vol. 55, no. 3, pp. 179–185, 2001.
[76] INRA. Expertise scientifique collective “Pesticides et environment,” “Vers une réduction de l’utilisation des pesticides et de leurs impacts environnementaux,” 2005.
[77] H. D. Bailey, C. Infante-Rivard, C. Metayer, J. Clavel, T. Lightfoot, P. Kaatsch, E. Roman, C. Magnani, L. G. Spector, E. Th. Petridou, E. Milne, J. D. Dockerty, L. Miligi, B. K. Armstrong, J. Rudant, L. Fritschi, J. Simpson, L. Zhang, R. Rondelli, M. Baka, L. Orsi, M. Moschovi, A. Y. Kang, and J. Schüz, “Home pesticide exposures and risk of childhood leukemia: Findings from the childhood leukemia international consortium,” *Int. J. Cancer*, vol. 137, no. 11, pp. 2644–2663, Dec. 2015.
[78] International Agency for Research on cancer, “Occupational exposures in insecticide application, and some pesticides,” 1991.
[79] IARC, “Some Organophosphate Insecticides and Herbicides: Diazinon, Glyphosate, Malathion, Parathion, and Tetrachlorvinphos,” 2015.
[80] M. Botsivali, · Soterios, and A. Kyrtopoulos, “Transplacental exposure to carcinogens and risks to children: evidence from biomarker studies and the utility of omic profiling,” *Arch. Toxicol.*, vol. 0, p. 3.
[81] G. R. Bunin, E. Ward, S. Kramer, C. A. Rhee, and A. T. Meadows, “Neuroblastoma and parental occupation.,” *Am. J. Epidemiol.*, vol. 131, no. 5, pp. 776–80, May 1990.
[82] A. F. Olshan, A. J. De Roos, K. Teschke, J. P. Neglia, D. O. Stram, B. H. Pollock, and R. P. Castleberry, “Neuroblastoma and parental occupation,” *Cancer Causes Control*, vol. 10, no. 18, pp. 776–780, 1990.
[83] M. A. Kerr, P. C. Nasca, K. A. Mundt, A. M. Michalek, M. S. Baptiste, and M. C. Mahoney, “Parental occupational exposures and risk of neuroblastoma: A case-control study (United States),” *Cancer Causes Control*, vol. 11, no. 7, pp. 635–643, 2000.
[84] A. MacCarthy, K. J. Bunch, N. T. Fear, J. C. King, T. J. Vincent, and M. F. G. Murphy, “Paternal occupation and neuroblastoma: A case-control study based on cancer registry data for Great Britain 1962-1999,” *Br. J. Cancer*, vol. 102, no. 3, pp. 615–619, 2010.
[85] P. Kristensen, A. Andersen, L. M. Irgens, A. S. Bye, and L. Sundheim, “Cancer in offspring of parents engaged in agricultural activities in Norway: Incidence and risk factors in the farm environment,” *Int. J. Cancer*, vol. 65, no. 1, pp. 39–50, Jan. 1996.
[86] K. B. Flower, C. Knott, D. L. Shore, C. F. Lynch, D. P. Sandler, A. Blair, and J. A. Hoppin, “Cancer risk and parental pesticide application in children of Agricultural Health Study participants.,” *Environ. Health Perspect.*, vol. 112, no. 5, pp. 631–635, Apr. 2003.
[87] A. Moore and D. A. Enquobahrie, “Paternal occupational exposure to pesticides and risk of neuroblastoma among children: a meta-analysis,” *Cancer Causes Control*, vol. 22, no. 11, pp. 1529–1536, Nov. 2011.

[88] IARC, “Monograph - Tobacco smoking,” 2010.
[89] C. Hansen, I. Asmussen, and H. Autrup, “Detection of carcinogen-DNA adducts in human fetal tissues by the 32P-postlabeling procedure.,” *Environ. Health Perspect.*, vol. 99, pp. 229–31, Mar. 1993.
[90] P. Esakky and K. H. Moley, “Paternal smoking and germ cell death: A mechanistic link to the effects of cigarette smoke on spermatogenesis and possible long-term sequelae in offspring,” *Mol. Cell. Endocrinol.*, vol. 435, pp. 85–93, 2016.
[91] IARC, “IARC Monographs on the Evaluation of Carcinogenic Risks to Humans VOLUME 96 Alcohol Consumption and Ethyl Carbamate,” 2010.
[92] S. Kramer, E. Ward, A. T. Meadows, and K. E. Malone, “Medical and drug risk factors associated with neuroblastoma: a case-control study.,” *J. Natl. Cancer Inst.*, vol. 78, no. 5, pp. 797–804, May 1987.
[93] J. A. Schwartzbaum, “Influence of the mother’s prenatal drug consumption on risk of neuroblastoma in the child,” *Am. J. Epidemiol.*, vol. 135, no. 12, pp. 1358–1367, 1992.
[94] E. Müller-Schulte, G. Kurlemann, and A. Harder, “Tobacco, alcohol and illicit drugs during pregnancy and risk of neuroblastoma: Systematic review,” *Arch. Dis. Child. Fetal Neonatal Ed.*, vol. 103, no. 5, pp. F467–F473, Nov. 2018.
[95] A. J. De Roos, A. F. Olshan, K. Teschke, C. Poole, D. A. Savitz, J. Blatt, M. L. Bondy, and B. H. Pollock, “Parental occupational exposures to chemicals and incidence of neuroblastoma in offspring,” *Am. J. Epidemiol.*, vol. 154, no. 2, pp. 106–114, Jul. 2001.
[96] J. E. Heck, A. S. Park, J. Qiu, M. Cockburn, and B. Ritz, “An exploratory study of ambient air toxics exposure in pregnancy and the risk of neuroblastoma in offspring,” *Environ. Res.*, vol. 127, pp. 1–6, 2013.
[97] S. V. Kumar, P. J. Lupo, L. A. Pompeii, and H. E. Danysh, “Maternal residential proximity to major roadways and pediatric embryonal tumors in offspring,” *Int. J. Environ. Res. Public Health*, vol. 15, no. 3, 2018.
[98] AUDIPOG, “Morphométrie néonatale Poids , taille et périmètre crânien à la naissance Calcul des Z scores et des centiles,” 2008.
[99] WHO, *ICD-10 5th Edition; International Statistical Classification of Diseases and Related Health Problems- 10th Revision*. 2016.
[100] EUROCAT, “EUROCAT Guide 1.4 Section 3.2 3.2 Minor Anomalies and other conditions for Exclusion,” 2013.
[101] D. Moher, L. Shamseer, M. Clarke, D. Ghersi, A. Liberati, M. Petticrew, P. Shekelle, L. A. Stewart, M. Estarli, E. S. A. Barrera, R. Martínez-Rodríguez, E. Baladia, S. D. Agüero, S. Camacho, K. Buhring, A. Herrero-López, D. M. Gil-González, D. G. Altman, A. Booth, A. W. Chan, S. Chang, T. Clifford, K. Dickersin, M. Egger, P. C. Gøtzsche, J. M. Grimshaw, T. Groves, M. Helfand, J. Higgins, T. Lasserson, J. Lau, K. Lohr, J. McGowan, C. Mulrow, M. Norton, M. Page, M. Sampson, H. Schünemann, I. Simera, W. Summerskill, J. Tetzlaff, T. A. Trikalinos, D. Tovey, L. Turner, and E. Whitlock, “Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement,” *Rev. Esp. Nutr. Humana y Diet.*, vol. 20, no. 2, pp. 148–160, 2016.
[102] G. Wells, B. Shea, D. O’Connell, J. Peterson, V. Welch, M. Losos, and P. Tugwell, “Newcastle-Ottawa Quality Assessment Scale,” 1993.
[103] S. Moola, Z. Munn, C. Tufanaru, E. Aromataris, K. Sears, R. Sfetcu, M. Currie, R. Qureshi, P. Mattis, K. Lisy, and P.-F. Mu, “Checklist for Case Control Studies,” 2017.
[104] E. P. Stavrou, D. F. Baker, and J. F. Bishop, “Maternal smoking during pregnancy and childhood cancer in New South Wales: A record linkage investigation,” *Cancer Causes Control*, vol. 20, no. 9, pp. 1551–1558, 2009.
[105] J. E. Heck, Z. A. Contreras, A. S. Park, T. B. Davidson, M. Cockburn, and B. Ritz,

"Smoking in pregnancy and risk of cancer among young children: A population-based study," *Int. J. Cancer*, vol. 139, no. 3, pp. 613–616, 2016.
[106] T. Sorahan, R. Lancashire, P. Prior, I. Peck, and A. Stewart, "Childhood cancer and parental use of alcohol and tobacco," *Ann. Epidemiol.*, vol. 5, no. 5, pp. 354–359, 2002.
[107] D. Pang, R. McNally, and J. M. Birch, "Parental smoking and childhood cancer: Results from the United Kingdom Childhood Cancer Study," *Br. J. Cancer*, vol. 88, no. 3, pp. 373–381, 2003.
[108] Insee, "Tableaux de l'économie française. Équipement des ménages," 2011.
[109] S. Wacholder, J. K. McLaughlin, D. T. Silverman, and J. S. Mandel, "Selection of Controls in Case-Control Studies," *Am. J. Epidemiol.*, vol. 135, no. 9, pp. 1019–1028, 2017.
[110] B. Blondel and M. Kermarrec, "Enquête nationale périnatale - Les maternités en 2010 et leur évolution depuis 2003," *Bur. santé la Popul. DREES*, p. 81, 2011.
[111] J. Schuz, "Non-response bias as a likely cause of the association between young maternal age at the time of delivery and the risk of cancer in the offspring," *Paediatr. Perinat. Epidemiol.*, vol. 17, no. 1, pp. 106–112, 2003.
[112] EUROCAT, "European Surveillance of Congenital Anomalies. Number of cases and prevalence per 10,000 births of all anomalies, for all full member countries, from 1980–2012." [Online]. Available: http://www.eurocat-network.eu/accessprevalencedata/prevalencetables. [Accessed: 14-Mar-2019].
[113] J. Tort, N. Lelong, C. Prunet, B. Khoshnood, and B. Blondel, "Maternal and health care determinants of preconceptional use of folic acid supplementation in France: Results from the 2010 National Perinatal Survey," *BJOG An Int. J. Obstet. Gynaecol.*, vol. 120, no. 13, pp. 1661–1667, 2013.
[114] H. D. Bailey, B. Lacour, L. Guerrini-Rousseau, A. I. Bertozzi, P. Leblond, C. Faure-Conter, I. Pellier, C. Freycon, F. Doz, S. Puget, S. Ducassou, L. Orsi, and J. Clavel, "Parental smoking, maternal alcohol, coffee and tea consumption and the risk of childhood brain tumours: the ESTELLE and ESCALE studies (SFCE, France)," *Cancer Causes Control*, vol. 28, no. 7, pp. 719–732, 2017.
[115] C. Evin, "Bulletin épidémiologique hebdomadaire Éditorial / Editorial Augmentation récente du tabagisme en France : principaux résultats du Baromètre santé , France , 2010," 2011.
[116] D. R. Groh, J. R. Ferrari, and L. A. Jason, "Self-reports of substance abusers: The impact of social desirability on social network variables," *J. Groups Addict. Recover.*, vol. 4, no. 1–2, pp. 51–61, 2009.
[117] K. A. O'Neill, M. F. Murphy, K. J. Bunch, S. E. Puumala, S. E. Carozza, E. J. Chow, B. A. Mueller, C. C. McLaughlin, P. Reynolds, T. J. Vincent, J. Von Behren, and L. G. Spector, "Infant birthweight and risk of childhood cancer: international population-based case control studies of 40 000 cases," *Int. J. Epidemiol.*, vol. 44, no. 1, pp. 153–168, 2015.
[118] N. Rahman, "Mechanisms predisposing to childhood overgrowth and cancer," *Curr. Opin. Genet. Dev.*, vol. 15, no. 3 SPEC. ISS., pp. 227–233, 2005.
[119] J. A. Ross, L. G. Spector, L. L. Robison, and A. F. Olshan, "Epidemiology of leukemia in children with down syndrome," *Pediatr. Blood Cancer*, vol. 44, no. 1, pp. 8–12, 2005.
[120] Y. Le Bouc, I. Netchine, L. Brugieres, C. Gicquel, A. Lacoste, S. Rossignol, M.-P. Vazquez, F. Auber, F. Brioude, M. Gauthier-Villars, and G. Audry, "Beckwith-Wiedemann Syndrome: Growth Pattern and Tumor Risk according to Molecular Mechanism, and Guidelines for Tumor Surveillance," *Horm. Res. Paediatr.*, vol. 80, no. 6, pp. 457–465, 2013.

[121] S. Dawson, A. K. Charles, C. Bower, N. H. de Klerk, and E. Milne, “Risk of cancer among children with birth defects: A novel approach,” *Birth Defects Res. Part A Clin. Mol. Teratol.*, vol. 103, no. 4, pp. 284–291, Apr. 2015.
[122] D. Wellesley, P. Boyd, H. Dolk, and S. Pattenden, “An aetiological classification of birth defects for epidemiological research,” *J. Med. Genet.*, vol. 42, no. 1, pp. 54–57, 2005.
[123] Y. I. Goh, E. Bollano, T. R. Einarson, and G. Koren, “Prenatal multivitamin supplementation and rates of pediatric cancers: A meta-analysis,” *Clinical Pharmacology and Therapeutics*, vol. 81, no. 5. pp. 685–691, 21-May-2007.
[124] C. L. Williams, K. J. Bunch, C. A. Stiller, M. F. Murphy, B. J. Botting, W. Hamish Wallace, M. Davies, and A. G. Sutcliffe, “Cancer Risk among Children Born after Assisted Conception,” *N Engl J Med*, vol. 19369, no. 7, pp. 1819–27, 2013.
[125] M. Hargreave, A. Jensen, T. S. S. Nielsen, E. P. Colov, K. K. Andersen, A. Pinborg, and S. K. Kjaer, “Maternal use of fertility drugs and risk of cancer in children - A nationwide population-based cohort study in Denmark,” *Int. J. Cancer*, vol. 136, no. 8, pp. 1931–1939, Apr. 2015.
[126] M. Hargreave, A. Jensen, A. Toender, K. K. Andersen, and S. K. Kjaer, “Fertility treatment and childhood cancer risk: A systematic meta-analysis,” *Fertil. Steril.*, vol. 100, no. 1, pp. 150–161, 2013.
[127] R. M. Martin, D. Gunnell, C. G. Owen, and G. D. Smith, “Breast-feeding and childhood cancer: A systematic review with metaanalysis,” *Int. J. Cancer*, vol. 117, no. 6, pp. 1020–1031, 2005.
[128] M. F. Greaves, “Aetiology of acute leukaemia.,” *Lancet (London, England)*, vol. 349, no. 9048, pp. 344–9, Feb. 1997.
[129] M. Alsaweed, C. T. Lai, P. E. Hartmann, D. T. Geddes, and F. Kakulas, “Human milk miRNAs primarily originate from the mammary gland resulting in unique miRNA profiles of fractionated milk,” *Sci. Rep.*, vol. 6, 2016.
[130] N. L. Carlsen, “Neuroblastomas presenting in the first year of life: epidemiological differences from those presenting at older ages.,” *Cancer Detect. Prev.*, vol. 20, no. 3, pp. 251–61, 1996.
[131] C. Chevrier, C. Petit, G. Limon, C. Monfort, G. Durand, and S. Cordier, “Biomarqueurs urinaires d’exposition aux pesticides des femmes enceintes de la cohorte Pélagie réalisée en),” 2002.
[132] N. C. Deziel, J. S. Colt, E. E. Kent, R. B. Gunier, P. Reynolds, B. Booth, C. Metayer, and M. H. Ward, “Associations between self-reported pest treatments and pesticide concentrations in carpet dust,” *Environ. Heal. A Glob. Access Sci. Source*, vol. 14, no. 1, 2015.
[133] E. M. Ostrea, D. M. Bielawski, N. C. Posecion, M. Corrion, E. Villanueva-Uy, R. C. Bernardo, Y. Jin, J. J. Janisse, and J. W. Ager, “Combined analysis of prenatal (maternal hair and blood) and neonatal (infant hair, cord blood and meconium) matrices to detect fetal exposure to environmental pesticides,” *Environ. Res.*, vol. 109, no. 1, pp. 116–122, Jan. 2009.
[134] R. C. Lewis, D. E. Cantonwine, L. V. A. Del Toro, A. M. Calafat, L. Valentin-Blasini, M. D. Davis, S. E. Baker, A. N. Alshawabkeh, J. F. Cordero, and J. D. Meeker, “Urinary biomarkers of exposure to insecticides, herbicides, and one insect repellent among pregnant women in Puerto Rico,” *Environ. Heal. A Glob. Access Sci. Source*, vol. 13, no. 1, p. 97, Dec. 2014.
[135] M. Bjørling-Poulsen, H. R. Andersen, and P. Grandjean, “Potential developmental neurotoxicity of pesticides used in Europe,” *Environmental Health: A Global Access Science Source*, vol. 7. p. 50, 2008.

[136] G. N., W. M.H., G. R., C. J.S., S. L. C., B. P.A., and M. C., "Characterization of residential pesticide use and chemical formulations through self-report and household inventory: The northern California childhood leukemia study," *Environ. Health Perspect.*, vol. 121, no. 2, pp. 276–282, 2013.
[137] Q. Wang, S. Horel, B. Li, S. E. Carozza, and S. Cooper, "Agricultural pesticides and risk of childhood cancers," *Int. J. Hyg. Environ. Health*, vol. 212, no. 2, pp. 186–195, 2008.
[138] F. Vinson, M. Merhi, I. Baldi, H. Raynal, and L. Gamet-Payrastre, "Exposure to pesticides and risk of childhood cancer: A meta-analysis of recent epidemiological studies," *Occupational and Environmental Medicine*, vol. 68, no. 9. pp. 694–702, Sep-2011.
[139] P. Chu, Y. Guo, J. Shi, J. Lu, X. Ni, H. Wang, W. Han, S. Han, and Y. Jin, "Maternal smoking during pregnancy and risk of childhood neuroblastoma: Systematic review and meta-analysis," *J. Cancer Res. Ther.*, vol. 12, no. 2, p. 999, 2016.
[140] M. A. Beal, C. L. Yauk, and F. Marchetti, "From sperm to offspring: Assessing the heritable genetic consequences of paternal smoking and potential public health impacts," *Mutation Research - Reviews in Mutation Research*, vol. 773. pp. 26–50, 2017.
[141] B. Coulm, C. Bonnet, and B. Blondel, "Enquête nationale périnatale Rapport 2016 Les naissances et les établissements Situation et évolution depuis 2010 Rapport rédigé par l'INSERM et la DREES Enquête réalisée avec la participation des services départementaux de Protection maternelle et infant," 2017.
[142] E. McKenna, A. Hure, A. Perkins, and E. Gresham, "Dietary supplement use during preconception: The Australian longitudinal study on women's health," *Nutrients*, vol. 9, no. 10, pp. 1–12, 2017.
[143] Inpes, "Folates et désir de grossesse : informer et prescrire au bon moment. Les essentiels de l'Inpes."
[144] C. Bower, M. Miller, J. Payne, and P. Serna, "Promotion of folate for the prevention of neural tube defects : Knowledge and ...," *Paediatr. Perinat. Epidemiol.*, pp. 435–444, 2005.
[145] J.-N. Aubertot, J.-M. Barbier, A. Carpentier, J.-J. Gril, L. Guichard, P. Lucas, S. Savary, I. Savini, and M. Voltz, "Expertise scientifique collective INRA-Cemagref Pesticides, agriculture et environnement Réduire l'utilisation des pesticides et en limiter les impacts environnementaux Rapport d'expertise," 2005.
[146] FOOD A N D AGRICULTURE ORGANIZATION O F T H E UNITED NATIONS, "International Code of Conducton the Distribution and Use of Pesticides. Adopted by the Hundred and Twenty-third Session of the FAO Council in November 2002.," Food and Agriculture Organization of the United Nations, 2005.
[147] C. Petit, M. Blangiardo, S. Richardson, and S. Cordier, "Original Contribution Association of Environmental Insecticide Exposure and Fetal Growth With a Bayesian Model Including Multiple Exposure Sources The PELAGIE Mother-Child Cohort."
[148] F. Mayhoub, T. Berton, V. Ronique Bach, K. Tack, C. Deguines, A. Floch-Barneaud, S. Desmots, E. Sté Phan-Blanchard, and K. Chardon, "Self-Reported Parental Exposure to Pesticide during Pregnancy and Birth Outcomes: The MecoExpo Cohort Study," 2014.
[149] ANSES, "Étude Pesti'home," 2008.

7 Supplementary material

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Risk of neuroblastoma, birth-related characteristics, congenital malformations and perinatal exposures: A pooled analysis of the ESCALE and ESTELLE French studies (SFCE)

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Cancer Epidemiology

Neuroblastoma (NB), an embryonic tumour arising from neural crest cells, is the most common malignancy among infants. The aetiology of NB is largely unknown. We conducted a pooled analysis to explore whether there is an association between NB and preconception and perinatal factors using data from two French national population-based case-control studies. The mothers of 357 NB cases and 1783 controls younger than 6 years, frequency-matched by age and gender, responded to a telephone interview that focused on demographic, socioeconomic and perinatal characteristics, childhood environment, life-style and maternal reproductive history. Unconditional logistic regression was used to estimate pooled odds ratios and 95% confidence intervals. After controlling for matching variables, study of origin and potential confounders, being born either small (OR 1.4 95% CI 1.0-2.0) or large (OR 1.5 95% CI 1.1–2.2) for gestational age and, among children younger than 18 months, having congenital malformations (OR 3.6 95% CI 1.3–8.9), were significantly associated with NB. Inverse associations were observed with breastfeeding (OR 0.7 95% CI 0.5–1.0) and maternal use of any supplements containing folic acid, vitamins or minerals (OR 0.5 95% CI 0.3–0.9) during the preconception period. Our findings reinforce the hypothesis that fetal growth anomalies and congenital malformations may be associated with an increased risk of NB. Further investigations are needed in order to clarify the role of folic acid supplementation and breastfeeding, given their potential importance in NB prevention.

Key words: childhood cancer, neuroblastoma, fetal growth, congenital malformations, risk factors

Abbreviations: AGA: Appropriate for Gestational Age; CI: Confidence Interval; EUROCAT: European Surveillance of Congenital Anomalies; ESCALE: Etude Sur les Cancers et les Leucémies de l'Enfant; ESTELLE: Etude Sur les Tumeurs Embryonnaires, Leucémies et Lymphomes de l'Enfant; ICD-10: International Classification of Diseases 10th Revision; LGA: Large for Gestational Age; NB: Neuroblastoma; MYCN: N-myc proto-oncogene; OR: Odds Ratio; RNCE: Registre National des Cancers de l'Enfant; SGA: Small for Gestational Age; SFCE: Société Française de lutte contre les Cancers de l'Enfant et de l'Adolescent

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What's new?

Neuroblastoma is the most common extracranial solid tumor in children, yet little is known about its etiology. The vast majority of neuroblastomas are not inherited, suggesting that neuroblastoma risk is influenced by other factors, particularly certain preconception or perinatal factors. Here, in a population-based case-control study of 357 neuroblastoma patients, congenital malformations and small or large size for gestational age were associated with increased neuroblastoma risk. By contrast, breastfeeding and preconception supplementation with vitamins or minerals were inversely associated with risk. The findings confirm previous links between neuroblastoma, abnormal fetal growth, and congenital malformations while highlighting potential protective factors.

Introduction

Neuroblastoma (NB) is the most common extra-cranial solid tumour in childhood. Every year, 140–150 cases aged 15 years or younger are diagnosed in France.[1] NB is the most frequently diagnosed neoplasm during infancy, 40% of cases are diagnosed before the age of 1 year and 85% before the age of 5 years.[2]

NB is a malignant embryonal tumour of the neural crest cells. The neural crest develops early during embryonal development and differentiates to create the sympathetic nervous system. The trademark of NB is its clinical heterogeneity, characterised by contrasting patterns of clinical behaviour. The tumour may spontaneously regress in some patients while progressing in others, despite intensive multimodality therapy.[3] The risk assessment algorithm combines clinical variables such as age at diagnosis, with specific biologic variables as histological category and the status of N-myc proto-oncogene (MYCN). MYCN amplification is a genetic aberration that occurs in about 20% of primary tumours and it is one of the strongest independent adverse prognostic factors.[4] Some predictor factors such as MYCN status and age at diagnosis may have aetiological relevance since some studies have found that some exposures in pregnancy were more strongly related to NB at an earlier age of diagnosis.[5–7]

The aetiology of NB is largely unknown. Familial NB is rare and accounts for only approximately 1% of all NB cases.[8] Genetic predisposition cannot fully explain the origin of NB. The embryonic nature of NB and the early age at diagnosis suggest a role of intrauterine and neonatal factors. Some previous studies have focused on newborn characteristics like gestational age[6,7,9–11] and birth weight, but the results are inconsistent and most of them do not distinguish between the effects of birth weight *per se*,[11–13] and rate of fetal growth.[6,14–18] Several co-occurring congenital conditions have been described: Beckwith-Wiedemann syndrome, Von Recklinghausen syndrome and Hirschsprung's disease. In addition, some studies have reported a positive association with congenital malformations,[10,15,19] while maternal use of folic acid, vitamins or minerals during pregnancy[20–22] and breastfeeding[23,24] may potentially be protective.

We evaluated whether there was an association between maternal and neonatal characteristics and the risk of NB, based on two population-based case-control studies: ESCALE and ESTELLE. The ESCALE study, conducted by our group in 2003–2004, reported a positive association with congenital malformations.[25] Because the numbers were limited, the ESTELLE study, conducted in 2010–2011, was designed for pooling with the ESCALE study in order to increase the power for the investigation of gestational age, fetal growth, congenital malformations, maternal use of fertility treatments, vitamin, mineral and folic acid supplementation in the periconceptional period, and breastfeeding, which were the focus of this paper.

Material and Methods

Study population

The ESCALE[26] and ESTELLE[27] studies were two nationwide population-based case-control studies using the French National registry of childhood cancers (RNCE), and supported by the Société Française de lutte contre les Cancers de l'Enfant et de l'Adolescent (SFCE).

The cases were children aged <15 years and residing in mainland France at the time they were newly diagnosed with cancer. The ESCALE study included cases of NB, leukaemia, lymphoma and malignant brain tumour diagnosed in 2003–2004, while the ESTELLE study included cases of NB, leukaemia, lymphoma, malignant brain tumour, Wilm's tumour and hepatoblastoma diagnosed in 2010–2011. The present paper focuses on NB in children under 6 years old.

In both studies, the cases were ineligible if they had been adopted or if their biological mother had died, was absent or did not speak French ($n = 21$), had a serious social problem or psychiatric disorder ($n = 5$), or could not be interviewed for ethical reasons because the child was in palliative care or had died ($n = 22$). Information on the MYCN amplification subtype was obtained subsequently from the RNCE. During the two study periods, 500 cases of NB were diagnosed, 276 in 2003–2004 and 224 in 2010–2011. The present analysis was limited to 357 cases aged less than six years (91.3% of included cases). Overall case participation rates were 81.2% for ESCALE and 92.2% for ESTELLE (Fig. 1).

The population controls were children free from cancer selected contemporaneously using quota-sampling methods. Quotas were used to obtain, overall, at least one control per case in ESCALE and at least one control per case for each year of age, gender, and type of cancer in ESTELLE, based on the expected numbers derived from the RNCE. In both studies, the quotas also ensured that the control group had the same distribution as the overall population for the number of children aged <15 years living in the household, conditionally on age. In the same way as for the cases, the children who had been adopted, or whose biological mother had died or did not speak

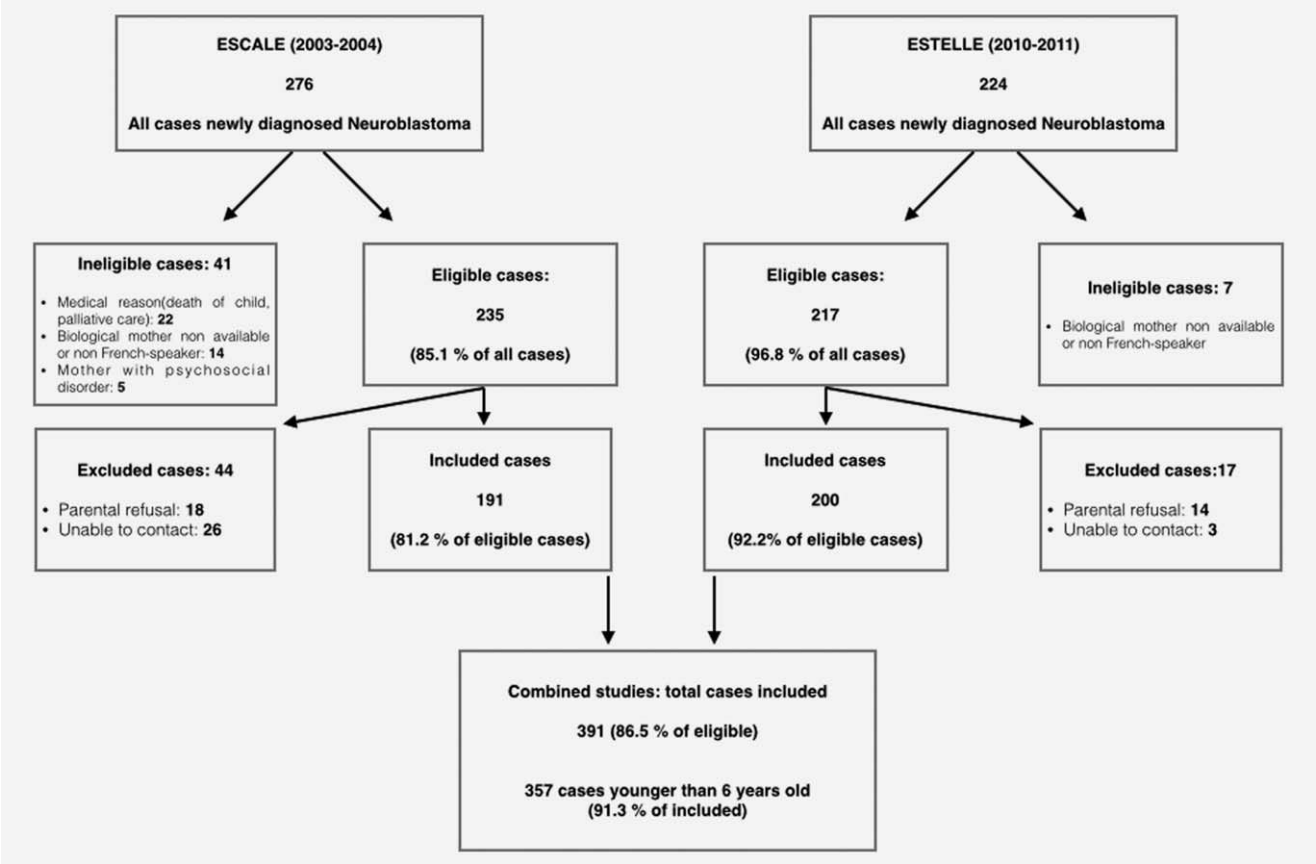

Figure 1. Case participation

French were not eligible as controls. The procedure for control sampling was slightly different for the two studies: in the ESCALE study, a base of 60,000 phone numbers was randomly extracted from the national telephone directory. The set was representative of the population in terms of the administrative regions and urbanization. By incrementing each number by 1, a new set of 60,000 numbers was generated. The new set included unlisted numbers and had geographic and demographic distributions similar to those of the initial set (same first six digits, which indicate the location of the line). In the ESTELLE study, telephone numbers were randomly generated and dialled over the 2-year subject recruitment period. The participation rates were 71.2% and 85.5%, respectively. The present analyses were limited to 1783 controls aged <6 years at the reference date (57.5% of those recruited). Figure 2 shows the steps in the control recruitment process in the ESCALE and ESTELLE studies.

Data Collection

Case and control mothers were interviewed using a standardized telephone interview conducted by trained interviewers and lasting ~50 min. In both studies, the interview included questions on demographic and socioeconomic characteristics, childhood environment and life-style, familial and personal medical history and parental occupational history.

Mothers were asked about their age at the index child's birth, the parity of that pregnancy, if they had difficulty becoming pregnant and if they took vitamin, mineral or folic acid supplements three months before pregnancy or in the 1st, 2nd or 3rd trimester of pregnancy. In ESTELLE, the mothers were also asked the name of the supplement. Gestational age and birth weight of the index children, breastfeeding duration, and whether the child was diagnosed with a congenital malformation and, if so, details of the type and site of malformations were also collected. As a memory aid, the mothers were advised to consult the relevant pages of the child's personal health record during the interview.

Data Management

Difficulty becoming pregnant was defined as taking more than one year to conceive the index child and/or the need to consult a doctor and/or the need for the mother or father to undergo fertility treatment. In the latter case, the mother was asked to specify the type of treatment. The use of vitamin, mineral or folic acid supplements was considered as a binary variable (ever/never), and by trimester from three months before pregnancy to birth, taking as reference category the absence of supplementation in any of those periods. We also used a more specific classification, defining 'substantiated' folic acid supplementation as supplementation reported for

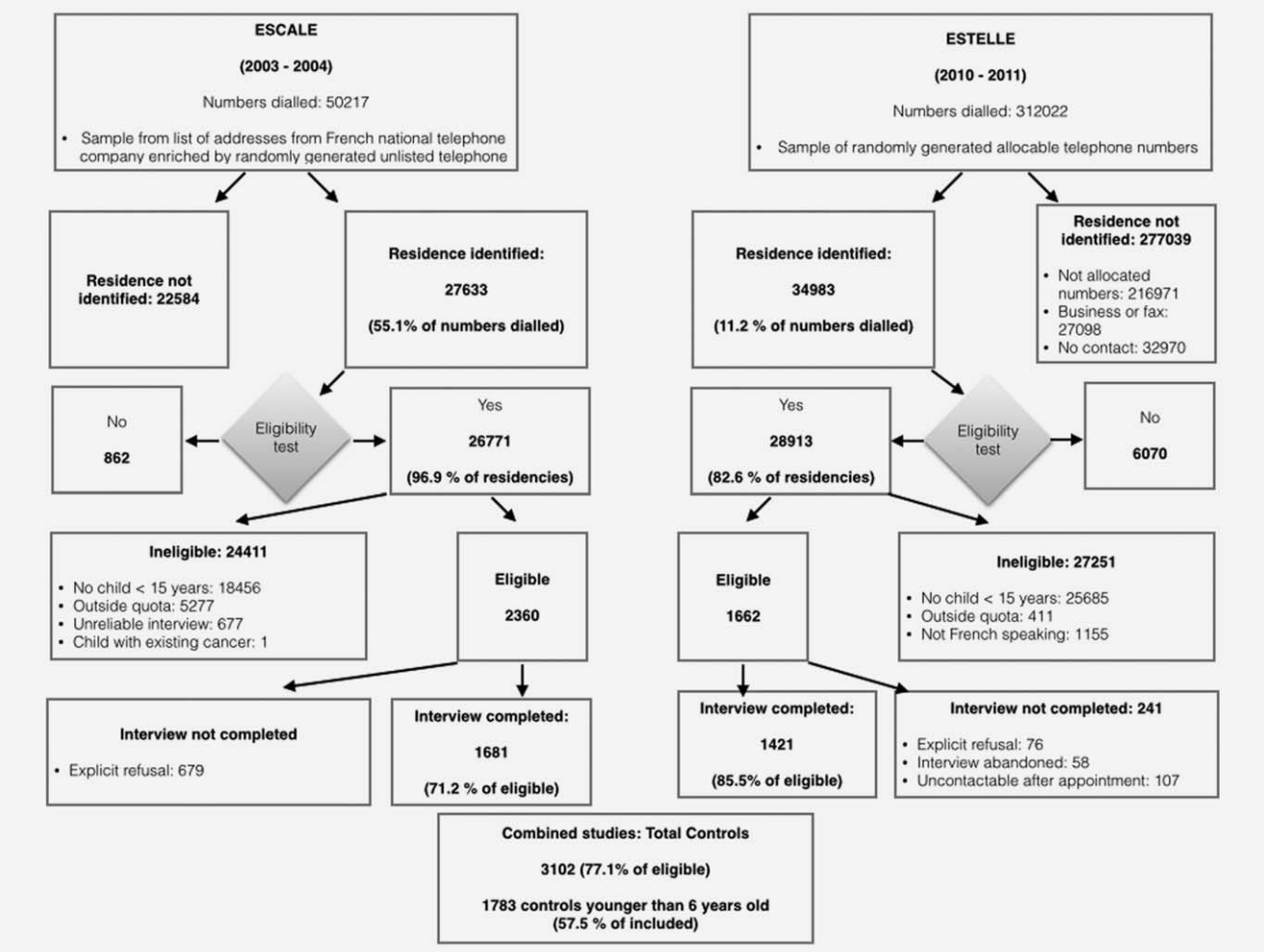

Figure 2. Control recruitment

the relevant time period with a valid product name, and 'unsubstantiated' supplementation as supplementation reported for the relevant time period but with no valid name given.

Gestational age was classified as: pre-term (<37 complete weeks of pregnancy), post-term (42 weeks or more) and two at-term categories (37–39 and 40–41 weeks). Birth weight was classified with cut-offs in grams (<2,500, 2,500–2,999, 3,000–3,499, 3,500–3,999, ≥4,000) comparable with those used by the French national perinatal surveys.[28] Fetal growth was estimated by using the birth weight *Z*-score based on gender-specific percentiles for birth weight by gestational age in weeks from a large French cohort.[29] The categories were defined using classical *Z*-score cut-offs, *i.e.* from the 10th through the 90th percentile for the category appropriate for gestational age (AGA), <10th percentile for small for gestational age (SGA) and >90th percentile for large for gestational age (LGA).

The congenital malformations were coded using the International Classification of Diseases, 10th Revision (ICD-10),[30] with the coder blind to case-control status. Then, children with only minor anomalies or unspecified anomalies were excluded in accordance with the European Surveillance of Congenital Anomalies (EUROCAT) recommendations.[31]

Breastfeeding was defined as having been breastfed for at least three days after birth and ever breastfed children were also categorized by duration (<3 months, 3–5 months and 6 months or more).

Statistical Analyses

Study-specific odds ratios (OR) and pooled OR and their 95% confidence intervals (95% CI) were estimated by unconditional logistic regression (STATA version 11, StatCorp LP, College Station TX). All the models included the stratification variables: child's age and gender, and, for the pooled analyses, the indicator of study of origin. To avoid possible residual confounding by age and because of the particular age distribution of NB, the first year of age was split into trimesters (< 3 months, 3–5 months, 6–8 months, 9–11 months), the second year into semesters (12–17 months, 18–23 months), and the other ages were kept as single years (2, 3, 4 and 5 years).

Between-study heterogeneity was tested by regression models with and without an interaction term between specific study and exposure. Because the findings of the individual studies were similar, only the results of the pooled analysis are presented herein.

The following variables were considered *a priori* to be potential confounders and were tested to determine whether they met the empirical definition of confounding (independent association with both the exposure and outcome): paternal and maternal age at the child's birth, maternal level of education, birth-order and degree of urbanization of the place of residence. Of these, maternal age, birth-order and degree of urbanization of the place of residence were retained in all the models.

In order to properly address breastfeeding in relation to the development of NB, analyses were limited to children aged six months or older at diagnosis or at the reference date for controls, so that the cases and controls would have had the opportunity of being breastfed for up to six months.

At the time of the ESCALE study, the frequency of folic acid supplementation in France was very low. Therefore, we only presented the data from the ESTELLE study in this paper.

Additional analyses were conducted by subgroups of NB defined by age at diagnosis (age <18 months and ≥ 18 months) and MYCN oncogene amplification status (MYCN amplified and MYCN not amplified).

Since controls were recruited from a sample of landline phone numbers, sensitivity analyses were performed excluding case mothers with no landline at home (restricted to ESTELLE as ESCALE cases were not asked this question). Finally, multiple births and children born following *in vitro* fertilization were excluded from the analysis of fetal growth and folic acid supplementation, in order to control for possible confounding by the use of fertility treatments.

Results

Overall, 357 NB cases (174 in ESCALE, 183 in ESTELLE) and 1783 controls (949 in ESCALE and 834 in ESTELLE) under six years of age were included in the pooled analysis. Table 1 shows the main characteristics of all participants by individual study and for the pooled sample. The MYCN oncogene was amplified in 64 cases (18%), not amplified in 270 cases (76%) and non-informative (NI) in 23 cases (6%). In both studies the cases and the controls were generally similar, except that the cases were more likely to be younger, be first born, to have younger mothers and more likely to live in an urban area than the controls. Because the sampling was performed to enable analyses of multiple childhood cancer diagnoses, the control group had the same age distribution as the whole ESCALE and ESTELLE case population. There were at least two controls for each case in each age stratum.

There was no association between NB and difficulty becoming pregnant or use of fertility treatments for the index pregnancy (Table 2). There was no significant association between gestational age or birth weight and NB. SGA and LGA were both associated with NB (ORs 1.4 95% CI 1.0–2.0 and 1.5 95% CI 1.1–2.2, respectively). The associations were specially marked for children younger than 18 months (ORs 1.7 95% CI 1.0–3.1 and 2.0 95% CI 1.2–3.3, respectively).

Overall, congenital malformations were reported slightly more often for cases (4%) than for controls (3%), and the association tended to be more marked, although based on small numbers, when the child had two or more malformations (OR 6.3 95% CI 1.3–29). The most frequently reported sites of malformations were the skeletal and genitourinary systems. Lastly, the association between malformations and NB was only observed in children aged <18 months (OR 3.6 95% CI 1.3–8.9). Results were similar when the analysis by subgroups defined by case MYCN amplification status was performed (results not shown).

The breastfeeding analysis was restricted to the 1,589 controls and 269 cases who were aged 6 months or older (Table 3). The cases were breastfed less often than the controls (OR 0.7 95% CI 0.5–1.0) but there was no evidence of a decrease in risk with increasing breastfeeding duration. The inverse trend was significant when the association was restricted to the cases with MYCN amplification (p values for trend < 0.01).

There was an inverse association with maternal use of any supplement containing folic acid, vitamins or minerals in the three months before conception (OR 0.5 95% CI 0.3–0.9; Table 4). There was little change when the analysis was restricted to substantiated supplements containing folic acid (OR 0.4 95% CI 0.2–1.1). There was no association with the use of supplements during the other trimesters of pregnancy (Table 4). The analysis of subgroups defined by age at diagnosis (dichotomised at 18 months) and by case MYCN amplification status showed only minor heterogeneity of effect (results not shown).

The results remained unchanged after exclusion of the ESTELLE cases with no telephone landline or after exclusion of multiple births and children born following *in vitro* fertilization (results not shown).

Discussion

The main findings of this study consist in the positive associations with being born either small or large for gestational age, the positive association with having a congenital malformation, and the inverse associations with breastfeeding and maternal use of supplements containing folic acid, vitamins or minerals in the preconception period. No association with difficulty becoming pregnant or with the use of fertility treatments was observed.

Harder et al[32] conducted a meta-analysis of 11 studies and reported that a birth weight $> 4{,}000$ g was consistently associated with an increased risk of NB across studies. In addition, six of the included studies[6,14–18] also analyzed fetal growth, with four studies reporting associations with fetal growth anomalies, which is in line with our findings. The

Table 1. Distribution of the cases and controls by sociodemographic characteristics and by study

	Cases						Controls							
	ESCALE (*n* = 174)		ESTELLE (*n* = 183)		TOTAL (*n* = 357)		ESCALE (*n* = 949)		ESTELLE (*n* = 834)		TOTAL (*n* = 1783)			
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*p*	Controls per case
MYCN status														
Amplified	34	*20*	30	*16*	64	*18*								
Non amplified	131	*75*	139	*76*	270	*76*								
Missing or unspecified	9	*5*	14	*8*	23	*6*								
Age (years)													*< 0.01*	
<1	74	*42*	74	*40*	148	*41*	187	*20*	196	*24*	383	*21*		2.6
1	36	*21*	33	*18*	69	*19*	182	*19*	122	*15*	304	*17*		4.4
2	30	*17*	36	*20*	66	*19*	153	*16*	145	*17*	298	*17*		4.5
3-5	34	*20*	40	*22*	74	*21*	427	*45*	371	*44*	798	*45*		10.7
Sex													*0.4*	
Male	86	*49*	100	*55*	186	*52*	520	*55*	446	*53*	966	*54*		5.2
Female	88	*51*	83	*45*	171	*48*	429	*45*	388	*47*	817	*46*		4.8
Birth-order													*0.08*	
Firstborn	89	*51*	91	*50*	180	*50*	402	*42*	345	*41*	747	*42*		4.1
Second or more	85	*49*	92	*50*	177	*50*	547	*58*	489	*59*	1036	*58*		5.8
Maternal age at index birth (years)													*<0.01*	
<25	31	*18*	25	*14*	56	*16*	80	*8*	86	*10*	166	*9*		2.9
25-29	61	*35*	70	*38*	131	*37*	314	*33*	250	*30*	564	*32*		4.3
30-34	54	*31*	50	*27*	104	*29*	360	*38*	293	*35*	653	*37*		6.3
≥ 35	28	*16*	38	*21*	66	*18*	195	*20*	205	*25*	400	*22*		6.1
Maternal education													*0.6*	
Less than secondary	55	32	50	27	105	29	319	33	203	24	522	29		5.0
Secondary	32	*18*	44	*24*	76	*21*	195	*21*	192	*23*	387	*22*		5.1
Tertiary	87	*50*	88	*48*	175	*49*	435	*46*	439	*53*	874	*49*		5.0
Missing			1	*0.5*	1	*0.3*								
Urban status of the area of residence (inhabitants)													*0.05*	
< 5,000	57	*33*	59	*32*	116	*33*	360	*38*	351	*42*	711	*40*		6.1
5,000-100,000	36	*21*	43	*23*	79	*22*	211	*22*	174	*21*	385	*22*		4.9
100,000-2,000,000	41	*23*	50	*27*	91	*25*	233	*25*	158	*19*	391	*22*		4.3
>2,000,000 (Paris)	37	*21*	30	*16*	67	*19*	145	*15*	149	*18*	294	*16*		4.4
Missing	3	*2*	1	*0.5*	4	*1*	-	-	2	*0.2*	2	*0.1*		

Table 2. Maternal reproductive history, birth-related characteristics, congenital malformations and risk of neuroblastoma. Pooled analysis of the ESCALE and ESTELLE studies

	All neuroblastomas							Age <18 months							Age ≥18 months						
	Controls *n* = 1783		Cases *n* = 357					Controls *n* = 544		Cases *n* = 188					Controls *n* = 1239		Cases *n* = 169				
	n	%	n	%	OR[1]	95%CI	*p-value*	n	%	n	%	OR[1]	95%CI	*p-value*	n	%	n	%	OR[1]	95%CI	*p-value*
Difficulty to get pregnant							*0.7*							*0.5*							*0.8*
No	1498	*84*	299	*84*	1.0	Ref		446	*82*	158	*84*	1.0	Ref		1052	*85*	141	*83*	1.0	Ref	
Yes	285	*16*	58	*16*	1.0	[0.6-1.3]		98	*18*	30	*16*	0.8	[0.5-1.3]		187	*15*	28	*17*	1.1	[0.7-1.7]	
Use of fertility treatment for the index child							*0.8*							*0.5*							*0.3*
No	1669	*94*	335	*94*	1.0	Ref		512	*94*	175	*93*	1.0	Ref		1157	93	160	*95*	1.0	Ref	
Yes	114	*6*	22	*6*	1.0	[0.6-1.6]		32	*6*	13	*7*	1.2	[0.6-2.4]		82	*7*	9	*5*	0.8	[0.4-1.3]	
Type of fertility treatment																					
No	1669	*94*	335	*94*	1.0	Ref		512	*94*	175	*93*	1.0	Ref		1157	93	160	*95*	1.0	Ref	
Stimulation only	47	*3*	12	*3*	1.2	[0.6-2.3]	*0.7*	13	*2*	8	4	1.7	[0.7-4.4]	*0.3*	34	3	4	*2*	0.8	[0.3-2.3]	*0.6*
In vitro fertilisation	33	*2*	4	*1*	0.6	[0.2-1.9]	*0.3*	11	*2*	2	1	0.5	[0.1-2.2]	*0.3*	22	2	2	*1*	0.7	[0.2-3.2]	*0.7*
Artificial insemination	15	*0.8*	1	*0.3*	0.4	[0.1-3.4]	*0.4*	2	*0.4*	1	0.5	1.7	[0.2-21]	*0.6*	13	1	-	-	-	-	-
Another technique	16	*0.9*	5	*1*	1.2	[0.4-3.7]	*0.7*	5	*0.9*	2	1	1.2	[0.3-6.9]	*0.8*	11	1	3	*2*	1.5	[0.5-5.7]	*0.9*
Missing	3	*0.2*						1	*0.2*						2	0.2					
Gestational age (weeks)							*0.2*							*0.2*							*0.7*
<37	126	*7*	32	*9*	1.2	[0.8-1.9]		39	*7*	17	*9*	1.0	[0.5-1.9]		87	*7*	15	*9*	1.4	[0.7-2.5]	
37-39	652	*37*	136	*38*	1.0	Ref		217	*40*	79	*42*	1.0	Ref		435	*35*	57	*34*	1.0	Ref	
40-41	942	*53*	176	*49*	0.9	[0.7-1.2]		270	*50*	89	*47*	0.8	[0.6-1.2]		672	*54*	87	*51*	1.1	[0.7-1.5]	
≥ 42	35	*2*	3	*1*	0.4	[0.1-1.3]		12	*2*	1	*0.5*	0.2	[0.1-1.4]		23	*2*	2	*1*	0.7	[0.1-3.0]	
Missing	28	*1*	10	*3*	-	-		6	*1*	2	*1*	-	-		22	*2*	8	*5*	-	-	
Birth-weight (grams)							*0.4*							*0.2*							*0.8*
<2500	105	*6*	25	*7*	1.2	[0.9-1.7]		31	*6*	11	*6*	1.3	[0.6-2.9]		74	*6*	14	*8*	1.4	[0.7-2.6]	
2500-2999	342	*19*	73	*20*	1.3	[0.8-2.2]		102	*19*	45	*24*	1.6	[1.0-2.6]		240	*19*	28	*17*	0.9	[0.6-1.5]	
3000-3499	683	*38*	123	*34*	1.0	Ref		210	*38*	59	*31*	1.0	Ref		473	*38*	64	*38*	1.0	Ref	
3500-3999	494	*28*	95	*27*	1.1	[0.8-1.5]		143	*26*	46	*25*	1.3	[0.8-2.1]		351	*28*	49	*29*	1.0	[0.6-1.5]	
≥ 4000	159	*9*	40	*11*	1.4	[0.9-2.2]		58	*11*	26	*14*	1.8	[1.0-3.2]		101	*8*	14	*8*	1.1	[0.6-2.1]	
Missing			1	*0.3*	-					1	*0.5*										
Fetal growth							*0.02*							*< 0.001*							*0.6*
Small for gestational age	200	*11*	46	*13*	1.4	[1.0-2.0]		47	*9*	25	*13*	1.7	[1.0-3.1]		153	*12*	21	*12*	1.2	[0.7-2.0]	
Appropriate for gestational age	1366	*77*	249	*70*	1.0	Ref		430	*79*	128	*68*	1.0	Ref		936	*75*	121	*72*	1.0	Ref	

Table 2. Maternal reproductive history, birth-related characteristics, congenital malformations and risk of neuroblastoma. Pooled analysis of the ESCALE and ESTELLE studies (Continued)

	All neuroblastomas							Age <18 months							Age ≥18 months						
	Controls *n* = 1783		Cases *n* = 357					Controls *n* = 544		Cases *n* = 188					Controls *n* = 1239		Cases *n* = 169				
	n	%	n	%	OR[1]	95%CI	*p-value*	n	%	n	%	OR[1]	95%CI	*p-value*	n	%	n	%	OR[1]	95%CI	*p-value*
Large for gestational age	189	*11*	51	*14*	1.5	[1.1-2.2]		61	*11*	32	*17*	2.0	[1.2-3.3]		128	*10*	19	*11*	1.1	[0.7-2.0]	
Missing	28	*1*	11	*3*	-	-		6	*1*	3	*2*	-	-		22	*2*	8	*5*	-	-	
Malformations							*0.2*							*0.01*							*0.6*
No	1736	*97*	344	*96*	1.0	Ref		535	*98*	179	*95*	1.0	Ref		1201	*97*	165	*98*	1.0	Ref	
Any	47	*3*	13	*4*	1.6	[0.8-3.0]		*9*	*2*	*9*	*5*	3.6	[1.3-8.9]		38	*3*	4	*2*	0.8	[0.3-2.3]	
Number of malformations							*0.1*							-							*0.2*
No	1736	*97*	344	*96*	1.0	Ref		535	*98*	179	*95*	1.0	Ref		1201	*97*	165	*98*	1.0	Ref	
1	43	*2*	10	*3*	1.3	[0.6-2.6]		*9*	*2*	8	*4*	2.9	[1.1-7.9]		34	*3*	2	*1*	0.4	[0.1-1.8]	
2+[2]	4	*0.2*	3	*0.8*	6.3	[1.3-29]		-	-	1	*0.5*	-	-		4	*0.3*	2	*1*	4.1	[0.7-23.3]	
Type of malformation[3]																					
Cardiovascular	13	*0.7*	1	*0.3*	[4]																
Digestive	1	*0.1*	1	*0.3*	[4]																
Genitourinary	15	*0.8*	6	*1.7*	2.2	[0.8-6.0]	*0.3*														
Skeleton	14	*0.8*	3	*0.9*	[4]																
Central nervous system	0	-	1	*0.3*	[4]																
Head and neck	4	*0.2*	1	*0.3*	[4]																

[1]Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by logistic regression models adjusted for age and sex, birth-order, maternal age, urban status of the area of residence and study
[2]1 case with hemihypertrophy + hepatomegaly + fetal macrosomia; 1 case with ventricular septum defect + auricular septum defect + bilateral ovarian hernia; 1 case with lumbar myelomeningocele + malformations of both hands; 1 control with Waardenburg syndrome; 1 control with aortic malformation + syndactyly + congenital scoliosis; 1 control with a Charge syndrome; 1 control with a Noonan syndrome
[3]The reference category were children with no malformation
[4]Too few cases to fit model.

association with high birth weight was also reported by a large recent meta-analysis of data from the UK and USA.[33] In this study, the odds ratios were slightly higher when gestational age was considered, suggesting that the risk of cancer may be principally related to fetal growth, rather that birth weight *per se*. The biological hypothesis regarding the mechanisms underlying associations between fetal growth anomalies and NB risk remains speculative. The association with fetal overgrowth may be related to the observation that embryonic tumours like NB are more frequent among children with overgrowth disorders, such as Beckwith-Wiedemann syndrome.[34] The syndrome is caused by overexpression of the gene for insulin-like growth factor IGF2, which is suspected to play a role in the development of several childhood malignancies.[35] However, the overexpression of IGF 2 would not explain the association with low birth weight that we and others[14,18] have observed. In the meta-analysis, Harder *et al.*[32] suggested that the observed association with low birth weight might be related to recall bias, since studies using interview data reported stronger associations than those using registries as the data source.

We found a positive association between congenital malformations and NB among children aged <18 months. This finding is consistent with previous publications.[10,15,19,36,37] Over diagnosis of malformations among NB cases or a differential recall bias in which case mothers are more likely to remember minor defects than control mothers, may introduce differential classification bias in case-control studies. In order to limit this potential bias, we have excluded minor anomalies that are not always truly congenital in origin, sometimes associated with immaturity at birth, and have lesser medical or functional consequences. The criteria for exclusion were based on EUROCAT group recommendations,[31] as the group's experience showed that the definition, diagnosis and reporting of minor malformations vary considerably, while major malformations are less liable to differential recall bias. Despite the potential influence of exposure misclassification and recall bias, other studies based on birth records provide support for the validity of our findings.[10,15] The association observed with congenital malformations strengthens the hypothesis that developmental factors could have an aetiological role in NB.

Our finding of an inverse relationship with breastfeeding is consistent with a large US study, which reported an association of the same order of magnitude (OR 0.63 95% CI 0.41–0.96).[24] The association between breastfeeding and NB has been less documented than the association with leukemia or brain tumors. A meta-analysis published in 2005,[38] which reported a 41% reduction in the risk of neuroblastoma, relied on only 3 studies,[23,39,40] two of which[39,40] involved <45 cases each. The hypotheses with respect to underlying biological mechanisms are unclear at present. Graves' hypothesis regarding childhood leukaemia[41] suggests that breast milk may play an important role in the prevention of childhood leukemia by actively stimulating or modulating the immune system and promoting its development in early life. However, an infectious aetiology and a role of immunological modifiers in NB development have not been prominent hypotheses. Epigenetic mechanisms have also been suggested based on the contribution of some human milk compounds to metabolic and differentiation processes, and to the development of the infant's immune system.[42]

Our study suggests that maternal folic acid; vitamin or mineral supplementation in the preconception period may reduce the incidence of NB. The main limitation with regard to the interpretation of this finding is the retrospective design of the study and its potential reporting bias. Only half of the mothers who reported folic acid supplementation during the preconception period could name a valid folic acid product. However, the imprecision was similar for case and control mothers, and there was little change in the estimates when only those mothers were analyzed.

Although various constituents of the multivitamin supplements may have been responsible for the protective effects, only 2% of controls and 1% of cases reported using a supplement not containing folic acid during the preconception period. In line with these findings, a significant reduction in NB incidence was observed in Canada after mandatory flour fortification with folic acid was instituted.[20] Moreover, two US case-control studies,[21,43] also reviewed in Goh *et al.*[22] concluded that periconceptional folic acid supplementation, three months before pregnancy and early in pregnancy, should be recommended. By contrast, a recent Norwegian data linkage study found no association between supplemental folic acid before and/or pregnancy and NB. However, with only 71 cases of NB, the study had insufficient power to specifically investigate preconceptional supplementation.[44]

Periconceptional folic acid supplementation has been shown to reduce the risk of neural tube defects by almost three-quarters.[45] Neural tube defects arise from the same embryonic structures as NB. The biological plausibility of the associations derives from evidence that folic acid derivatives are essential for the synthesis of nucleic acids and amino acids, cell division, tissue growth, and DNA methylation. In our study, the potential protective effect was only observed when supplementation started before pregnancy. One explanation for this finding could be that the inception, migration, divergence and maturation of neural crest progenitors occurs early in pregnancy (between 3rd and 5th week of gestation)[46], a critical period of time when most women may still not know they are pregnant. The association may also result from confounding since the women who began supplementation prior to pregnancy may differ in terms of profile and lifestyle from those who did not use supplements or started them after discovering they were pregnant. The French 2010 National Perinatal Survey[47] showed that women who took folic acid supplements before pregnancy were more likely to be: older; married or cohabiting; European; non-smokers; with a body mass index less than 25. Folic acid use during preconception was also higher in low-parity highly-educated

Table 3. Breast-feeding and risk of neuroblastoma

	Controls $n = 1589$		All neuroblastomas $n = 269$						MYCN amplification								< 18 months						≥ 18 months			
							MYCN -				MYCN+				Controls		Cases				Controls		Cases			
	n	%	n	%	OR[1]	95% CI	*n*	%	OR[1]	95% CI	*n*	%	OR[1]	95% CI	*n*	%	*n*	%	OR[1]	95% CI	*n*	%	*n*	%	OR[1]	95% CI
Breastfeeding						*p-value 0.03*				*p-value 0.1*				*p-value 0.07*						*p-value 0.05*						
Never	642	*40*	118	*44*	1.0	Ref	78	*42*	1.0	Ref	31	*51*	1.0	Ref	118	*34*	40	*40*	1.0	Ref	524	*42*	78	*46*	1.0	Ref
Ever	947	*60*	151	*56*	0.7	[0.5-1.0]	109	*58*	0.8	[0.6-1.1]	30	*49*	0.6	[0.3-1.0]	232	*66*	60	*60*	0.6	[0.4-1.0]	715	*58*	91	*54*	0.8	[0.6-1.1]
Breastfeeding duration																										
Never breastfed	642	*40*	118	*44*	1.0	Ref	78	*42*	1.0	Ref	31	*51*	1.0	Ref	118	*34*	40	*40*	1.0	Ref	524	*42*	78	*46*	1.0	Ref
< 3 months	348	*22*	64	*24*	0.8	[0.6-1.1]	40	*21*	0.7	[0.5-1.1]	17	*28*	0.9	[0.5-1.6]	88	*25*	23	*23*	0.5	[0.3-1.0]	260	*21*	41	*24*	1.0	[0.6-1.5]
3 - 5 months	293	*18*	42	*16*	0.6	[0.4-1.0]	31	*17*	0.7	[0.4-1.1]	8	*13*	0.5	[0.2-1.2]	82	*23*	21	*21*	0.5	[0.3-1.1]	211	*17*	21	*13*	0.7	[0.4-1.1]
≥ 6 months	287	*18*	42	*16*	0.7	[0.5-1.1]	35	*19*	0.9	[0.6-1.4]	5	*8*	0.3	[0.1-0.9]	51	*15*	13	*13*	0.7	[0.3-1.5]	236	*19*	29	*17*	0.7	[0.4-1.2]
Missing	19	*1*	3	*1*	-	-	3	*1*	-	-	-				11	*3*	3	*3*	-	-	8	*1*		-	-	-
						p-trend 0.06				*p-trend 0.4*				*p-trend < 0.01*						*p-trend 0.3*						*p-trend 0.1*

[1]Odds ratios (OR) and 95% confidence intervals (95%CI) estimated by logistic regression models adjusted for age and sex, birth-order, maternal age, urban status of the area of residence and study. Pooled analysis of the ESCALE and ESTELLE studies (children older than 6 months).

Table 4. Maternal use of folic acid, vitamin or mineral supplements around the index pregnancy and risk of neuroblastoma

	Preconception period							First trimester							Second trimester						
	Controls (*n* = 834)		Cases (*n* = 183)					Controls (*n* = 834)		Cases (*n* = 183)					Controls (*n* = 834)		Cases (*n* = 183)				
	n	*%*	*n*	*%*	OR[1]	95% CI	*p-value*	*n*	*%*	*n*	*%*	OR[1]	95% CI	*p-value*	*n*	*%*	*n*	*%*	OR[1]	95% CI	*p-value*
Any supplement containing folic acid, vitamins or minerals[2]																					
No supplements, ever	496	*59*	107	*58*	1.0	Ref		496	*59*	107	*58*	1.0	Ref		496	*59*	107	*58*	1.0	Ref	
Any supplement (substantiated or not)	107	*13*	16	*9*	0.5	[0.3-0.9]	*0.04*	206	*25*	53	*29*	0.9	[0.6-1.4]	*0.4*	201	*24*	43	*23*	0.8	[0.5-1.2]	*0.6*
Folic acid substantiated	45	*5*	7	*4*	0.4	[0.2-1.1]	*0.07*	86	*10*	24	*13*	0.9	[0.5-1.5]	*0.7*	71	*9*	19	*10*	0.9	[0.5-1.6]	*0.7*
Folic acid unsubstantiated	48	*6*	8	*4*	0.6	[0.3-1.4]	*0.2*	95	*12*	25	*14*	1.0	[0.6-1.7]	*0.9*	79	*9*	18	*10*	0.9	[0.5-1.7]	*0.9*
No folic acid	14	*2*	1	*1*				25	*3*	4	*2*				51	*6*	6	*3*			
No, but yes in another period	231	*28*	60	*33*				132	*16*	23	*13*				137	*16*	33	*18*			

	Third trimester							At any time						
	Controls (*n* = 834)		Cases (*n* = 183)					Controls (*n* = 834)		Cases (*n* = 183)				
	n	*%*	n	*%*	OR[1]	95% CI	*p-value*	n	*%*	n	*%*	OR[1]	95% CI	*p-value*
Any supplement containing folic acid, vitamins or minerals[2]														
No supplements, ever	496	*59*	107	*58*	1.0	Ref		496	*59*	107	*58*	1.0	Ref	
Any supplement (substantiated or not)	176	*21*	40	*22*	0.8	[0.6-1.3]	*0.6*	338	*41*	76	*42*	0.8	[0.6-1.2]	*0.3*
Folic acid substantiated	63	*7*	18	*10*	0.9	[0.5-1.7]	*0.8*	118	*14*	30	*16*	0.8	[0.5-1.4]	*0.5*
Folic acid unsubstantiated	75	*9*	17	*9*	0.9	[0.5-1.7]	*0.8*	150	*18*	35	*19*	0.9	[0.6-1.5]	*0.8*
No folic acid	38	*5*	5	*3*				70	*9*	11	*7*			
No, but yes in another period	162	*19*	36	*20*										

[1]Odds ratios (OR) and 95% Confidence interval estimated by logistic regression models adjusted for children age, sex and birth-order, maternal age and urban status of the area of residence
[2]Variable coded as following: "No supplements ever" if the mother said no or the named product did not contain folic acid, vitamins or minerals from three months before pregnancy to birth; "any supplement" if mother said she took any supplement containing folic acid, vitamins or minerals in the relevant period, regardless whether she gave a valid product name or not; "Folic acid substantiated" if the mother said she took folic acid in the relevant period or named a folic acid containing product; "Folic acid unsubstantiated "mother said yes in the relevant period but she did not give a name; "No folic acid" if the mother did not take folic acid in the relevant period but did take a product containing vitamins or minerals; "No, but yes in another period" if she did not take folic acid, vitamins or minerals in the relevant period time but took it in another period from three months before pregnancy to birth.
(ESTELLE only).

women. However, the cases and controls in our study were similar in terms of maternal education and parity, and the estimates were adjusted for maternal age. In France, health promotion campaigns encouraging prenatal folic acid supplementation began in 2004, after the ESCALE study period. The low frequency of supplementation in both cases and controls in that study (only two cases and three controls reported the use of folic acid or vitamin/mineral supplements in the pre-conception period) prevented supplementation being addressed in that study.

This study did not find any association between fertility treatments and NB and as such is consistent with the results of a large British cohort study.[48] In our study the estimates were very close to unity and do not suggest any increased risk of NB. However, the results are based on small numbers of exposed subjects and cannot be interpreted as meaning there is no risk. The literature remains discordant. A Danish cohort study suggested that maternal fertility treatment with progesterone before childbirth might increase the risk of sympathetic nervous system tumours.[49] Additionally, a meta-analysis by the same author, based on five studies of NB, found an increased risk of NB among children born after fertility treatment (OR= 4.04 95% CI 1.24–13.18).[50]

Our study has the same limitations as all case-control interview-based studies, which are inherently exposed to a risk of selection bias and recall bias. However, during the interview, women were asked to consult the child's personal health record, which was available for over 95% of cases and 98% of controls.

Despite the number of controls our study was underpowered for some analysis and findings based on small subgroups of cases, like MYCN amplified cases, may be due to chance. At the same time, as evidence from previous studies showed that age at diagnosis and MYCN status are highly correlated,[51] our findings about congenital malformations and fetal growth anomalies being more strongly associated with NB among children aged <18 months may be related to MYCN status or other genetic or biologic risks that are yet undiscovered or unproven, and not necessarily with the age at diagnosis.

However, our study also has several strengths. Since the ESCALE and ESTELLE studies were designed to be pooled, with uniformly defined exposures, this is one of the largest studies of NB at present. In this population-based study, cases were ascertained by a nationwide cancer registry, which has a high degree of completeness. The overall participation rate of the controls was high and we also adjusted our analysis for factors that might be associated with control participation like maternal age and degree of urbanization. The controls were very similar to the French general population, when compared with the national perinatal surveys,[28] with regard to maternal level of education, perinatal characteristics such as birth weight and gestational age, and the use of fertility treatments. The use of a complete and standardized questionnaire enabled investigation of several exposures and potential confounders in detail, while reducing potential differential misclassifications.

In conclusion, the results from this pooled analysis of the ESCALE and ESTELLE studies support the hypotheses that fetal growth anomalies and congenital malformations are related to NB. The results also suggest protective effects of breastfeeding and preconception use of folic acid supplements, which need further investigation. If these findings were replicated, intensifying current health policies with regard to those practices could be important for NB prevention.

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References

1. INCa report. Les cancers en France. Edition 2013 [Internet]. 2014;Available from: http://www.e-cancer.fr/Expertises-et-publications/Catalogue-des-publications/Les-cancers-en-France-Edition-2013
2. Lacour B, Guyot-Goubin A, Guissou S, et al. Incidence of childhood cancer in France: National Children Cancer Registries, 2000-2004. *Eur J Cancer Prev* 2010;19:173–81.
3. Brodeur GM, Bagatell R. Mechanisms of neuroblastoma regression. *Nat Rev Clin Oncol* 2014;11: 704–13.
4. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG task force report. *J Clin Oncol* 2009;27:289–297.;:–.
5. Carlsen NL. Neuroblastomas presenting in the first year of life: epidemiological differences from those presenting at older ages. *Cancer Detect Prev* 1996;20:251–61.
6. Urayama KY, Von Behren J, Reynolds P. Birth characteristics and risk of neuroblastoma in young children. *Am J Epidemiol* 2006;165:486–95.
7. Bluhm E, McNeil DE, Cnattingius S, et al. Prenatal and perinatal risk factors for neuroblastoma. *Int J Cancer* 2008;123:2885–90.
8. Schleiermacher G, Janoueix-Lerosey I, Delattre O. Recent insights into the biology of neuroblastoma. *Int J Cancer* 2014;135:2249–61.
9. Hamrick SE, Olshan AF, Neglia JP, et al. Association of pregnancy history and birth characteristics with neuroblastoma: a report from the Children's Cancer Group and the Pediatric Oncology Group. *Paediatr Perinat Epidemiol* 2001;15:328–37.
10. Bjørge T, Engeland A, Tretli S, et al. Birth and parental characteristics and risk of neuroblastoma in a population-based Norwegian cohort study. *Br J Cancer* 2008;99:1165–9.
11. Parodi S, Merlo DF, Ranucci A, et al. Risk of neuroblastoma, maternal characteristics and perinatal exposures: The SETIL study. *Cancer Epidemiol* 2014;38:686–94.
12. Neglia JP, Smithson WA, Gunderson P, et al. Prenatal and perinatal risk factors for neuroblastoma. A case-control study. *Cancer* 1988;61:2202–6.
13. Buck GM, Michalek AM, Chen CJ, et al. Perinatal factors and risk of neuroblastoma. *Paediatr Perinat Epidemiol* 2001;15:47–53.
14. Johnson KJ, Puumala SE, Soler JT, et al. Perinatal characteristics and risk of neuroblastoma. *Int J Cancer* 2008;123:1166–72.
15. Chow EJ, Friedman DL, Mueller BA. Maternal and perinatal characteristics in relation to neuroblastoma. *Cancer* 2007;109:983–92.
16. Schüz J, Forman MR. Birthweight by gestational age and childhood cancer. *Cancer Causes Control* 2007;18:655–63.
17. McLaughlin CC, Baptiste MS, Schymura MJ, et al. Perinatal risk factors for neuroblastoma. *Cancer Causes Control 2009*;20:289–301.
18. Johnson CC, Spitz MR. Neuroblastoma: case-control analysis of birth characteristics. *J Natl Cancer Inst* 1985;74:789–92.
19. Menegaux F, Olshan AF, Reitnauer PJ, et al. Positive association between congenital anomalies and risk of neuroblastoma. *Pediatr Blood Cancer* 2005;45:649–55.
20. French A. Folic acid food fortification is associated with a decline in neuroblastoma. *Clin Pharmacol Ther* 2003;74:288–94.
21. Olshan AF, Smith JC, Bondy ML, et al. Maternal vitamin use and reduced risk of neuroblastoma. *Epidemiology* 2002;13:575–580.
22. Goh YI, Bollano E, Einarson TR, et al. Prenatal multivitamin supplementation and rates of pediatric cancers: a meta-analysis. *Clin Pharmacol 38 Ther* 2007;81:685–91.
23. Daniels JL, Olshan AF, Pollock BH, et al. Breastfeeding and neuroblastoma, USA and Canada. *Cancer Causes Control* 2002;13:401–5.
24. Menegaux F, Olshan AF, Neglia JP, et al. Day care, childhood infections, and risk of neuroblastoma. *Am J Epidemiol* 2004;159:843–51.
25. Munzer C, Menegaux F, Lacour B, et al. Birth-related characteristics, congenital malformation, maternal reproductive history and neuroblastoma: the ESCALE study (SFCE). *Int J Cancer* 2008;122: 2315–21.
26. Amigou A, Rudant J, Orsi L, et al. Folic acid supplementation, MTHFR and MTRR polymorphisms, and the risk of childhood leukemia: the ESCALE study (SFCE). *Cancer Causes Control* 2012;23:1265–77.
27. Orsi L, Rudant J, Ajrouche R, et al. Parental smoking, maternal alcohol, coffee and tea consumption during pregnancy, and childhood acute leukemia: the ESTELLE study. *Cancer Causes Control* 2015;26:1003–17.
28. Blondel B, Kermarrec M. Les naissances en 2010 et leur évolution en 2003. 2012 [cited 2015 Jun 14];Available at: http://www.epsilon.insee.fr/jspui/handle/1/14305
29. Association des Utilisateurs de Dossiers Informatisés en Pédiatrie, Obstetrique et Gynécologie (AUDIPOG). Morphométrie néonatale. Poids, taille et périmètre crânien à la naissance. 2008;
30. World Health Organisation. International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) [Internet]. 2015;Available at: http://www.who.int/classifications/icd/en/
31. EUROCAT- European Surveillance of congenital anomalies. Eurocat Guide 1.4 and Reference Documents [Internet]. 2013 [cited 2016 Feb 2]; Available at: www.eurocat-network.eu/aboutus/datacollection/guidelinesforregistration/guide1_4
32. Harder T, Plagemann A, Harder A. Birth weight and risk of neuroblastoma: a meta-analysis. *Int J Epidemiol* 2010;39:746–56.
33. O'Neill KA, Murphy MF, Bunch KJ, et al. Infant birthweight and risk of childhood cancer: international population-based case control studies of 40 000 cases. *Int J Epidemiol* 2015;44:153–68.
34. Rahman N. Mechanisms predisposing to childhood overgrowth and cancer. *Curr Opin Genet Dev* 2005;15:227–33.
35. Callan AC, Milne E. Involvement of the IGF system in fetal growth and childhood cancer: an overview of potential mechanisms. *Cancer Causes Control* 2009;20:1783–98.
36. Foulkes WD, Buu PN, Filiatrault D, et al. Excess of congenital abnormalities in French-Canadian children with neuroblastoma: a case series study from Montréal. *Med Pediatr Oncol* 1997;29:272–9.
37. Narod SA, Hawkins MM, Robertson CM, et al. Congenital anomalies and childhood cancer in Great Britain. *Am J Hum Genet* 1997;60:474–85.
38. Martin RM, Gunnell D, Owen CG, et al. Breast-feeding and childhood cancer: a systematic review with metaanalysis. *Int J Cancer* 2005;117:1020–31.
39. Smulevich VB, Solionova LG, Belyakova SV. Parental occupation and other factors and cancer risk in children. I. Study methodology and non-occupational factors. *Int J Cancer* 1999;83:712–7.
40. Hardell L, Dreifaldt AC. Breast-feeding duration and the risk of malignant diseases in childhood in Sweden. *Eur J Clin Nutr* 2001;55:179–85.
41. Greaves MF. Aetiology of acute leukaemia. *Lancet* 1997;349:344–9.
42. Alsaweed M, Lai CT, Hartmann PE, et al. Human milk miRNAs primarily originate from the mammary gland resulting in unique miRNA profiles of fractionated milk. *Sci Rep* 2016;6:20680
43. Michalek AM, Buck GM, Nasca PC, et al. Gravid health status, medication use, and risk of neuroblastoma. *Am J Epidemiol* 1996;143:996–1001.
44. Mortensen JHS, Øyen N, Fomina T, et al. Supplemental folic acid in pregnancy and childhood cancer risk. *Br J Cancer* 2016;114:71–5.
45. De-Regil LM, Fernández-Gaxiola AC, Dowswell T, et al. Effects and safety of periconceptional folate supplementation for preventing birth defects. *Cochrane Libr [Internet]* 2010; cited 2015 Jun 4];Available at: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD007950.pub2/full
46. Marshall GM, Carter DR, Cheung BB, et al. The prenatal origins of cancer. *Nat Rev Cancer* 2014; 14:277–89.
47. Tort J, Lelong N, Prunet C, et al. Maternal and health care determinants of preconceptional use of folic acid supplementation in France: results from the 2010 National Perinatal Survey. 2013; 1661–7.
48. Williams CL, Bunch KJ, Stiller CA, et al. Cancer risk among children born after assisted conception. *N Engl J Med* 2013;369:1819–27.
49. Hargreave M, Jensen A, Nielsen TSS, et al. Maternal use of fertility drugs and risk of cancer in children—a nationwide population-based cohort study in Denmark. *Int J Cancer* 2015;136: 1931–9.
50. Hargreave M, Jensen A, Toender A, et al. Fertility treatment and childhood cancer risk: a systematic meta-analysis. *Fertil Steril* 2013;100:150–61.
51. London WB, Castleberry RP, Matthay KK, et al. Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. *J Clin Oncol* 2005;23: 6459–65.

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ORIGINAL PAPER

Maternal use of household pesticides during pregnancy and risk of neuroblastoma in offspring. A pooled analysis of the ESTELLE and ESCALE French studies (SFCE)

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Abstract

Purpose Neuroblastoma (NB) is an embryonic tumor that occurs almost exclusively in infancy and early childhood. While considerable evidence suggests that it may be initiated during embryonic development, the etiology of NB is still unknown. The aim of this study was to explore whether there is an association between maternal use of household pesticides during pregnancy and the risk of NB in the offspring.

Methods We conducted a pooled analysis of two French national-based case–control studies. The mothers of 357 NB cases and 1,783 controls younger than 6 years, frequency-matched by age and gender, responded to a telephone interview that focused on sociodemographic and perinatal characteristics, childhood environment, and lifestyle. Unconditional logistic regression was used to estimate pooled odds ratios and 95% confidence intervals.

Results After controlling for matching variables, study of origin, and potential confounders, the maternal use of any type of pesticide during pregnancy was associated with NB (OR 1.5 [95% CI 1.2–1.9]). The most commonly used type of pesticides were insecticides and there was a positive association with their use alone (OR 1.4 [95% CI 1.1–1.9]) or with other pesticides (OR 2.0 [95% CI 1.1–3.4]).

Conclusions Although there is the potential for recall bias due to the study design, our findings add to the evidence of an association between the household use of pesticides and NB. Until a better study design can be found, our findings

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add yet another reason why to advise pregnant women to limit pesticide exposure during the periconceptional period.

Keywords Neuroblastoma · Pesticides · Risk factors · Childhood cancer · Case–control study

Abbreviations

ESCALE	Etude Sur les Cancers et les Leucémies de l'Enfant
ESTELLE	Etude Sur les Tumeurs Embryonnaires, Leucémies et Lymphomes de l'Enfant
MYCN	N-myc proto-oncogene
NB	Neuroblastoma
RNCE	Registre National des Cancers de L'Enfant
SFCE	Société Française de lutte contre les Cancers de l'Enfant et de l'Adolescent

Introduction

Neuroblastoma (NB) is a malignant embryonal tumor of the neural crest cells that occurs almost exclusively in infancy and early childhood. Forty percent of NB cases are diagnosed before the age of one year and 85% before the age of five years [1].

The etiology of NB remains unknown. A genetic predisposition of NB has been suggested after the observation of rare familial cases (1% of NB cases) and a possible association with congenital malformations [2, 3]. Considerable progress has been made recently in the germ line and somatic genetic characterisation of patients and tumors [4]. However, genetic predisposition cannot fully explain its origin and other factors may be involved in NB development [5].

Considerable evidence suggests that NB may be initiated in utero during sympathoadrenal development [6]. The undifferentiated stem cells from the neural crest progenitors may persist in crest-derived tissues awaiting stimulation or reactivation in response to environmental or cellular cues [7]. Prenatal exposure to pesticides may be one such risk factor. A possible association between pesticides and NB was first suggested more than 30 years ago in relation to exposures to chlordane or heptachlor [8], which are active ingredients in many household and garden pesticides. An association between pesticide exposure and NB is plausible as more than 25 chemical compounds used in pesticides have been classed as potential human carcinogens [9, 10]. In addition, previous studies have showed that they pass through the placenta leading to fetal exposure [11, 12].

Literature about the subject is scarce. Only two previous studies [13, 14] have shown associations between NB and use of household pesticides during the preconception period, pregnancy, or in early childhood. Studies looking at parental occupational exposure to pesticides are heterogeneous in exposure definition and in findings. Some studies have suggested an increased risk of NB with maternal occupational exposure to pesticides during pregnancy [15] or at any time during the preconception-pregnancy period or childhood [14, 16], while a meta-analysis found no association with paternal occupational exposure to pesticides at any time [17]. A large cohort study [18] suggested an increased risk of NB in offspring of farm holders, while a registry-based study using geographic information [19] did not support an association with residence exposure to pesticides related to neighboring agricultural activities.

The aim of this study was to investigate whether the maternal use of household pesticides during pregnancy was associated with the risk of NB in her child. For these analyses, we pooled data from two large case–control studies, ESCALE and ESTELLE, which were conducted by the same investigators in France.

Materials and methods

Study population

The ESCALE and ESTELLE studies have been described in detail elsewhere [2]. Briefly, they were two nationwide population-based case–control studies, which were conducted with the support of the Société Française de lutte contre les Cancers de L'Enfant et l'Adolescent (SFCE).

The cases, which were directly identified from the French national registry of childhood cancer (RNCE), were children younger than 15 years old who lived in France at the time they were newly diagnosed with cancer. The ESCALE study included cases of NB, lymphoma, leukemia, and malignant brain tumor diagnosed in 2003–2004. The ESTELLE study included cases of NB, leukemia, lymphoma, childhood brain tumor, Wilms' tumor, and hepatoblastoma diagnosed in 2010–2011.

Overall case participation rates were 81.2% for ESCALE and 92.2% for ESTELLE. Information on the MYCN amplification subtype was obtained subsequently from the RNCE.

The population controls were children free from cancer randomly selected from the French population using quota-sampling methods who were recruited by telephone during the same time periods. The participation rates were 71.2 and 85.5%, respectively.

In both studies, the cases and the controls were ineligible if their biological mother was unavailable, did not speak French, or had a serious psychosocial problem. In

addition, 22 cases, 9 out of 248 cases under 18 months (3.6%) and 13 out of 252 cases aged 18 months or more (5.2%), were not eligible to maternal interview for ethical reasons because they had died or were in palliative care.

The present paper focuses on NB in children under six years old (91.3% of cases).

Data collection

Trained interviewers conducted standardized telephone interviews with the biological mothers of cases and controls, which lasted approximately 50 min. The interviews used similar scripts and were performed in the same conditions in the two studies. They focused on socioeconomic characteristics, prenatal and childhood environment exposures, familial and personal medical history.

In regards to pesticide exposures during pregnancy, the mother was asked if she used herbicides ("weed killers"), fungicides, or insecticides (and whether they were used indoors, for gardening or outdoors, or on pets). The mother was also asked if she was exposed to any type of pesticides in the workplace.

The ESTELLE study included additional questions about maternal pesticide use in the three months prior to conception and after birth, and whether there had been any professional pest control treatments of the home.

Data analysis

Study-specific odds ratios (ORs) and pooled ORs and 95% confidence intervals (95% CIs) were estimated by unconditional logistic regression (SAS version 9; SAS Institute Inc., Cary, NC, USA). All the models included the study matching factors: child's age and gender, and, for the pooled analyses, the indicator of the study of origin.

The socioeconomic variables tested as potential confounders were: maternal level of education, size of the urban unit of residence, maternal age at child's birth, birth order, and the type of housing during pregnancy. We also tested fetal growth, congenital malformations, and breastfeeding that were significantly associated with NB in previous analyses [2]. In the final model, only maternal age, birth order, size of the urban unit of residence, and the type of housing were retained.

Between-study heterogeneity was systematically tested by fitting an interaction term between the study and the exposure of interest.

We performed additional analyses stratified by age at diagnosis (<18 months/≥18 months), MYCN status (amplified/non-amplified), urban/rural status of the area of residence, and maternal level of education (less than baccalaureate/baccalaureate or higher).

Due to a small number of children with a congenital malformation, possible confounding was accounted for by excluding these children rather than by adjustment. Finally, sensitivity analyses were also conducted in the ESTELLE study by excluding the cases whose mother did not have a landline at home since they could not have been selected as controls (the information was not available in the ESCALE study).

Results

The pooled analysis included 357 cases (174 from ESCALE and 183 from ESTELLE) and 1,783 controls (949 from ESCALE and 834 from ESTELLE) younger than six years old (Table 1). Among cases, MYCN was amplified in 17.9% (11% of the cases under 18 months of age and in 25% of the older cases), non-amplified in 75.6%, and non-informative in 6.4%.

Case–control comparability

In both studies the cases were more likely to be younger, first born, to live in an urban area, and to have younger mothers than the controls (Table 1).

Between-study heterogeneity

Control mothers in the ESTELLE study lived more often in less populated areas and were more highly educated than those in the ESCALE study (Table 1). They were also more likely to have used pesticides at home ($p < 0.01$) (results not tabulated).

Pesticides use

Overall, maternal use of any pesticide during pregnancy was reported for 43.7% of the cases and 35.7% of the controls. Insecticides were the most commonly used (40.6% of cases and 33.9% of the controls) and they were mostly used alone. Their use was mainly reported indoors (80.0% of insecticides use in cases and 81.0% in controls). Mothers rarely used herbicides or fungicides and they often also used insecticides.

The maternal use of any type of pesticide during pregnancy was associated with the risk of NB (OR 1.5 [95% CI 1.2–1.9]). There was a positive association with the use of insecticides alone (OR 1.4 [95% CI 1.1–1.9]) or insecticides with other pesticides (OR 2.0 [95% CI 1.1–3.4]). There was no between-study heterogeneity except for herbicide use (pooled OR 2.0 [95% CI 1.1–3.7]); ESCALE (OR 3.8 [95% CI 1.8–8.0]); ESTELLE (OR 1.1 [95% CI 0.4–3.2]); p value for interaction = 0.07).

Table 1 Characteristics of the cases and controls of the ESCALE and ESTELLE studies

	ESCALE (2003–2004)				ESTELLE (2010–2011)				POOLED			
	Cases ($n = 174$)		Controls ($n = 949$)		Cases ($n = 183$)		Controls ($n = 834$)		Cases ($n = 357$)		Controls ($n = 1,783$)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
MYCN status												
Non-amplified	131	75.3			139	76.0			270	75.6		
Amplified	34	19.5			30	16.4			64	17.9		
Missing	9	5.2			14	7.6			23	6.4		
Age (years)												
<1	74	42.5	187	19.7	70	38.2	188	22.5	144	40.3	375	21.0
1	36	20.7	182	19.2	37	20.2	123	14.7	73	20.4	305	17.0
2	30	17.2	153	16.1	35	19.1	148	17.7	65	18.2	301	16.9
3	13	7.5	166	17.5	21	11.5	139	16.7	34	9.5	305	17.1
4	11	6.3	145	15.3	12	6.6	131	15.7	23	6.4	276	15.5
5	10	5.7	116	12.2	8	4.4	105	12.6	18	5.0	221	12.4
Sex												
Boys	88	50.6	429	45.2	83	45.4	388	46.5	171	47.9	817	45.8
Girls	86	49.4	520	54.8	100	54.6	446	53.5	186	52.1	966	54.2
Birth order												
1	89	51.1	402	42.4	91	49.7	345	41.4	180	50.4	747	41.9
2 or more	85	48.9	547	57.6	92	50.3	489	58.6	177	49.6	1,036	58.1
Maternal age at child's birth (years)												
<25	31	17.8	80	8.4	25	13.7	86	10.3	56	15.7	166	9.3
25–29	61	35.1	314	33.1	70	38.2	250	30.0	131	36.7	564	31.6
30–34	54	31.0	360	37.9	50	27.3	293	35.1	104	29.1	653	36.6
≥35	28	16.1	195	20.5	38	20.8	205	24.6	66	18.5	400	22.4
Maternal education												
<Baccalaureate	55	31.6	319	33.6	50	27.3	203	24.3	105	29.4	522	29.3
Baccalaureate	32	18.4	195	20.5	44	24.0	192	23.0	76	21.3	387	21.7
>Baccalaureate	87	50.0	435	45.8	88	48.1	439	52.6	175	49.0	874	49.0
Missing	0	0	0	0	1	0.5	0	0	1	0.3	0	0
Size of urban unit of residence (population)												
<5,000	57	32.8	360	37.9	59	32.2	351	42.1	116	32.5	711	39.9
5,000–99.999	36	20.1	211	22.2	43	23.5	174	20.9	79	22.1	385	21.6
100.000–1.999.999	41	23.6	233	24.5	50	27.3	158	18.9	91	25.5	391	21.9
Paris unit	37	21.3	145	15.3	30	16.4	149	17.9	67	18.8	294	16.5
Missing	3	1.7	0	0	1	0.5	2	0.2	4	1.1	2	0.1
Type of housing during pregnancy												
Apartment	78	44.8	379	39.9	77	42.1	324	38.8	155	43.4	703	39.4
House or farm	96	55.1	570	60.1	105	57.3	506	60.7	201	56.3	1,076	60.3
Missing	0	0	0	0	1	0.5	4	0.5	1	0.3	4	0.2

Maternal occupational pesticide exposure during pregnancy was associated with the risk of NB (OR 2.0 [95% CI 1.0–4.0), although the frequency of exposure was low (3.6% of cases and 1.8% of controls) (data not tabulated).

The results were similar whether or not MYCN was amplified (Table 2), and when the analyses were stratified by age at diagnosis (Table 3). Among the controls, the prevalence of pesticide use varied by urban/rural status, but not by maternal level of education, and the results did not change when stratification for either of these factors was used instead of adjustment (Supplementary Table 1);

Table 2 Maternal use of pesticides during pregnancy and risk of neuroblastoma

	Controls $n = 1,783$		All NB cases $n = 357$		OR[a]	95% CI	MYCN— $n = 270$		OR[a]	95% CI	MYCN + $n = 64$		OR[a]	95% CI
	n	%	*n*	%			*n*	%			*n*	%		
Maternal use of any pesticides														
None	1,112	62.4	198	55.5	1.0	Reference	155	57.4	1.0	Reference	32	50.0	1.0	Reference
Any pesticide	636	35.7	156	43.7	1.5	1.2–1.9	113	41.8	1.4	1.1–1.9	31	48.4	1.7	1.0–2.9
Any insecticide	604	33.9	145	40.6	1.5	1.1–1.9	105	38.9	1.4	1.1–1.9	28	43.7	1.6	0.9–2.7
Any herbicide	61	3.4	20	5.6	2.0	1.1–3.7	14	5.2	1.9	0.9–3.6	3	4.7	1.8	0.5–6.5
Any fungicide	50	2.8	14	3.9	1.6	0.8–3.1	7	2.6	1.1	0.5–2.6	6	9.4	4.3	1.6–11.5
Only insecticides	537	30.1	125	35	1.4	1.1–1.9	93	34.4	1.4	1.0–1.9	23	35.9	1.5	0.8–2.6
Only herbicides	13	0.7	5	1.4	1.5	0.4–4.8	5	1.8	1.9	0.6–6.2	0	0		
Only fungicides	19	1.1	6	1.7	1.7	0.7–4.6	3	1.1	1.2	0.3–4.4	3	4.7	5.2	1.4–19.8
Insecticides + other pesticides	67	3.8	20	5.6	2.0	1.1–3.4	12	4.4	1.6	0.8–3.1	5	7.8	2.4	0.9–6.8
Only herbicides and fungicides	0		0											
Use of insecticides														
Indoor use	489	27.4	116	32.5	1.5	1.1–2.0	81	30.0	1.4	1.0–1.9	25	39.1	1.8	1.0–3.1
Gardening and outdoor use	57	3.2	13	3.6	1.3	0.7–2.5	7	2.6	0.9	0.4–2.1	3	4.7	1.6	0.5–5.8
For pets	224	12.6	49	13.7	1.3	0.9–1.9	37	13.7	1.3	0.9–2.0	10	15.6	1.2	0.5–2.8
Missing	35	1.9	3	0.8			2	0.7			1	1.6		

Pooled analyses of the ESCALE and ESTELLE studies

[a] Odds ratios (OR) and 95% confident intervals (CI) estimated by unconditional logistic regression models adjusted for children age and sex, study, maternal age, birth order, size of the urban unit of residence, and type of housing during pregnancy

Table 3 Maternal use of pesticides during pregnancy and risk of neuroblastoma by age at diagnosis

	Age <18 months						Age ≥18 months					
	Cases		Controls		OR[a]	95% CI	Cases		Controls		OR[a]	95% CI
	$n = 188$	%	$n = 544$	%			$n = 169$	%	$n = 1,239$	%		
Maternal use of pesticides												
None	114	60.6	369	67.8	1.0	Reference	84	49.7	743	59.9	1.0	Reference
Any pesticide	73	38.8	166	30.5	1.5	1.0–2.1	83	49.1	470	37.9	1.6	1.1–2.2
Any insecticide	65	34.6	155	28.5	1.4	0.9–2.0	80	47.3	449	36.2	1.6	1.1–2.3
Any herbicide	7	3.7	22	4.0	1.1	0.4–3.1	13	7.7	39	3.1	3.4	1.6–7.1
Any fungicide	5	2.7	15	2.8	1.0	0.3–3.3	9	5.3	35	2.8	2.3	1.0–5.1
Only insecticides	62	32.9	134	24.6	1.5	1.0–2.2	63	37.3	403	32.5	1.4	1.0–2.0
Only herbicides	4	2.1	7	1.3	1.4	0.3–5.9	1	0.6	6	0.5	1.6	0.2–14.7
Only fungicides	4	2.1	4	0.7	3.1	0.7–13.7	2	1.2	15	1.2	1.1	0.3–5.0
Insecticides + other pesticides	3	1.6	21	3.9	0.5	0.1–1.9	17	10.0	46	3.7	3.4	1.8–6.5
Only herbicides and fungicides	0		0				0		0			
Use of insecticide												
Indoor use	50	26.6	121	22.2	1.5	1.0–2.2	66	39.0	368	29.7	1.6	1.1–2.3
Gardening and outdoor use	8	4.3	23	4.2	1.5	0.6–3.6	5	2.9	34	2.7	1.2	0.4–3.2
For pets	23	12.2	58	10.7	1.3	0.7–2.3	26	15.4	166	13.4	1.3	0.8–2.2
Missing	1	0.5	9	1.6			2	1.2	26	2.1		

[a] Odds ratios (OR) and 95% confident intervals (CI) estimated by unconditional logistic regression models adjusted for children age and sex, study, maternal age, birth order, size of the urban unit of residence, and type of housing during pregnancy

neither did the exclusion of children with congenital malformations change the results.

Maternal use of pesticides before and after pregnancy and the use of professional pest control treatments at home were not collected in the ESCALE study. In the ESTELLE study, more than a quarter of the mothers reported pesticide use during all three time periods (29.0% of the cases and 27.6% of the controls) and very few mothers reported the use during pregnancy only (6.0% of cases and 3.5% of controls). This precluded specific analyses by time window.

No association was found with professional pest control treatments at home during pregnancy (OR 1.2 [95% CI 0.5–2.8]) (data not tabulated).

Finally, in the ESTELLE study, the results were unchanged in sensitivity analysis excluding the cases with no telephone landline.

Discussion

Our findings suggest that the maternal use of pesticides during pregnancy may be associated with an increased risk of NB.

In a large US case–control study, Daniels et al. [13] also reported modest associations (OR around 1.5) with the use of pesticides at home or in the garden during the preconception-pregnancy period or childhood. Consistent with our findings, this study showed similar estimates irrespective of the MYCN status, which does not support the potential for pesticides to act through different pathways in the two subtypes. In that study, the estimates were stronger among older children. Since the use of pesticides at home may be associated with life-patterns, the stronger associations observed in older children may reflect the effect of longer period of exposures to pesticides. Previous studies have also suggested that different etiologic factors may be specific to age at NB diagnosis [14, 20, 21]. Our study found no difference by age for the most prevalent exposures, pesticides and specifically insecticides. While there was a suggestion of differences by age with the use of herbicides and fungicides, this could be a chance finding since based on small numbers, the confidence intervals in the two groups overlapped substantially.

Another study conducted in Germany by Schüz et al. [14] found an association between NB and the use of household pesticides after the child's birth, but did not investigate pesticide exposure during pregnancy.

Consistent with our findings, associations between NB and maternal occupational exposure to pesticides were reported in two case–control studies in the USA [15, 16]. In a cohort study in Norway, an increased risk of NB was observed in offspring of parents having worked in field vegetable [18]. However, these findings should be interpreted with caution since they are based on small numbers. In two of these studies [16, 18], like in ours, estimates were based on less than ten exposed cases and could represent a chance finding. Literature on paternal occupational exposure to pesticides is not supportive of an association with NB as shown in a meta-analysis conducted by Moore et al. [17]. A large Texan case–control study estimated residential exposures to pesticides due to neighboring agricultural activities and found no association [19]. However, estimates were inconsistent between low and high level of exposure and were based on less than 15 exposed cases.

It is biologically plausible that maternal pesticide exposure during pregnancy could be associated with the risk of NB. Pregnancy represents a critical window of exposure since some explanatory hypotheses suggest that NB is initiated in utero during the sympathoadrenal development from the neural crest [6]. It has been shown that maternal exposure during pregnancy can lead to fetal exposure since these compounds pass through the placenta and can be found in cord blood, infant hair, and meconium [11, 22]. The potential underlying mechanisms are still unknown. Many pesticides are suspected to have different mutagenic or immunotoxic properties and some individual pesticides have been classed as "probable or possible carcinogens" by the International Agency for Research on Cancer [9, 10]. In addition, because of the similarity of brain biochemistry, some insecticides that target the nervous system of insects may also be neurotoxic to humans [23]. However, in our study, because the use of pesticides was correlated between time periods (preconception, pregnancy, and childhood) we cannot identify pregnancy as a true critical period of exposure. It is possible that patterns of exposure are related to lifetime habits, which are normally consistent throughout the periconceptional period and later in childhood.

A limitation of our study is that the majority of mothers who reported any pesticide use (94%) actually used insecticides, either alone or combined with herbicides or fungicides, which limited our ability to investigate associations with pesticides other than insecticides. In addition, we could not identify the active ingredients in the products used as in our study we only asked women about the category of pesticide as we thought this would be recalled with greater accuracy than the actual product name. Commercial household pesticides often contain multiple active ingredients, all which may have different properties including potentially carcinogenic actions. In our study we mainly focused on maternal use of pesticides at home. However, the mothers may be exposed to other direct or indirect sources of pesticides which we did not account for, like the paternal use of household pesticides or other sources of environmental exposure.

As expected, the proportion of cases ineligible because they had died or were receiving palliative care was smaller under 18 months of age than for those aged 18 months or more. This may have introduced a selection bias if age was related to opportunity of pesticide exposure. However, the age-stratified analyses did not provide evidence of substantial differences in the associations between NB and maternal use of pesticides. As no information on exposure was available on cases and controls that refused to participate, we do not know if these children were comparable to the study sample. To overcome these limitations, we stratified our analysis for different factors that may have been related to control participation to limit selection bias.

Finally, the study of pesticides exposure relied on maternal self-report, which may involve both non-differential and differential bias. Because of the particular distribution of NB (52.7% of the cases younger than 18 months) the lapse of time between the exposure time and the interview was short, which may have limited non-differential measurement error, and which may not be the case for other childhood cancers. In our study, trained interviewers using highly structured questionnaires conducted interviews with the aim of reducing potential differential misclassifications. Despite this, it cannot be excluded because case mothers may have tended to think more deeply about their possible exposures than control mothers. Previous studies have found consistency between self-reported pesticide treatments and pesticides concentrations in dust [24] and that agreement about pesticides exposure between parents did not differ by case–control status [13], suggesting no differential recall based on motivation of case parents. Furthermore, our findings stratified by urban status were similar, despite differences in the reported prevalence of pesticide use across these strata.

Our studies also have several strengths. The ESCALE and ESTELLE studies were designed to be pooled with uniformly defined exposures, which facilitated the pooling process, making this one of the largest investigations of NB at present. In these population-based studies, the control participation rate was high and the cases were identified from a nationwide cancer registry, which has a high degree of completeness.

In conclusion, this pooled analysis adds to the evidence of an association between NB and maternal use of pesticides during pregnancy, in household or occupation. Because data were obtained retrospectively by questionnaire, recall bias is possible, particularly for domestic use. Replication by other large epidemiological studies with different designs is important. However, until a better study design can be found, our findings add yet another reason why to advise pregnant women to limit pesticide exposure during the periconceptional period.

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References

1. Lacour B, Guyot-Goubin A, Guissou S et al (2010) Incidence of childhood cancer in France: National Children Cancer Registries, 2000–2004. Eur J Cancer Prev 19:173–181
2. Rios P, Bailey HD, Orsi L et al (2016) Risk of neuroblastoma, birth-related characteristics, congenital malformations and perinatal exposures: a pooled analysis of the ESCALE and ESTELLE French studies (SFCE). Int J Cancer. doi:10.1002/ijc.30239
3. Menegaux F, Olshan AF, Reitnauer PJ et al (2005) Positive association between congenital anomalies and risk of neuroblastoma. Pediatr Blood Cancer 45:649–655. doi:10.1002/pbc.20263
4. Schleiermacher G, Janoueix-Lerosey I, Delattre O (2014) Recent insights into the biology of neuroblastoma 135:2249–2261. doi:10.1002/ijc.29077
5. Heck JE, Ritz B, Hung RJ et al (2009) The epidemiology of neuroblastoma: a review. Paediatr Perinat Epidemiol 23:125–143. doi:10.1111/j.1365-3016.2008.00983.x
6. Marshall GM, Carter DR, Cheung BB et al (2014) The prenatal origins of cancer. Nat Publ Gr. doi:10.1038/nrc3679
7. Maguire LH, Thomas AR, Goldstein AM (2015) Tumors of the neural crest: common themes in development and cancer. Dev Dyn 244:311–322. doi:10.1002/dvdy.24226
8. Infante PF, Epstein SS, Newton WAJ (1978) Blood dyscrasias and childhood tumors and exposure to chlordane and heptachlor. Scand J Work Environ Health 4:137–150. doi:10.5271/sjweh.2718
9. International Agency for Research on Cancer (2015) Some organophosphate insecticides and herbicides: diazinon, glyphosate, malathion, parathion, and tetrachlorvinphos
10. International Agency for Research on cancer (1991) Occupational exposures in insecticide application, and some pesticides
11. Ostrea EM, Bielawski DM, Posecion NC et al (2009) Combined analysis of prenatal (maternal hair and blood) and neonatal (infant hair, cord blood and meconium) matrices to detect fetal exposure to environmental pesticides. Environ Res 109:116–122. doi:10.1016/j.envres.2008.09.004
12. Fisher M, Arbuckle TE, Liang CL et al (2016) Concentrations of persistent organic pollutants in maternal and cord blood from the

maternal-infant research on environmental chemicals (MIREC) cohort study. Environ Health 15:59. doi:10.1186/s12940-016-0143-y
13. Daniels JL, Olshan AF, Teschke K et al (2001) Residential pesticide exposure and neuroblastoma. Epidemiology 12:20–27
14. Schüz J, Kaletsch U, Meinert R et al (2001) Risk factors for neuroblastoma at different stages of disease. Results from a population-based case-control study in Germany. J Clin Epidemiol 54:702–709
15. Kerr MA, Nasca PC, Mundt KA et al (2000) Parental occupational exposures and risk of neuroblastoma: a case–control study (United States). Cancer Causes Control 11:635–643. doi:10.1023/A:1008951632482
16. Olshan AF, De Roos AJ, Teschke K et al (1999) Neuroblastoma and parental occupation. Cancer Causes Control 10:539–549. doi:10.1023/A:1008998925889
17. Moore A, Enquobahrie DA (2011) Paternal occupational exposure to pesticides and risk of neuroblastoma among children: a meta-analysis. Cancer Causes Control 22:1529–1536. doi:10.1007/s10552-011-9829-1
18. Kristensen P, Andersen A, Irgens LM et al (1996) Cancer in offspring of parents engaged in agricultural activities in Norway: incidence and risk factors in the farm environment. Int J Cancer 65:39–50. doi:10.1002/(SICI)1097-0215(19960103)65:1<39:AID-IJC8>3.0.CO;2-2
19. Carozza SE, Li B, Wang Q, et al Agricultural pesticides and risk of childhood cancers. doi: 10.1016/j.ijheh.2008.06.002
20. Carlsen NL (1996) Neuroblastomas presenting in the first year of life: epidemiological differences from those presenting at older ages. Cancer Detect Prev 20:251–261
21. Urayama KY, Von Behren J, Reynolds P (2007) Birth characteristics and risk of neuroblastoma in young children. Am J Epidemiol 165:486–495. doi:10.1093/aje/kwk041
22. Lewis RC, Cantonwine DE, Anzalota Del Toro LV et al (2014) Urinary biomarkers of exposure to insecticides, herbicides, and one insect repellent among pregnant women in Puerto Rico. Environ HealTH 13:97. doi:10.1186/1476-069X-13-97
23. Bjørling-Poulsen M, Andersen HR, Grandjean P (2008) Potential developmental neurotoxicity of pesticides used in Europe. Environ Health 7:50. doi:10.1186/1476-069X-7-50
24. Deziel NC, Colt JS, Kent EE, Gunier RB, Reynolds P,7, Booth B, Metayer C9 WM (2015) Associations between self-reported pest treatments and pesticide concentrations in carpet dust. Environ Health 14:27

Supplementary Table 1: Maternal use of pesticides during pregnancy and risk of neuroblastoma – Pooled analyses of the ESCALE and ESTELLE studies stratified by socio-demographic characteristics.

	Cases		Controls		OR	95% CI
	n	%	n	%		
Size of urban unit[1]						
<5000 inhabitants						
No use of pesticides	63	*54.3*	414	*58.2*	1.0	Reference
Any pesticide	52	*44.8*	284	*39.9*	1.3	0.8-1.9
Any insecticide	50	*43.1*	271	*38.1*	1.3	0.8-1.9
Missing	1	*0.9*	13	*1.8*		
≥5000 inhabitants						
No use of pesticides	135	*57.0*	697	*65.1*	1.0	Reference
Any pesticide	100	*42.2*	351	*32.8*	1.6	1.2-2.3
Any insecticide	92	*38.8*	332	*31.0*	1.6	1.2-2.2
Missing	2	*0.8*	22	*2.1*		
Maternal education[2]						
≤ Baccalaureate						
No use of pesticides	81	*44.5*	325	*35.7*	1.0	Reference
Any pesticide	99	*54.4*	566	*44.5*	1.5	1.1-2.2
Any insecticide	76	*41.8*	309	*34.0*	1.5	1.0-2.2
Missing	2	*1.1*	18	*2.0*		
> Baccalaureate						
No use of pesticides	75	*42.9*	311	*35.6*	1.0	Reference
Any pesticide	99	*56.6*	546	*42.9*	1.4	1.0-2.0
Any insecticide	69	*39.4*	295	*33.7*	1.4	1.0-2.0
Missing	1	*0.6*	17	*1.9*		

[1] Odds ratios (OR) and 95% confident intervals (CI) estimated by unconditional logistic regression models adjusted for age, sex, study, maternal age, birth order and type of housing during pregnancy.

[2] OR and 95% CI estimated by unconditional logistic regression models adjusted for age, sex, study, maternal age, birth order, size of the urban unit of residence and type of housing during pregnancy.

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Parental smoking, maternal alcohol consumption during pregnancy and the risk of neuroblastoma in children. A pooled analysis of the ESCALE and ESTELLE French studies

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Neuroblastoma (NB) is the most common extra-cranial tumour in children. Little is known about the aetiology of NB. The early age at onset and the embryonic nature suggest a role for perinatal exposures. We conducted a pooled analysis of two French national population-based case–control studies to explore whether there was an association between parental smoking and alcohol consumption and the risk of NB. The mothers of 357 NB cases and 1,783 controls from general population, frequency matched by age and sex, were interviewed on demographic, socioeconomic and perinatal characteristics, maternal reproductive story, and life-style and childhood environment. Unconditional logistic regression was used to estimate pooled odds ratios and 95% confidence intervals. A meta-analysis of our findings with those of previous studies was also conducted. Maternal smoking during pregnancy was slightly more often reported for the cases (24.1%) than for the controls (19.7%) (OR 1.3 [95% CI 0.9–1.7]; summary OR from meta-analysis 1.1 [95% CI 1.0–1.3]. Paternal smoking in the year before child's birth were not associated with NB as independent exposure (OR 1.1 [95% CI 0.9–1.4] but the association was stronger when both parents reported having smoked during pregnancy (OR 1.5 [95% CI 1.1–2.1]. No association was observed with maternal alcohol intake during pregnancy (OR 1.0 [95% CI 0.8–1.4], summary OR from meta-analysis 1.0 [95% CI 0.9–1.2]. Our findings provide some evidence of an association between maternal smoking during pregnancy and NB and add another reason to recommend that women refrain from smoking during pregnancy.

Introduction

Neuroblastoma (NB) is a malignant tumour that arises from the embryonal neural crest cells during the sympathetic nervous system development. It is the most common diagnosed cancer during infancy and the majority of cases are diagnosed before the age of 5 years.[1] The trademark of NB is its clinical heterogeneity characterised by contrasted patterns of prognosis and clinical behaviour. Younger children at diagnosis have

Key words: Neuroblastoma, tobacco, alcohol, risk factors, childhood cancer, case–control study

Abbreviations: ESCALE: Etude Sur les Cancers et les Leucémies de l'Enfant; ESTELLE: Etude Sur les Tumeurs Embryonnaires, Leucémies et Lymphomes de l'Enfant; MYCN: N-myc proto-oncogene; NB: Neuroblastoma; RNCE: Registre National des Cancers de L'Enfant; SFCE: Société Française de lutte contre les Cancers de l'Enfant et de l'Adolescent

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What's new?

Neuroblastoma strikes early in life, which suggests an influence from risk factors that occur before birth. Here, the authors looked at parental smoking and alcohol drinking in a large population sample in France. Cases were collected by a nationwide registry, so the sample was very complete, and included 357 cases of neuroblastoma. The analysis revealed no association between maternal alcohol drinking and the cancer, nor between neuroblastoma and paternal smoking. They did identify a slight positive association with maternal smoking, and the effect was stronger if both parents smoked.

better survival rates and less often overexpress the MYCN oncogene.[2] MYCN amplification is a genetic aberration that occurs in about 20% of primary tumours and it is one of the strongest independent adverse prognostic factors.

Little is known about the aetiology of NB. In rare cases, neuroblastoma can occur in a context of malformation syndromes (like Hirschsprung's disease). NB might also occur in the context of genetic predisposition, with constitutional mutations in ALK or PHOXB having been reported.[3] The early onset of NB after birth and its embryonal characteristics suggest a role of perinatal factors and environmental exposures during the periconceptional period. Tobacco smoke and alcohol consumption are of interest as potential risk factors as the International Agency on Research on Cancer has classed tobacco smoke and its metabolites as proven carcinogens to humans (Group 1).[4,5] Tobacco smoke is a human germ cell mutagen and its compounds also can cross the placenta leading to foetal exposure.[6] Teratogen and carcinogenic effects have also been shown with regards to alcohol consumption.[5]

Previous studies have shown associations between maternal smoking or alcohol consumption during pregnancy and the risk of childhood cancer, however only a small number addressed NB.[7–19] The studies on NB and maternal smoking, which mainly had less than 200 cases were included in two recent meta-analyses[20,21] which suggested a positive association between maternal smoking during pregnancy and NB. In an effort to improve precision, we pooled data from two nationwide case–control studies conducted in France by the same investigators. The aim of these analyses was to evaluate if there was an association between parental smoking, maternal alcohol consumption during pregnancy and the risk of NB.

Methods

The ESCALE and ESTELLE studies were two separate nationwide population-based case–control studies conducted by the same investigators in France. The study designs have been previously described elsewhere.[22] Both studies included cases of NB, lymphoma, leukaemia and malignant brain tumour. The ESTELLE study additionally included cases of Wilm's tumour, hepatoblastoma and non-malignant brain tumours. This article focuses on NB.

Study population

The cases were children, younger than 15 years old who were living in mainland France and were diagnosed with a childhood cancer in 2003–2004 (ESCALE) and 2010-2011(ESTELLE).

Eligible cases were directly identified from the French National Registry of Childhood Cancer (RNCE). The definition of a case was any child diagnosed with NB (Group 4 of the International Classification of Childhood Cancer Third edition).[23] Ineligible cases were those who had died or were receiving palliative care (n = 22) and those whose biological mother were not available (orphans and adopted children), could not speak French (n = 21) or had major psychosocial problems (n = 5). Information on the MYCN amplification subtype was obtained subsequently from the RNCE.

Controls were recruited by telephone and the methods have been previously described in detail.[24] They were children free from cancer randomly selected from the general French population and frequency matched by sex and age. Quota-sampling methods were applied to ensure the sample was representative of children aged less than 15 years. Like cases, controls were ineligible if the biological mother was not available for interview or did not speak French.

Because of the particular age distribution of NB cases, the present paper focuses on NB in children under 6 years old (91.3% of cases).

Data collection

Data were collected from the biological mothers of cases and controls by trained interviewers using computer assisted telephone interviews. The interview included questions on sociodemographic characteristics, prenatal and childhood environment exposures, familial and personal medical history.

In both studies, the mothers were asked whether they had smoked cigarettes during the pregnancy with the index child, and if yes, their average daily consumption. They were asked the same questions with regards to the paternal consumption of cigarettes. The ESTELLE study included additional questions about their smoking habits in the 3 months before the pregnancy and in each trimester of pregnancy. A convenience subset of ESTELLE fathers was also interviewed about their smoking habits in order to validate the maternal responses about paternal exposures[24]. Mothers were asked about alcohol consumption (wine, beer/cider, and spirits) during pregnancy and to quantify their consumption if applicable. In the Estelle study, they were also asked specifically about consumption in the first trimester.

Data management

Maternal and paternal tobacco smoking were analysed as dichotomous variables (ever/never) and also as quantitative

variables depending on the reported quantity of cigarettes. Cigarettes smoked per day were grouped in three categories based on the tertiles among control parents who smoked. The cut-offs were: nil; <5; 5–9 and ≥10 cigarettes per day (CPD) for mothers and nil; <10; 10–15 and >15 CPD for fathers.

For ESTELLE mothers, we also analysed the tobacco exposures by time window (three months before the index pregnancy and for each trimester of pregnancy). Finally, the joint effect of maternal and paternal smoking was analysed (neither parent, only mother, only father, both).

Alcohol consumption was examined in different forms: a dichotomous variable (ever/never), a quantitative variable (cut-offs defined *a priori* as following: nil, <1, 1–2 or >2 glasses per week), and by type (wine, beer or cider, spirits).

Statistical analyses

The odds ratios (OR) and their 95% confidence intervals (95% CI) were estimated using unconditional logistic regression models (SAS software; version 9; SAS Institute Inc., Cary, NC, USA).

All the analyses were adjusted for the matching variables age and sex, and for the study of origin. We tested the following factors to see if they met the empirical criteria for confounding (i.e. if they were independently associated with the exposure and the outcome): maternal age, paternal age, and maternal education, degree of urbanisation of the area of residency, birth-weight and birth-order. Only maternal age was retained in the final model. All the analyses on maternal and paternal smoking were mutually adjusted. The number of cigarettes smoked per day (CPD) was also analysed as continuous

Table 1. Characteristics of the cases and controls of the ESCALE and ESTELLE studies

	ESCALE (2003–2004)				ESTELLE (2010–2011)				POOLED			
	Cases		Controls		Cases		Controls		Cases		Controls	
	(n = 174)		(n = 949)		(n = 183)		(n = 834)		(n = 357)		(n = 1,783)	
	N	%	N	%	N	%	N	%	N	%	N	%
MYCN status												
Non-amplified	131	*75.3*			139	*76.0*			270	75.6		
Amplified	34	*19.5*			30	*16.4*			64	17.9		
Missing	9	*5.2*			14	*7.6*			23	6.4		
Age (years)												
‹1	74	*42.5*	187	*19.7*	70	*38.2*	188	*22.5*	144	*40.3*	375	*21.0*
1	36	*20.7*	182	*19.2*	37	*20.2*	123	*14.7*	73	*20.4*	305	*17.0*
2	30	*17.2*	153	*16.1*	35	*19.1*	148	*17.7*	65	*18.2*	301	*16.9*
3	13	*7.5*	166	*17.5*	21	*11.5*	139	*16.7*	34	*9.5*	305	*17.1*
4	11	*6.3*	145	*15.3*	12	*6.6*	131	*15.7*	23	*6.4*	276	*15.5*
5	10	*5.7*	116	*12.2*	8	*4.4*	105	*12.6*	18	*5.0*	221	*12.4*
‹18 months	93	*53.4*	283	*29.8*	95	*51.9*	261	*31.3*	188	*52.7*	544	30.5
≥18 months	81	*46.5*	666	*70.2*	88	*48.1*	573	*68.7*	169	*47.3*	1,239	*69.5*
Maternal age at child's birth (years)												
‹25	31	*17.8*	80	*8.4*	25	*13.7*	86	*10.3*	56	*15.7*	166	*9.3*
25–29	61	*35.1*	314	*33.1*	70	*38.2*	250	*30.0*	131	*36.7*	564	*31.6*
30–34	54	*31.0*	360	*37.9*	50	*27.3*	293	*35.1*	104	*29.1*	653	*36.6*
≥35	28	*16.1*	195	*20.5*	38	*20.8*	205	*24.6*	66	*18.5*	400	*22.4*
Maternal education												
‹Baccalaureate	55	*31.6*	319	*33.6*	50	*27.3*	203	*24.3*	105	*29.4*	522	*29.3*
Baccalaureate	32	*18.4*	195	*20.5*	44	*24.0*	192	*23.0*	76	*21.3*	387	*21.7*
›Baccalaureate	87	*50.0*	435	*45.8*	88	*48.1*	439	*52.6*	175	*49.0*	874	*49.0*
Missing	0	*0*	0	*0*	1	*0.5*	0	*0*	1	*0.3*	0	*0*
Size of urban unit of residence (population)												
‹5,000	57	*32.8*	360	*37.9*	59	*32.2*	351	*42.1*	116	*32.5*	711	*39.9*
5,000–99.999	36	*20.1*	211	*22.2*	43	*23.5*	174	*20.9*	79	*22.1*	385	*21.6*
100.000–1.999.999	41	*23.6*	233	*24.5*	50	*27.3*	158	*18.9*	91	*25.5*	391	*21.9*
Paris unit	37	*21.3*	145	*15.3*	30	*16.4*	149	*17.9*	67	*18.8*	294	*16.5*
Missing	3	*1.7*	0	*0*	1	*0.5*	2	*0.2*	4	*1.1*	2	*0.1*

Table 2. Association between neuroblastoma and parental smoking for the whole sample and according to age group and to MYCN amplification status. Pooled analysis of the ESCALE and ESTELLE studies.

	Total			<18 months			≥18 months			MYCN amplification (0-6 years)			
	Cases (%) N=357	Controls (%) N=1783	OR[2] [95% CI]	Cases (%) N =188	Controls (%) N=544	OR[2] [95% CI]	Cases (%) N=169	Controls (%) N=1239	OR[2] [95% CI]	Yes (%) N=64	OR[1] [95% CI]	No (%) N=270	OR[1] [95% CI]
Maternal smoking during pregnancy													
None	75.9	80.3	Reference	75.0	82.2	Reference	76.9	79.4	Reference	73.4	Reference	76.7	Reference
Any	24.1	19.7	1.3 [0.9-1.7]	25.0	17.8	1.4 [0.9-2.2]	23.1	20.5	1.1 [0.7-1.7]	26.6	1.5 [0.8-2.7]	23.3	1.2 [0.8-1.7]
<5 CPD	9.0	7.2	1.2 [0.7-1.8]	10.1	7.5	1.3 [0.7-2.4]	7.7	7.0	1.1 [0.6-2.1]				
5-9 CPD	7.3	6.0	1.3 [0.8-2.2]	7.4	4.6	1.8 [0.9-3.8]	7.1	6.6	1.1 [0.6-2.0]				
≥10 CPD	5.3	4.8	1.3 [0.7-2.2]	5.3	4.0	1.4 [0.6-3.1]	5.3	5.2	1.1 [0.5-2.3]				
Per 5 CPD increase			1.1 [1.0-1.3]			1.03 [0.98-1.09]			1.01 [0.96-1.07]				
Paternal smoking in the year before birth													
None	53.5	57.9	Reference	52.7	62.3	Reference	54.4	55.9	Reference	51.6	Reference	54.1	Reference
Any	44.5	40.8	1.1 [0.9-1.4]	45.7	37.1	1.2 [0.8-1.8]	43.2	42.4	1.0 [0.7-1.4]	46.9	1.3 [0.8-2.1]	43.7	1.2 [0.9-1.5]
<10 CPD	12.3	9.0	1.3 [0.9-2.0]	13.3	10.5	1.4 [0.8-2.4]	11.2	8.1	1.4 [0.8-2.4]				
10-15 CPD	14.8	16.1	0.9 [0.6-1.3]	16.0	15.6	1.2 [0.7-1.9]	13.6	16.4	0.8 [0.5-1.3]				
>15 CPD	18.5	16.3	1.3 [0.9-1.8]	17.5	11.2	1.8 [1.0-2.9]	19.5	18.5	1.1 [0.7-1.7]				
Per 10 CPD increase			1.00 [0.99-1.02]			1.01 [0.98-1.03]			0.99 [0.97-1.01]				
Maternal smoking during pregnancy and paternal smoking in the year before birth													
Neither parent	48.7	52.6	Reference	47.9	56.4	Reference	49.7	50.0	Reference	46.9	Reference	49.3	Reference
Only mother	4.8	6.0	0.9 [0.5-1.6]	4.8	5.9	1.0 [0.5-2.3]	4.7	6.0	0.8 [0.4-1.7]	4.7	1.0 [0.3-2.9]	4.8	0.9 [0.5-1.7]
Only father	25.8	27.8	1.0 [0.8-1.4]	26.1	25.4	1.2 [0.8-1.8]	25.4	28.3	0.9 [0.6-1.3]	25.0	1.0 [0.5-1.9]	25.9	1.0 [0.7-1.4]
Both parents	18.8	13.5	1.5 [1.1-2.1]	19.7	11.8	1.9 [1.2-3.1]	17.7	14.0	1.2 [0.8-1.9]	21.9	1.8 [0.9-3.5]	17.8	1.4 [1.0-2.1]

Pooled analysis of the ESCALE and ESTELLE studies.
[1]Odd ratio (OR) and 95 confident intervals (95% CI) estimated by unconditional logistic regression adjusted for age, sex, maternal age and study of origin.
[2]OR and 95%CI estimated by Polytomus logistic regression, adjusted for age, sex, maternal age and study of origin.

Table 3. Association between neuroblastoma and maternal alcohol consumption during pregnancy

	Cases (n = 357)		Controls (n = 1,783)		
	N	%	N	%	OR [95% CI]
Maternal alcohol drinking during pregnancy					
Never	268	*75.1*	1,293	*72.5*	Reference
Ever	88	*24.6*	490	*27.2*	1.0 [0.8–1.4]
Missing	1	0.3	*0*		
Glasses per week					
None	268	*75.1*	1,293	*72.5*	Reference
‹1	50	*14*	225	*12.6*	1.1 [0.8–1.6]
1–2	12	*3.4*	110	*6.2*	0.6 [0.3–1.2]
›2	22	*6.2*	143	*8.0*	1.0 [0.6–1.6]
	4	*1.1*	*12*	*0.7*	
Types of alcohol					
Wine	54	*15.1*	330	*18.5*	0.9 [0.6–1.3]
Beer or cider	34	*9.5*	167	*9.4*	1.2 [0.8–1.9]
Spirits	31	*8.7*	189	*10.6*	0.8 [0.5–1.2]

Pooled analysis of the ESCALE and ESTELLE studies.
Odd ratio (OR) and 95 confident intervals (95% CI) estimated by unconditional logistic regression adjusted for age, sex, maternal age and study of origin.

variable for an increase of 5 CPD for mothers and 10 CPD for fathers.

We systematically tested between-study heterogeneity by fitting an interaction term between the study and the exposure of interest and we explored the differences of each of the key exposure variables by study among the controls to see if there had been changes in parental behaviours between the study time periods. Because the findings by individual studies were similar, only the results for the pooled analyses are presented in this paper.

The tests for trend were computed for each quantitative variable of interest (number of cigarettes per day and number of glasses of alcohol per week). First, the deviation from linearity was tested by a likelihood ratio test, comparing the model with the newly generated quantitative variable, where subjects in each class of the categorical variables were assigned the median value of that class, to the full model with the categorical variable. If linearity was not rejected, the *p* value of the trend was determined by testing the slope of the quantitative variable using a Wald-test.

Additional analyses were conducted among subgroups of NB defined by age at diagnosis (<18 months/ ≥18 months) and by tumour MYCN oncogene amplification status (MYCN- / MYCN+), which are established clinically relevant entities. Polytomus logistic regression was used to estimate ORs by NB MYCN status.

Sensitivity analyses were conducted excluding cases whose families did not have a landline since controls were recruited from a sample of landline telephone numbers (data only collected for the ESTELLE study).

We then conducted a meta-analysis of our findings on maternal smoking and alcohol consumption during pregnancy with published findings of relevant previous studies performed flowing the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (see Supporting Information Material 1: Systematic Review study protocol and findings). We searched PubMed and Embase for original studies published from inception to October 2018. We used random effects, precision-based weighting to calculate the summary OR with our results. Statistical heterogeneity between studies was assessed using the Cochrane Q test. We assessed publication bias *via* inspection of the funnel plot and formal testing for funnel plot asymmetry using Egger's test.

Results

The study included 357 NB cases younger than 6 years (174 from ESCALE and 183 from ESTELLE) and 1,783 controls (949 from ESCALE and 834 from ESTELLE). The proportion of cases and controls aged less than 18 months was 53 and 30%, respectively (Table 1). MYCN was amplified in 11% of the cases younger than 18 months at diagnosis and 25% of the older cases.

Case–control comparability

Because the control sampling was performed in both studies to enable analyses of multiple childhood cancer diagnoses, the NB cases were younger than the controls (Table 1). There were at least two controls for each case in each age stratum. The cases had younger mothers and were more often living in an urban area than the controls.

Parental smoking

Maternal smoking during pregnancy was slightly more often reported for the cases (24.1%) than for the controls (19.7%),

Table 4. Characteristics of studies included in meta-analyses of maternal smoking and alcohol consumption during pregnancy and risk of neuroblastoma

Study	Cases and controls selection				Maternal consumption during pregnancy (ever/never)				
Author, country,	Cases		Controls		Smoking		Alcohol drinking		
year of case accrual	Source	n	Source	n	Crude OR [95% CI][1]	Adjusted OR [95% CI]	Crude OR [95% CI][1]	Adjusted OR [95% CI]	Matched factors/Adjustments
Data obtained by record linkage									
Johnson *et al.*,2008 [9]	Cancer Registry	155	Birth Registry	8,752	1.4 [0.9–2.2]	1.4 [0.9–2.3]	1.1 [0.4–3.5]	-	Year of birth, sex
USA (1976–2004)	Minnesota state								
Chow *et al.*, 2003 [13]	Cancer Registry	240	Birth Registry	2,400	0.8 [0.6–1.2]	0.8 [0.6–1.2]	-	-	Year of birth, sex/ gestational age, birth weight, parental age, ethnicity, maternal residence
USA (1980–1992)	Washington state								
McLaughlin *et al.*, 2009	Cancer Registry	529	Birth Records	12,010	-	1.0 [0.7–1.3]	-	1.2 [0.5–2.6]	Date of birth, sex
USA (1985–2001) [12]	New York state								
Stavrou *et al.*, 2009 [17]	Cancer Registry	122	Midwives data collection	1,045,966	0.8 [0.5–1.3]	1.0 [0.6–1.7]	-	-	Children age and sex, maternal age, birth weight, gestational age, socioeconomics, maternal hypertension, gestational diabetes, preeclampsia
Australia, (1994–2005)	New South Wales								
Heck *et al.*, 2016 [19]	Cancer Registry	238	Birth certificates	40,356	1.1 [0.5–2.4]	1.2 [0.5–2.5]	-	-	Year of birth/ maternal ethnicity, maternal education
USA (2007–2013)	California state								
Data obtained by Interview									
Kramer *et al.*, 1987 [7]	Cancer Registry	93	General population (RDD)	93	1.3 [0.8–2.1]		1.4 [0.9–2.2]		Date of birth, race, area code,
USA (1970–1979)	Great Delaware valley								
Buck *et al.*, 2001 [8]	Cancer Registry	155	Birth Registry	310	1.3 [0.8–2.1]	1.4 [0.9–2.1]	1.2 [0.8–1.9]	1.2 [0.8–1.9]	Year of birth, parity, maternal age, smoking and alcohol consumption
USA (1976–1987)	New York state								
Sorahan *et al.*, 1994	Cancer Registry	93	Birth Registry	93	-	1.0 [0.8–1.3]	-	-	Date of birth, sex
UK (1977–1981) [10]									
Schwartzbaum *et al.*, 1992 [11]	Hospital Cancer Registry	101	Hospital Cancer Registry	690	-	1.9 [1.1–3.2]	-	0.7 [0.4–1.1]	Age, race, maternal age, social class, exposure to x-ray, miscarriage, others (not specified)
USA (1979–1986)									
Schuz *et al.*, 2001 [14]	Cancer Registry	183	Residents database	1,785	1.4 [1.0–1.9]	-	0.9 [0.6–1.3]	-	age, sex, year of birth /SES, degree of urbanisation
Germany (1988–1993)									
Pang *et al.*, 2003 [15]	Cancer Registry	188	Family Health Services database	6,987	-	0.9 [0.6–1.3]	-	-	age, sex, parental age, deprivation score
UK (1992–1994)									

(Continues)

Table 4. Characteristics of studies included in meta-analyses of maternal smoking and alcohol consumption during pregnancy and risk of neuroblastoma (Continued)

Study	Cases and controls selection				Maternal consumption during pregnancy (ever/never)			
Author, country,	Cases		Controls		Smoking		Alcohol drinking	
year of case accrual	Source	n	Source	n	Crude OR	Adjusted OR [95% CI]	Crude OR	Adjusted OR [95% CI]	Matched factors/Adjustments
Yang *et al.*, 2000 [16]	Oncology Group	538	General population (RDD)	538	1.2 [0.9–1.6]	1.1 [0.8–1.4]	1.1 [0.9–1.4]	1.1 [0.8–1.4]	date of birth /sex, race, maternal education, household income in the birth year
USA (1992–1994)									
Parodi *et al.*, 2014 [18]	Oncology Group	153	National health service database	1,044	1.4 [0.9–2.2]	1.2 [0.7–2.1]	-	-	Gender, date of birth, area of residence/ maternal age and maternal education
Italy (1998–2001)									
France (2003–2004 and 2010–2011)	Cancer Registry	357	General population (RDD)	1,783	1.3 [1.0–1.7]	1.3 [0.9–1.7]	0.9 [0.7–1.1]	1.0 [0.8–1.4]	age, sex, maternal age, study of origin

[1]Calculated when raw numbers were stated in the published paper.

with an OR of 1.3 [95% CI 0.9–1.7] (Table 2). There was no trend with the average number of cigarettes smoked per day (OR for 5 CPD increase was 1.1 [95% CI 1.0–1.3]). The prevalence of smoking among mothers was similar between the ESCALE and ESTELLE studies. In the ESTELLE study, most of the mothers that reported having smoked during pregnancy started before conception and smoked during the whole pregnancy. Maternal smoking before and during pregnancy were highly correlated which precluded specific analysis by time window (Spearman's rho =0.69).

Paternal tobacco consumption during pregnancy was not associated with the risk of NB as an independent exposure (OR 1.1 [95% CI 0.9–1.4], but having both parents reported as having smoked during pregnancy was associated with NB (OR 1.5 [95% CI 1.1–2.1]) (Table 2). The maternal average daily consumption of cigarettes did not differ significantly according to whether only the mother or both parents were reported smokers (mean 5.7 CPD *vs.* 6.1 CPD, respectively). However, the percentage of mothers who smoked was higher when the fathers also smoked (34.4 *vs.* 10%, results not tabulated). The associations seemed to be only present among children younger than 18 months (OR 1.4 [95% CI 0.9–2.2]) *vs.* (OR 1.1 [95% CI 0.7–1.7]) among older children, but interaction with age was not significant (p-value for interaction 0.4). Although based on small numbers, the analyses did not reveal differences by MYCN status (Table 2).

Maternal alcohol consumption

Maternal alcohol consumption during pregnancy was not associated with the risk of NB (OR 1.0 [95% CI 0.8–1.4] (Table 3). There was not interaction between maternal smoking and alcohol consumption (p-value = 0.4). The results were similar with regards to different types of beverages and there was no increasing risk with increasing alcohol consumption or differences by age at diagnosis or MYCN status.

Compared to the ESCALE study, fewer controls mothers reported alcohol intake during pregnancy in the ESTELLE study (34 *vs.* 20%; p-value <0.01).

Sensitivity analyses

Results remain unchanged in sensitivity analysis performed for the ESTELLE study excluding case children with no landline phone (see Supporting Information Table S1).

Meta-analysis

We identified 13 studies that provided data on maternal smoking during pregnancy and seven of then also presented data on maternal alcohol consumption. Their details and main findings are summarised in Table 4.

The summary OR for maternal smoking during pregnancy was 1.1 [95% CI 1.0–1.3] (Fig. 1). Between-study heterogeneity was low (I^2 = 17.3%). The funnel plot did not provide any evidence of publication bias and Egger test was not significant (p-value = 0.2).

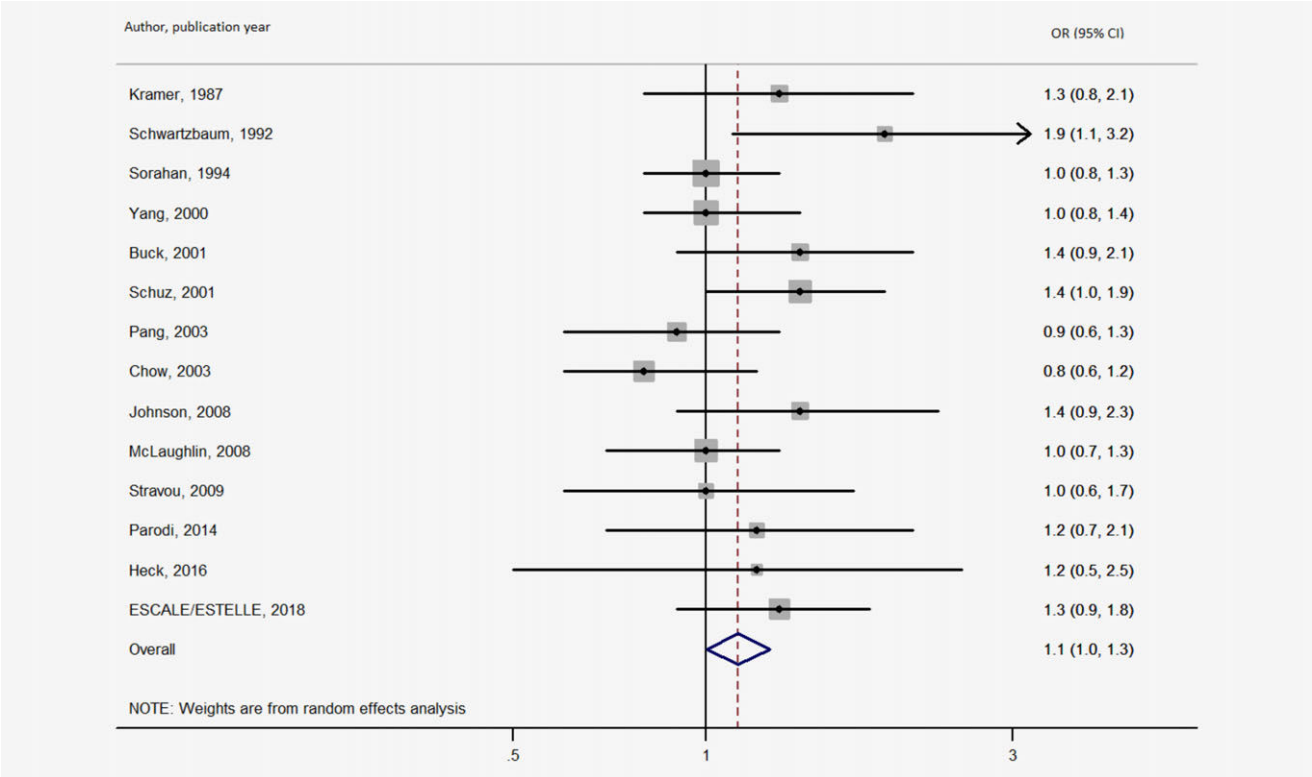


Figure 1. Forest plot of studies related to maternal smoking and neuroblastoma. [Color figure can be viewed at wileyonlinelibrary.com]

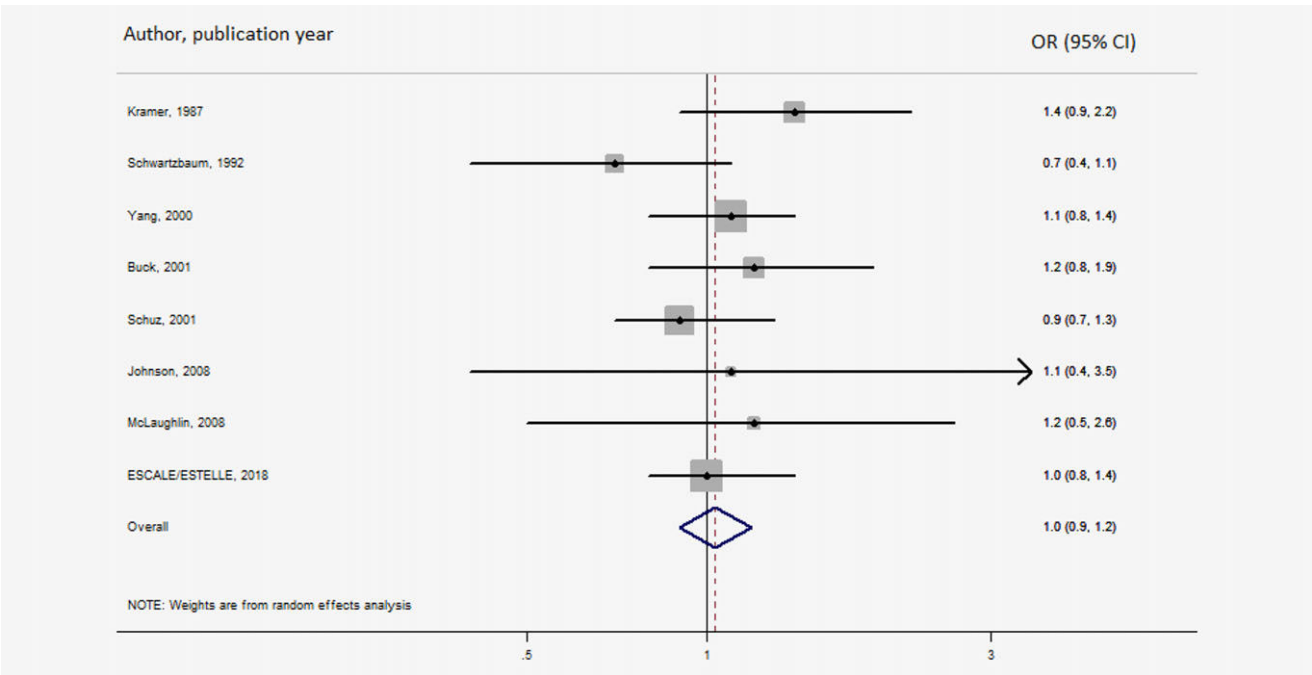


Figure 2. Forest plot of studies related to maternal alcohol consumption and neuroblastoma. [Color figure can be viewed at wileyonlinelibrary.com]

The analyses did not suggest any association between maternal alcohol consumption during pregnancy and neuroblastoma, with a summary OR of 1.0 [95% CI 0.9–1.2] (Fig. 2).

Discussion

The main finding of the ESCALE and ESTELLE studies was the slight positive association between maternal smoking during pregnancy and the risk of NB. By contrast, no association with maternal alcohol consumption or paternal smoking around pregnancy was observed. Findings are consistent between the ESCALE and ESTELLE studies with regards to overall analysis and also subgroup analysis by MYCN status and age at diagnosis.

Our meta-analysis also suggested a slight but significant association between NB and maternal smoking during pregnancy. This is consistent with the two previous meta-analyses by Chu *et al.*[21] (OR = 1.3 [95% CI 1.0–1.6]) and Müller-Schulte *et al.*[20] (OR = 1.2 [95% CI 1.0–1.4]), which included almost the same studies. Our meta-analysis adds nearly 500 cases from a recent Australian study[17] and the ESCALE and ESTELLE studies.

Given the rarity of NB, case–control is the most feasible design. While there is potential risk of measurement error with self-report, this may not be a major problem in the present context where the delay between exposure and interview is short. A Swedish validation study performed by Mattson *et al.*[25] found high level of agreement between mothers' self-reported smoking habits during pregnancy and their levels of serum cotinine.

Our study is the first to report stratified analysis by age at diagnosis and that the association with maternal smoking during pregnancy seemed to be stronger among younger children. These findings should be interpreted with caution given the small numbers and overlapping on the confident intervals. However, further investigation on these differences is needed given the clinical heterogeneity of NB at different ages, which could indicate differences in the aetiology. MYCN status was also accounted for by Yang *et al.*[16], whose findings were consistent with ours, which does not support the potential for maternal smoking to act through different pathways in the two subtypes.

Paternal tobacco smoking has been shown to be associated with increases in DNA damage, aneuploidies, and mutations in sperm and may act as a human germ cell mutagen.[26] Despite this, our study did not support the link between paternal smoking around pregnancy and the risk of NB as did two previous studies.[15,16] In our study, paternal smoking was assessed through the maternal report of the father's tobacco consumption and we cannot exclude misclassification bias. However, the extent of the bias is limited since in the subset used for validation, agreement between maternal and paternal responses was high with regards to both ever smoking and number of cigarettes smoked per day.[24,27]

The meaning of the apparent stronger association between maternal smoking and NB when both parents smoked is still unclear. We first hypothesised that when both parents smoked the mothers may have been heavier smokers, but there was no difference with regards to the quantity of cigarettes smoked per day. However, the prevalence of tobacco consumption was higher among mothers when the fathers also smoke. Yang *et al.*[16] also found stronger association when both parents smoked (OR 1.3 [95% CI 0.9–2.0]) compared to only mothers (OR 1.1 [95% CI 0.8–1.4].

Consistent with our findings, previous studies did not support the link between maternal alcohol intake during pregnancy and NB.[8,9,11,16,17]

Our study has several strengths. Since the ESCALE and ESTELLE studies were designed to be pooled, with uniformly defined exposures, this is one of the largest studies of NB at present. Cases were ascertained by a nationwide cancer registry, which has a high degree of completeness. The overall participation rate of the controls was high (77% of eligible controls) and we also adjusted our analysis for factors that might be associated with control participation.

However, our study has the same limitations as all case–control interview-based studies, with an inherent risk of selection and recall bias. Although there is potential for selection bias, the prevalence of reported smoking among control parents appears to be representative of the source population, both in terms of the prevalence and time trends among women and men of similar ages in the Perinatal National surveys conducted during the same time periods.[28]

We attempted to reduce recall bias by the use of computer-assisted standardised interviews conducted by trained interviewers. This may not totally prevent mothers of cases thinking more deeply and reporting the exposures more frequently than control mothers. However, the opposite may also be possible. Deleterious effects of smoking and alcohol consumption during pregnancy are well known and case mothers may under-report such behaviour. However, a recent validation study found high level of agreement between self-reported data on smoking during pregnancy and medical records.[29]

In conclusion, our findings provide some evidence of an association between maternal smoking during pregnancy and NB. These findings are consistent with literature and add another reason to recommend that women refrain from smoking during pregnancy.

References

1. Lacour B, Guyot-Goubin A, Guissou S, et al. Incidence of childhood cancer in France: National Children Cancer Registries, 2000-2004. *Eur J cancer Prev* 2010;19:173–81.
2. Thompson D, Vo KT, London WB, et al. Identification of patient subgroups with markedly disparate rates of MYCN amplification in neuroblastoma: a report from the international Neuroblastoma risk group project. *Cancer* 2016; 122:935–45.
3. Shohet J, Foster J. Neuroblastoma. *BMJ* 2017;357: j1863.

4. IARC Working Group on the Evaluation of Carcinogenic Risks In Humans. A review of human carcinogens. Part E: personal habits and indoor combustions. *IARC Monogr Eval Carcinog Risks Hum* 2012;100:43–309.
5. Lyon F. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans VOLUME 96 Alcohol Consumption and Ethyl Carbamate. 2010;
6. Hansen C, Asmussen I, Autrup H. Detection of carcinogen-DNA adducts in human fetal tissues by the 32P-postlabeling procedure. *Environ Health Perspect* 1993;99:229–31.
7. Kramer S, Ward E, Meadows AT, et al. Medical and drug risk factors associated with neuroblastoma: a case-control study. *J Natl Cancer Inst* 1987;78:797–804.
8. Buck GM, Michalek AM, Chen CJ, et al. Perinatal factors and risk of neuroblastoma. *Paediatr Perinat Epidemiol* 2001;15:47–53.
9. Johnson KJ, Puumala SE, Soler JT, et al. Perinatal characteristics and risk of neuroblastoma. *Int J Cancer* 2008;123:1166–72.
10. Sorahan T, Lancashire R, Prior P, et al. Childhood cancer and parental use of alcohol and tobacco. *Ann Epidemiol* 1995;5:354–9.
11. Schwartzbaum JA. Influence of the mother's prenatal drug consumption on risk of neuroblastoma in the child. *Am J Epidemiol* 1992;135:1358–67.
12. McLaughlin CC, Baptiste MS, Schymura MJ, et al. Perinatal risk factors for neuroblastoma. *Cancer Causes Control* 2009;20:289–301.
13. Chow EJ, Friedman DL, Mueller BA. Maternal and perinatal characteristics in relation to neuroblastoma. *Cancer* 2007;109:983–92.
14. Schüz J, Kaletsch U, Meinert R, et al. Risk factors for neuroblastoma at different stages of disease. Results from a population-based case-control study in Germany. *J Clin Epidemiol* 2001;54:702–9.
15. Pang D, McNally R, Birch JM. Parental smoking and childhood cancer: results from the United Kingdom childhood cancer study. *Br J Cancer* 2003;88:373–81.
16. Yang Q, Olshan AF, Bondy ML, et al. Parental smoking and alcohol consumption and risk of neuroblastoma. *Cancer Epidemiol Biomarkers Prev* 2000;9:967–72.
17. Stavrou EP, Deborah A, Baker F, Bishop JF. Maternal smoking during pregnancy and childhood cancer in New South Wales: a record linkage investigation.
18. Parodi S, Merlo DF, Ranucci A, et al. Risk of neuroblastoma, maternal characteristics and perinatal exposures: the SETIL study. *Cancer Epidemiol* 2014;38:686–94.
19. Heck JE, Contreras ZA, Park AS, et al. Smoking in pregnancy and risk of cancer among young children: a population-based study. *Int J Cancer* 2016;139:613–6.
20. Müller-Schulte E, Kurlemann G, Harder A. Tobacco, alcohol and illicit drugs during pregnancy and risk of neuroblastoma: Systematic review. *Arch Dis Child Fetal Neonatal Ed* 2018; 103:F467–73.
21. Chu P, Wang H, Han S, et al. Maternal smoking during pregnancy and risk of childhood neuroblastoma: systematic review and meta-analysis. *J Cancer Res Ther* 2016;12:999–1005.
22. Rios P, Bailey HD, Orsi L, et al. Risk of neuroblastoma, birth-related characteristics, congenital malformations and perinatal exposures: a pooled analysis of the ESCALE and ESTELLE French studies (SFCE). *Int J Cancer* 2016;139: 1936–48.
23. Steliarova-Foucher E, Stiller C, Lacour B, et al. International classification of childhood cancer, third edition. *Cancer* 2005;103:1457–67.
24. Bailey HD, Lacour B, Guerrini-Rousseau L, et al. Parental smoking, maternal alcohol, coffee and tea consumption and the risk of childhood brain tumours: the ESTELLE and ESCALE studies (SFCE, France). *Cancer Causes Control* 2017;28: 719–32.
25. Mattsson K, Källén K, Rignell-Hydbom A, et al. Cotinine validation of self-reported smoking during pregnancy in the Swedish medical birth register. *Nicotine Tob Res* 2015;18:ntv087.
26. Beal MA, Yauk CL, Marchetti F. From sperm to offspring: assessing the heritable genetic consequences of paternal smoking and potential public health impacts. *Mutat Res Mutat Res* 2017;773: 26–50.
27. Orsi L, Rudant J, Ajrouche R, et al. Parental smoking, maternal alcohol, coffee and tea consumption during pregnancy, and childhood acute leukemia: the ESTELLE study. *Cancer Causes Control* 2015;26:1003–17.
28. Blondel B, Kermarrec M. Enquete nationale perinatale 2010 les naissances en 2010 et leur evolution depuis 2003 Rapport rédigé par Enquête réalisée avec la participation, 2011.
29. Mattsson K, Källén K, Rignell-Hydbom A, et al. Cotinine validation of self-reported smoking during pregnancy in the Swedish medical birth register. *Nicotine Tob Res* 2016;18:79–83.

SUPPLEMENTARY MATERIAL

Parental smoking, maternal alcohol consumption during pregnancy and the risk of neuroblastoma in children: Systematic Review and Meta-analyses

METHODS

This systematic review and meta-analysis was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Table 1). Please note that the Table and Figure numbering refers to material included in this document and does not refer to material presented in the main paper.

Search Strategy

The main author (PR) worked in study selection, data abstraction and assessment of study quality of the systematic review process. In case of doubts about study inclusions or estimates extraction, these were discussed with co-authors (JC and HB).

The Medline and Embase database were searched from inception to October 2018 using the following combination of appropriate key words in a search algorithm:

(Maternal exposure OR prenatal exposure OR perinatal exposure OR perinatal characteristics OR perinatal risk factors OR prenatal risk factors OR risk factors OR exposure during pregnancy OR parental exposure OR smoking OR tobacco OR air toxics OR alcohol consumption OR maternal smoking OR parental smoking) AND (childhood cancer OR neuroblastoma OR risk of neuroblastoma) NOT neuroblastoma cells.

Studies were included if they were published in English, French or Spanish. In addition, all references cited in original studies and reviews were manually searched.

Table 1: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
Title			
	1	Identify the report as a systematic review, meta-analysis, or both.	Identified as pooled analysis
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	# 3 MD
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	# 4 MD
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	# 5 SM
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Study protocol provided as supplementary material
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	#3 #6 SM
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	# 7 MD # 1 SM

Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	#2 SM
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Figure 1 SM
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	#1 #6 SM
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	# 5 MD
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	#7 SM and Table 5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Table 4 MD
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	# 7 and #8 SM
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Figure 5 SM
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	# 7
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1 SM
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 4 MD
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 5 SM
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figures 1 and 2 MD
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	#9 and 10 MD
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	

Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	#9 SM and Figure 6 SM
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	#10 MD
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	#11 MD
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	#11 MD
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

MD= Main document

SM= Supplementary material study protocol

Eligibility:

Selection criteria are summarized in Table 2:

Table 2: Selection criteria

Item	Description
Patient	All children < 19 years old diagnosed with neuroblastoma
Exposure	Maternal smoking or alcohol consumption during pregnancy
Comparison	Children without a diagnosis of neuroblastoma
Outcomes	OR, RR estimates with 95% CI or provided data that allowed these to be calculated)

OR= Odds ratio, RR= Relative risk, CI= Confidence interval

To be included in the meta-analysis, each study was required to:

1. Be an original report.

2. Be a cohort or case–control study that presented ORs and corresponding 95% CIs for the association between maternal smoking or alcohol consumption during pregnancy and risk of childhood neuroblastoma (or provide data that allowed these to be calculated).

3. Include pediatric neuroblastoma cases (less than 19 years old)

Exclusion criteria for studies were:

1. Studies reporting "parental" consumption without specifying whether this was maternal or paternal exposure.

2. Studies exclusively reporting maternal smoking or alcohol consumption but not related to around the pregnancy time window.

3. Studies reporting estimates on "All childhood cancers" but not specifying numbers or estimates for neuroblastoma.

Data extraction

Extracted study characteristics encompassed: publication year, study time frame, study design, study size, recruitment source, and source of exposure information, age at diagnosis, matching variables, effect measures and confounders.

Quality assessment

In the assessment of the quality of included studies, and as to evaluate comparability of cases and controls on the basis of design, age at diagnosis was set a priori as the most important matching or adjusting factor in the Newcastle-Ottawa Quality scale.

Bias assessment

In the assessment of the possibility of bias in study designs, conduct and analysis, we used the Joanna Briggs checklists for case-control studies.

Statistical analyses

A meta-analysis of the ESCALE and ESTELLE data on maternal smoking during pregnancy with published findings of relevant previous studies was conducted. We used random effects, precision-based weighting to calculate the summary OR with our results.

Statistical heterogeneity between studies was assessed using the Cochrane Q test.

The publication bias were assessed via inspection of the funnel plot and formal testing for funnel plot asymmetry using Egger's test.

Sensitivity analysis were performed by stratifying our analysis by study type (studies bases on record linkage/interview) were performed. Finally, studies with lower quality scores (Newcastle-Ottawa Quality scale <7) were excluded.

RESULTS

Results of Search Strategy

The results of the search strategy are summarized in Figure 1. Out of the 973 articles rendered from the algorithm-based search, 929 were deemed irrelevant according to their title or abstract. The full texts of the possibly eligible 41 remaining publications were obtained and assessed according to the eligibility criteria, leading to the exclusion of 28 studies for various reasons (Table 3).

Data synthesis and assessment of quality of studies

There were 13 eligible studies included in this meta-analysis (5 record linkage and 8 case-control studies). All 13 studies assessed maternal smoking during pregnancy, and were jointly analyzed with our original data from the ESCALE and ESTELLE French studies. The same procedure was performed with seven studies (2 record linkage and 5 case-control studies) that also reported information on maternal alcohol consumption.

Ratings according to the Newcastle-Ottawa Quality scale spanned between 6 and 9 (Table 4). Briefly, all the included studies ensured comparability of cases and controls on the basis of study design. Despite the variability of exposure ascertainment among included studies, the same data collection method was used for cases and controls within individual studies; no study, however, had validated alcohol consumption records or used structured interviews blinded as to case/control status. With regards to risk of bias assessment, five studies presented insufficient data regarding the comparability of cases and controls (Table 5). Evaluation of publication bias was analyzed using funnel plots, which did not provide any evidence of publication bias and Egger test was not significant (p-value= 0.2), but these findings should be interpreted with care because of small numbers of studies (Figure 3).

The summary OR for maternal smoking during pregnancy was 1.1 [95% CI 1.0-1.3] (Figure 2a). Between-study heterogeneity was low (I^2= 17.3 %). The summary ORs

were similar for the analysis including studies reporting unadjusted and adjusted ORs (Figure 2 b and c).

The analyses did not suggest any association between maternal alcohol consumption during pregnancy and neuroblastoma, with a summary OR of 1.0 [95% CI 0.9–1.2] (Figure 3 a) with similar findings among studies with unadjusted ORs only (Figure 3 b).

With regards to sensitivity analysis, slightly lower estimates were observed when only record linkage studies were included, compared with interview based studies (Figure 6). However, results may be interpreted with caution because based in only five studies. Results did not change when studies with less than 7 in the Newcastle-Ottawa Quality scale were excluded.

Figure 1: Flow diagram of search strategy

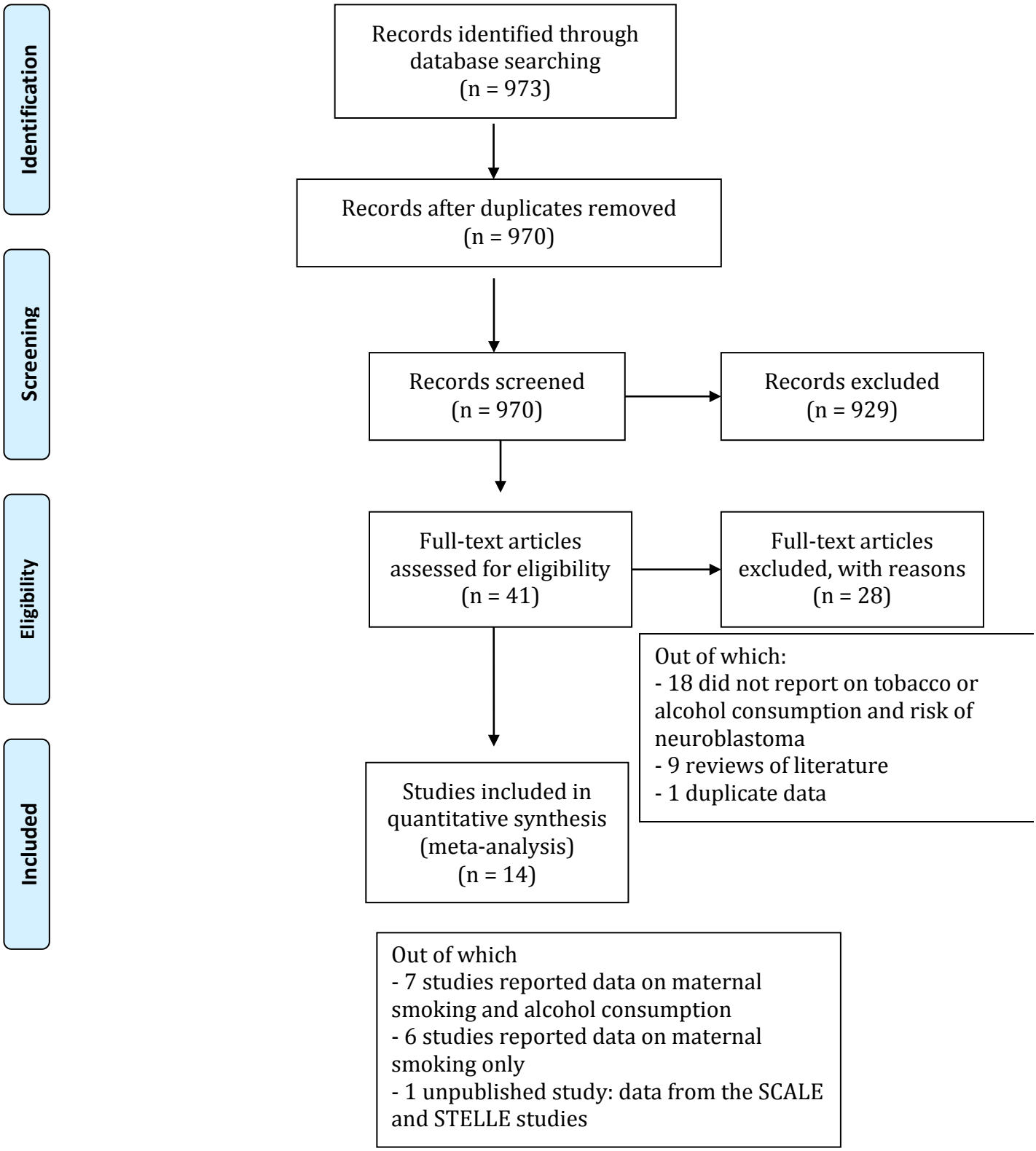


Table 3: excluded studies and reason for exclusion

Reason for exclusion	Excluded study
Did not report on tobacco or alcohol consumption during pregnancy and risk of neuroblastoma	Lavigne É, 2017. PMID: 28108116
	De Paula Silva N, 2016. PMID: 27768709
	Momen NC, 2016. PMID: 26689564
	Bhattacharya S, 2014. PMID: 24394797
	Magnani C, 2014. PMID: 25539823
	Heck JE, 2013. PMID: 24139061
	Heck JE, 2013. PMID: 24021746
	Ghosh JK, 2013. PMID: 23989198
	Massamba D, 2012. PMID: 22891530
	Bluhm E, 2008. PMID: 18798548
	Bluhm EC, 2006.PMID: 16633913
	Kerr MA, 2000.PMID: 10977108
	Olshan AF, 1999.PMID: 10547138
	Klebanoff MA, 1996PMID: 8942433
	Neglia JP, 1988.PMID: 3365650
	Grufferman S, 1983.PMID: 6646007
	Ortega-García JA, 2010PMID: 20412413
Review	Müller-Schulte E, 2017.PMID: 29162685
	Chu P, 2016. PMID: 27461688
	Infante-Rivard C, 2007. PMID: 18074306
	Polańska K, 2006. PMID: 17219803
	Ferrís i Tortajada J, 2005PMID: 15989872
	Ross JA, 2000. PMID: 11122848
	Sasco AJ, 1999. PMID: 10333301
	Bolande RP, 1999.PMID: 10191343
	McBride ML, 1998.PMID: 9654794
Duplicate data	Michaelis J, 1996. PMID: 8776703

Table 4: Study quality assessment using the Newcastle-Ottawa Quality scale.

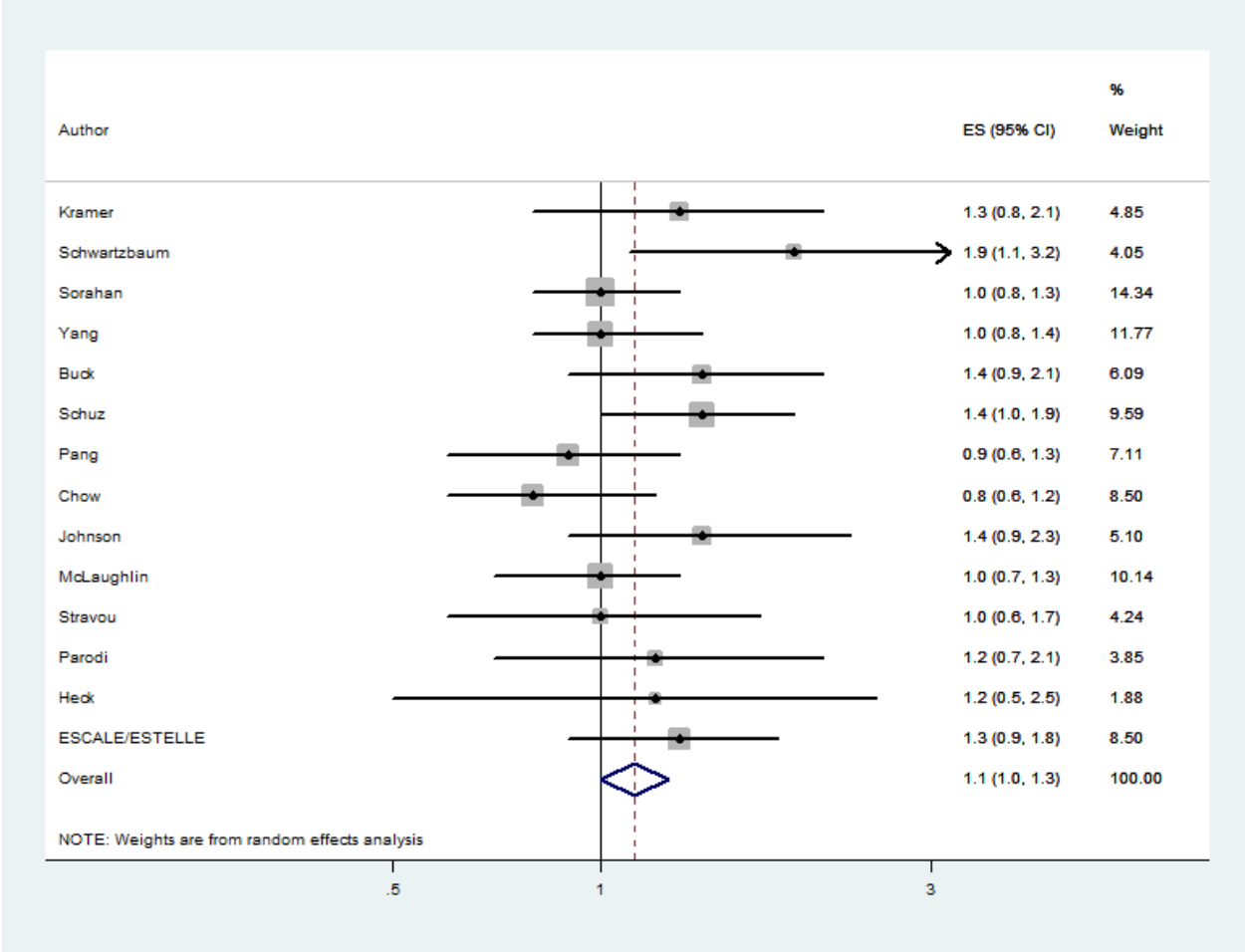
Study	Selection				Comparability		Outcome			Total
	Case definition	Representativeness of the cases	Selection of controls	Definition of controls	On age	On other risk factors	Assessment of exposure	Same method of ascertainment for cases and controls	Non-response rate	
Kramer et al, 1987	1	1	1	1	1	0	0	1	0	6
Buck et al, 2001	1	1	1	1	1	1	0	1	1	8
Johnson et al, 2008	1	1	1	1	1	0	1	1	1	8
Sorahan et al, 1994	1	1	1	1	1	0	0	1	1	7
Schwartzbaum et al, 1992	1	0	1	0	1	1	0	1	1	6
McLaughlin et al, 2009	1	1	1	1	1	0	1	1	1	8
Chow et al, 2003	1	1	1	1	1	1	1	1	1	9
Schuz et al, 2001	1	1	1	1	1	1	0	1	0	7
Pang et al, 2003	1	1	1	1	1	1	0	1	0	7
Yang et al, 2000	1	1	1	1	1	1	0	1	1	8
Stavrou et al, 2009	1	1	1	1	1	1	1	1	1	9
Parodi et al, 2014	1	1	1	1	1	1	0	1	1	8
Heck et al, 2016	1	1	1	1	1	1	1	1	1	9
Escale/Estelle	1	1	1	1	1	1	0	1	1	8

Table 5: Risk of bias assessment using the Joanna Briggs Institue Critical Appraisal tool for case-control studies.

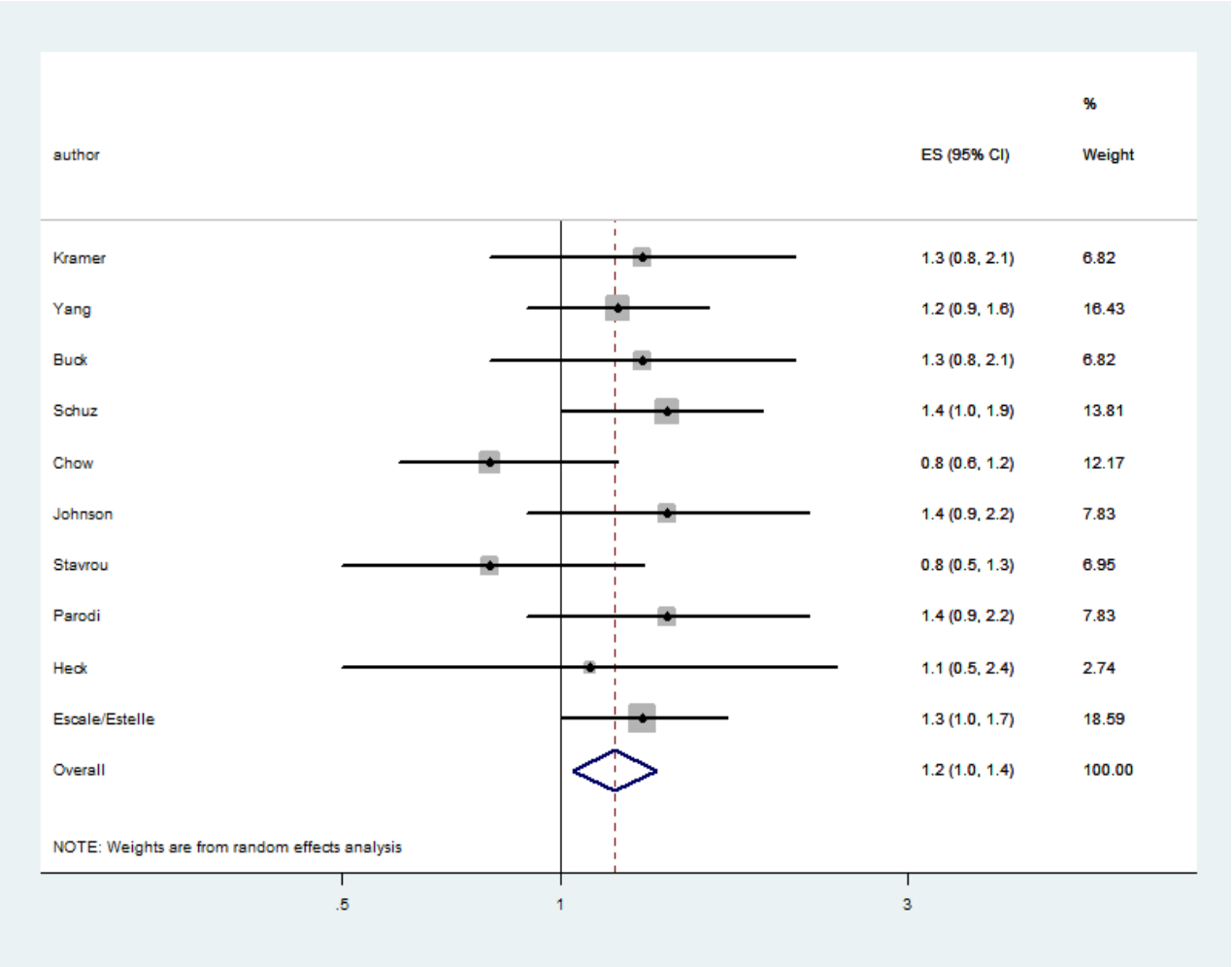
	Comparable groups Ca/Co	Ca/Co matched appropriately	Same criteria for identification of Ca/Co	Standard, valid and reliable measure of exposure	Exposure measured in the same way for Ca/Co	Confounding factors identified	Confounding factors stated	Standard, valid and reliable assessment of outcomes	Exposure period of interest long enough	Appropriate statistical analysis used
Kramer, 1987	Unclear	yes	yes	yes	yes	no	no	yes	yes	yes
Schwartzbaum, 1992	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Sorahan, 1994	Unclear	yes	yes	yes	yes	no	no	yes	yes	yes
Yang, 2000	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Buck, 2001	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Schuz, 2001	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Chow, 2003	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Pang, 2003	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Johnson, 2008	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
McLaughlin, 2008	Unclear	yes	yes	yes	yes	yes	yes	yes	yes	yes
Stavrou, 2009	Unclear	yes	yes	yes	yes	yes	yes	yes	yes	yes
Parodi, 2014	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Heck, 2016	Unclear	yes	yes	yes	yes	yes	yes	yes	yes	yes
ESCALE/ESTELLE 2018	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes

Figure 2. Forest plots describing the association between maternal smoking during pregnancy and risk of childhood neuroblastoma: (a) all studies (b) studies reporting unadjusted studies (c) studies reporting adjusted studies only.

(a)



(b)


author
ES (95% CI)
%
Weight
Kramer
1.3 (0.8, 2.1)
6.82
Yang
1.2 (0.9, 1.6)
16.43
Buck
1.3 (0.8, 2.1)
6.82
Schuz
1.4 (1.0, 1.9)
13.81
Chow
0.8 (0.6, 1.2)
12.17
Johnson
1.4 (0.9, 2.2)
7.83
Stavrou
0.8 (0.5, 1.3)
6.95
Parodi
1.4 (0.9, 2.2)
7.83
Heck
1.1 (0.5, 2.4)
2.74
Escale/Estelle
1.3 (1.0, 1.7)
18.59
Overall
1.2 (1.0, 1.4)
100.00
NOTE: Weights are from random effects analysis
.5
1
3

(c)

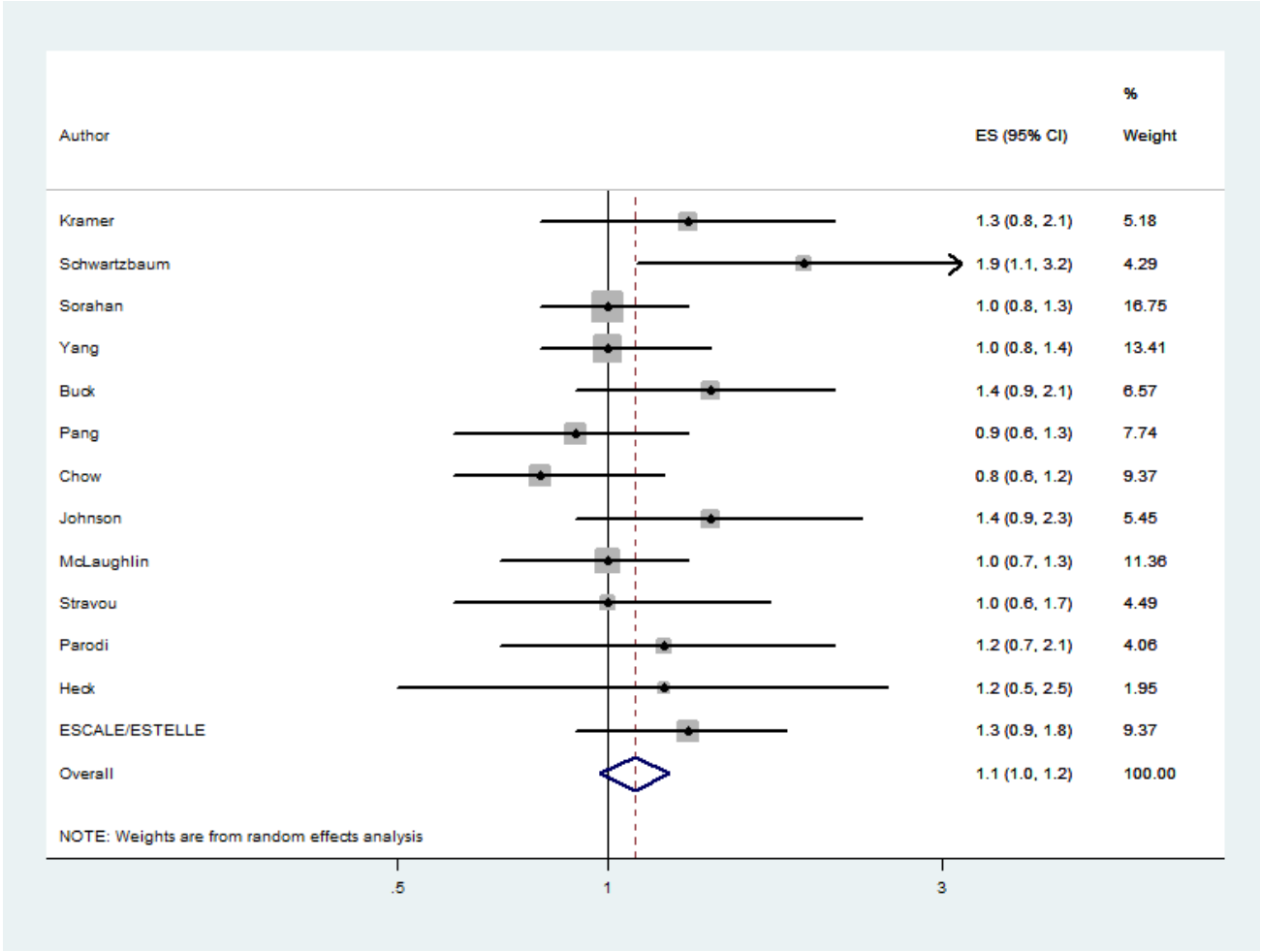
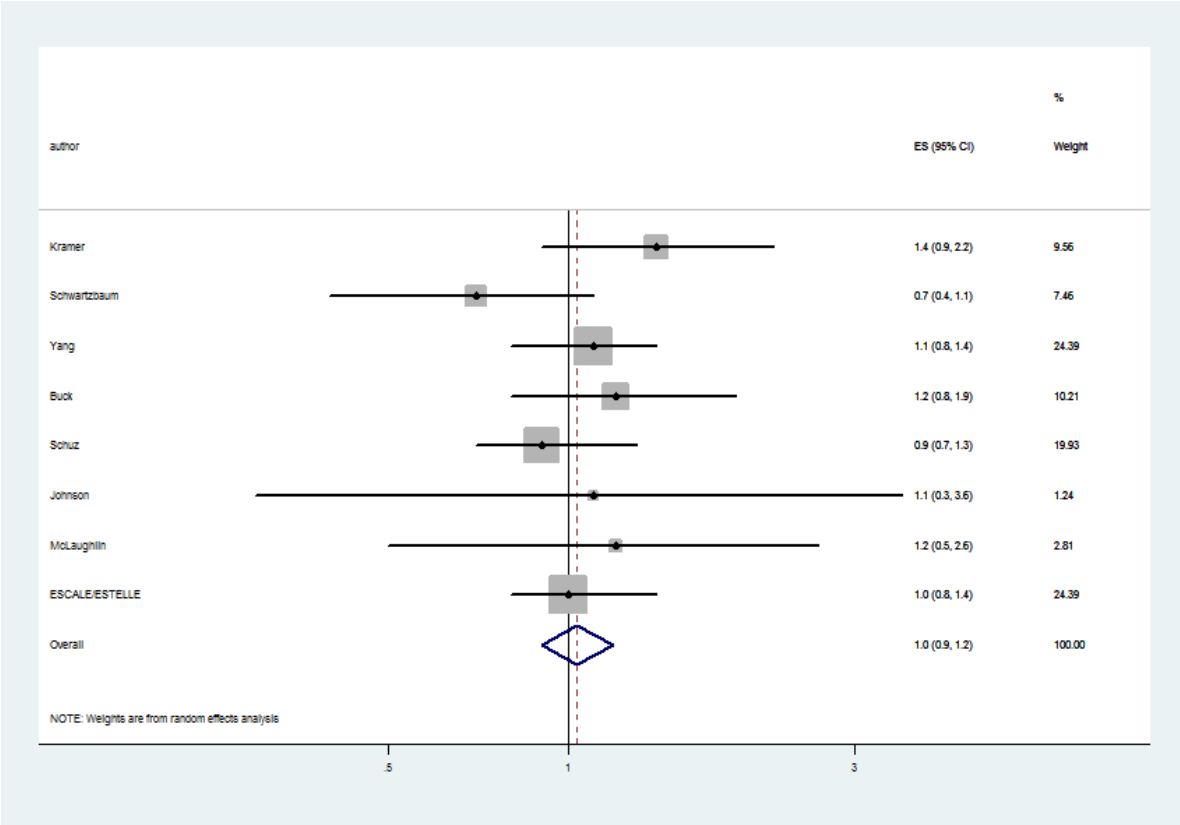

Author
ES (95% CI)
%
Weight
Kramer
1.3 (0.8, 2.1)
5.18
Schwartzbaum
1.9 (1.1, 3.2)
4.29
Sorahan
1.0 (0.8, 1.3)
16.75
Yang
1.0 (0.8, 1.4)
13.41
Buck
1.4 (0.9, 2.1)
6.57
Pang
0.9 (0.6, 1.3)
7.74
Chow
0.8 (0.6, 1.2)
9.37
Johnson
1.4 (0.9, 2.3)
5.45
McLaughlin
1.0 (0.7, 1.3)
11.36
Stravou
1.0 (0.6, 1.7)
4.49
Parodi
1.2 (0.7, 2.1)
4.06
Heck
1.2 (0.5, 2.5)
1.95
ESCALE/ESTELLE
1.3 (0.9, 1.8)
9.37
Overall
1.1 (1.0, 1.2)
100.00
NOTE: Weights are from random effects analysis
.5
1
3

Figure 4. Forest plots describing the association between maternal alcohol consumption during pregnancy and risk of childhood neuroblastoma: (a) all studies (b) studies reporting unadjusted studies.

(a)



(b)

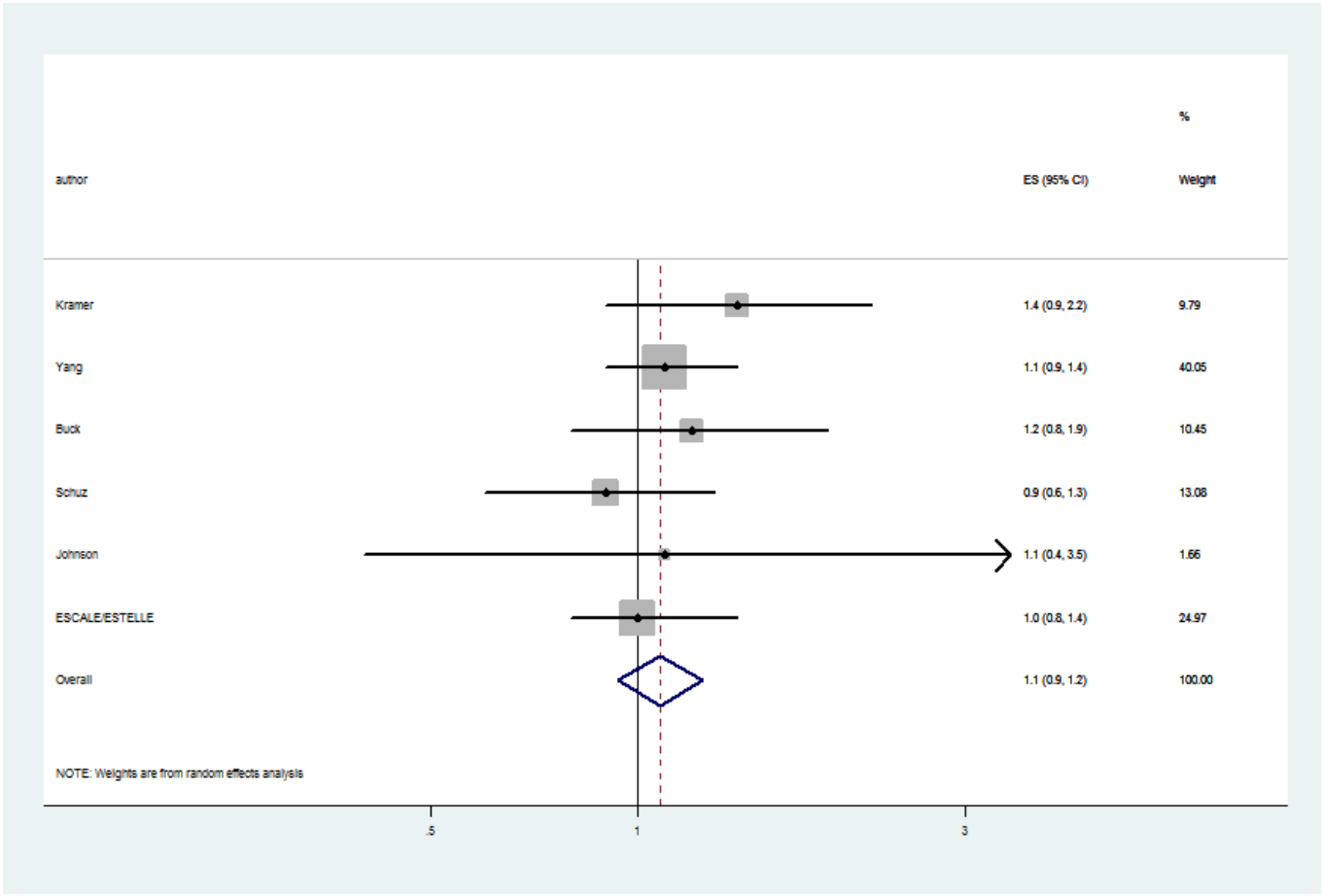
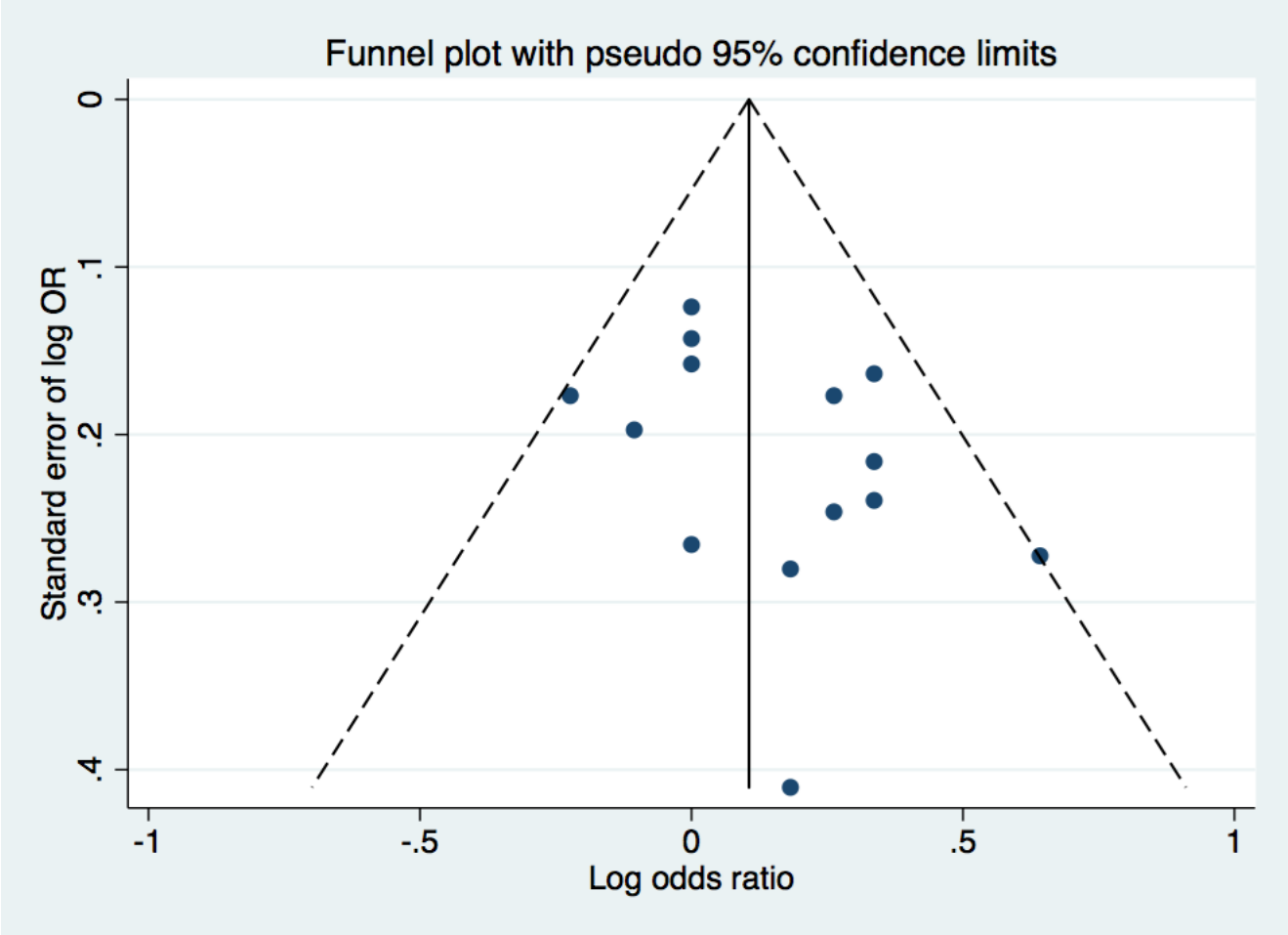

author
ES (95% CI)
%
Weight
Kramer
1.4 (0.9, 2.2)
9.79
Yang
1.1 (0.9, 1.4)
40.05
Buck
1.2 (0.8, 1.9)
10.45
Schuz
0.9 (0.6, 1.3)
13.08
Johnson
1.1 (0.4, 3.5)
1.66
ESCALE/ESTELLE
1.0 (0.8, 1.4)
24.97
Overall
1.1 (0.9, 1.2)
100.00
NOTE: Weights are from random effects analysis
.5
1
3

Figure 5: Funnel plot in studies reporting (a) maternal smoking or (b) alcohol consumption during pregnancy.

(a)



(b)

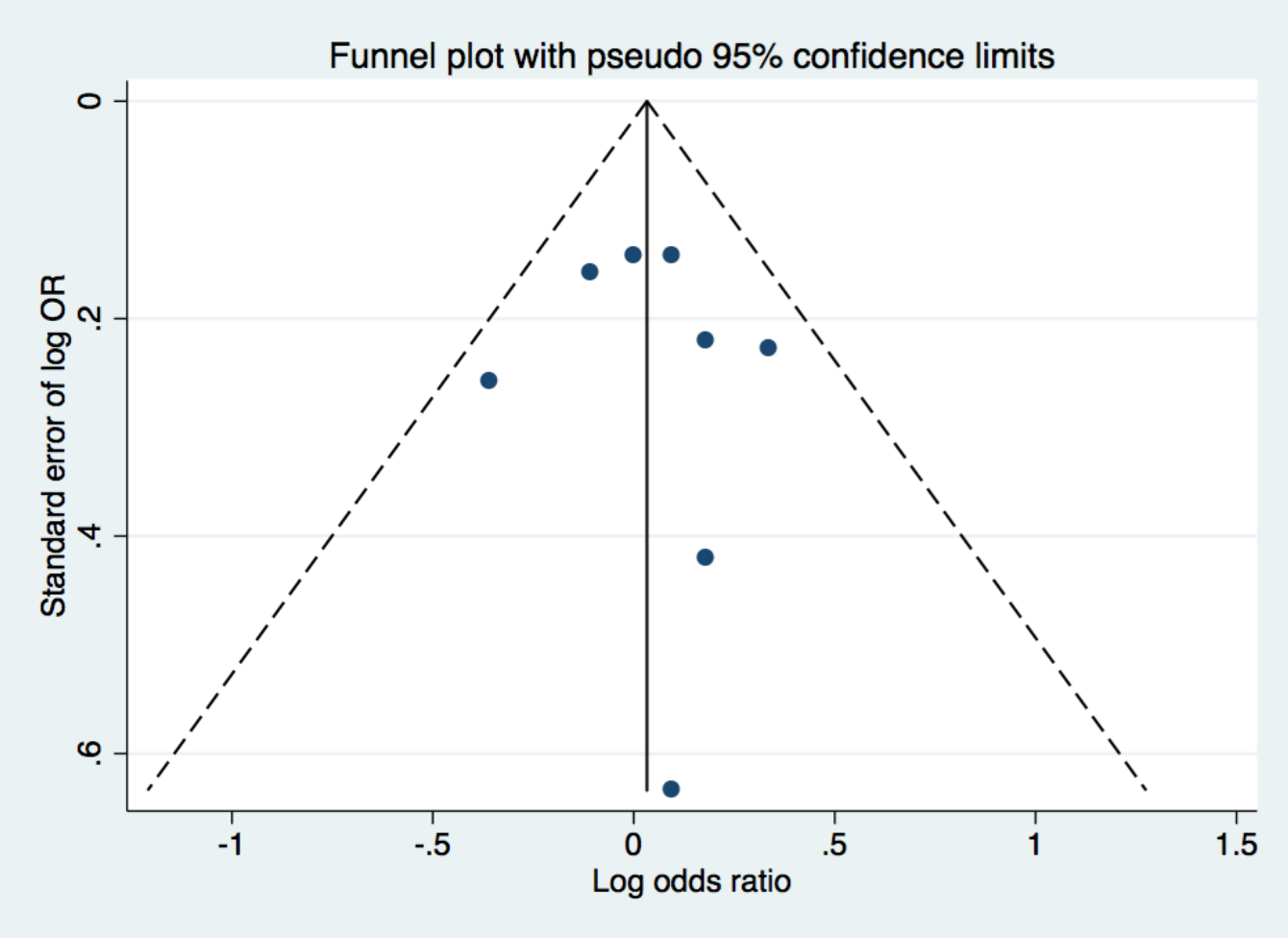
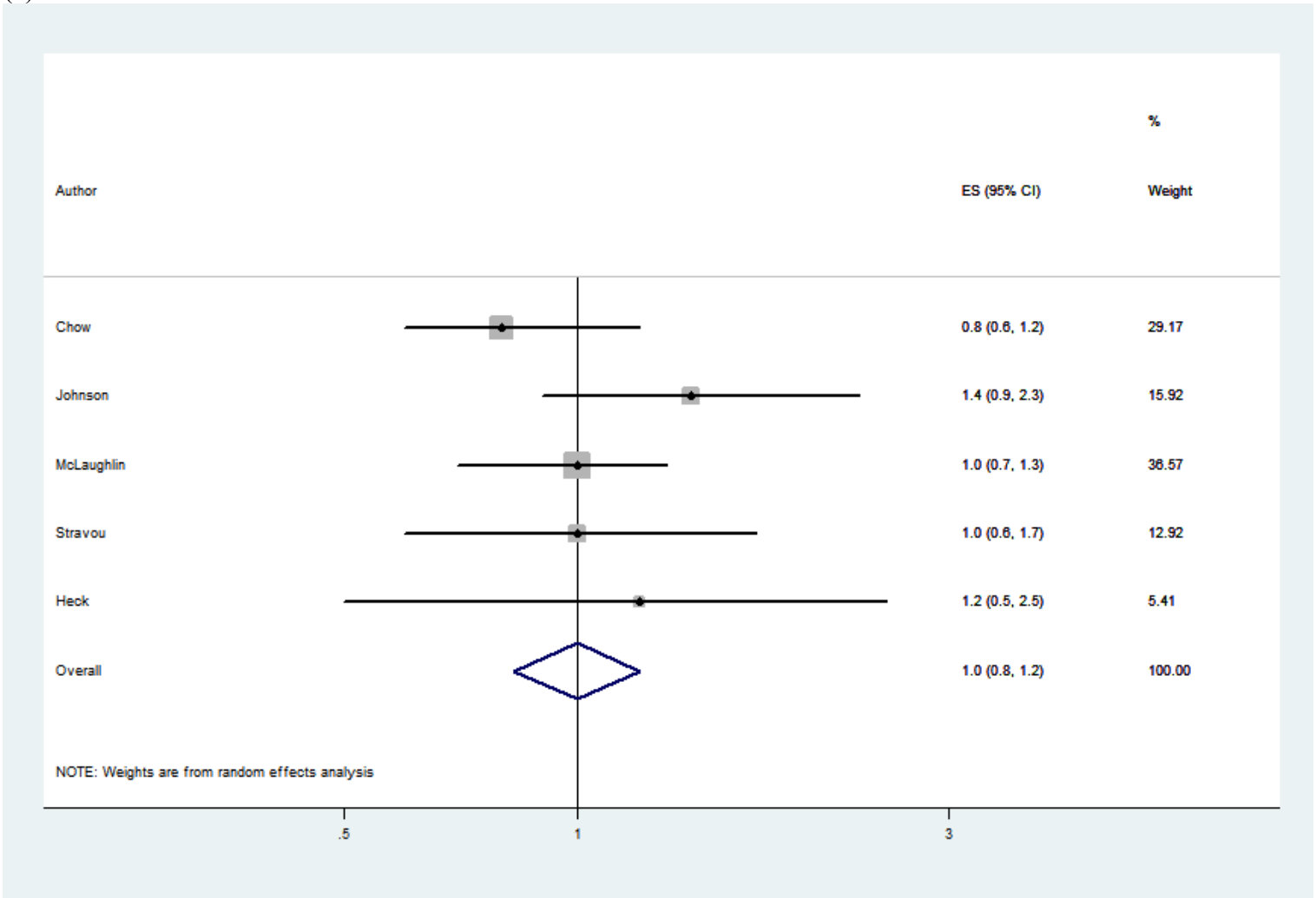
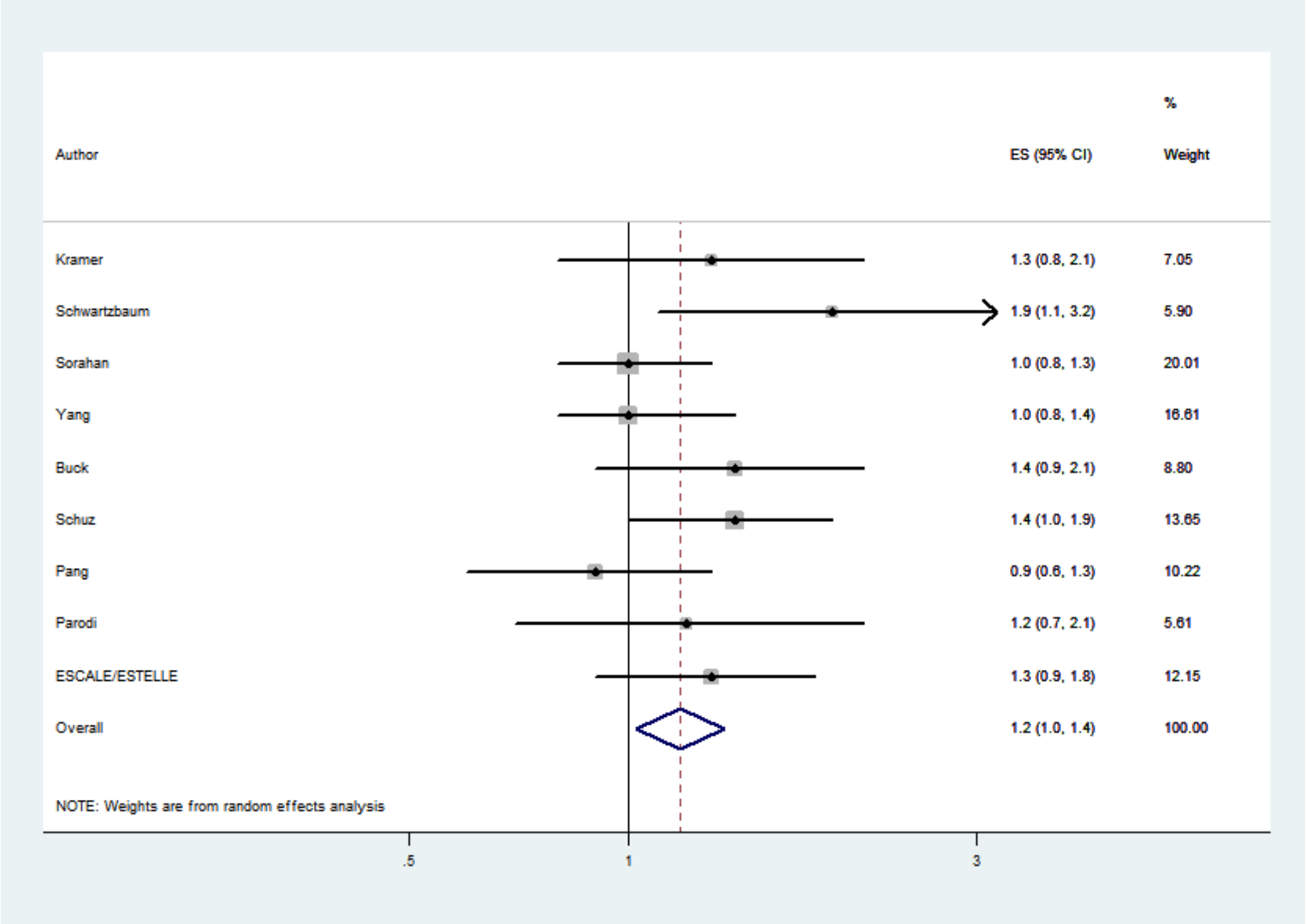

Funnel plot with pseudo 95% confidence limits
Standard error of log OR
0
.2
.4
.6
-1
-.5
0
.5
1
1.5
Log odds ratio

Figure 6: Sensitivity analysis. Stratified analysis by (a) record linkage or (b) interview based studies. (c) only studies with New Castle scale ≥ 7

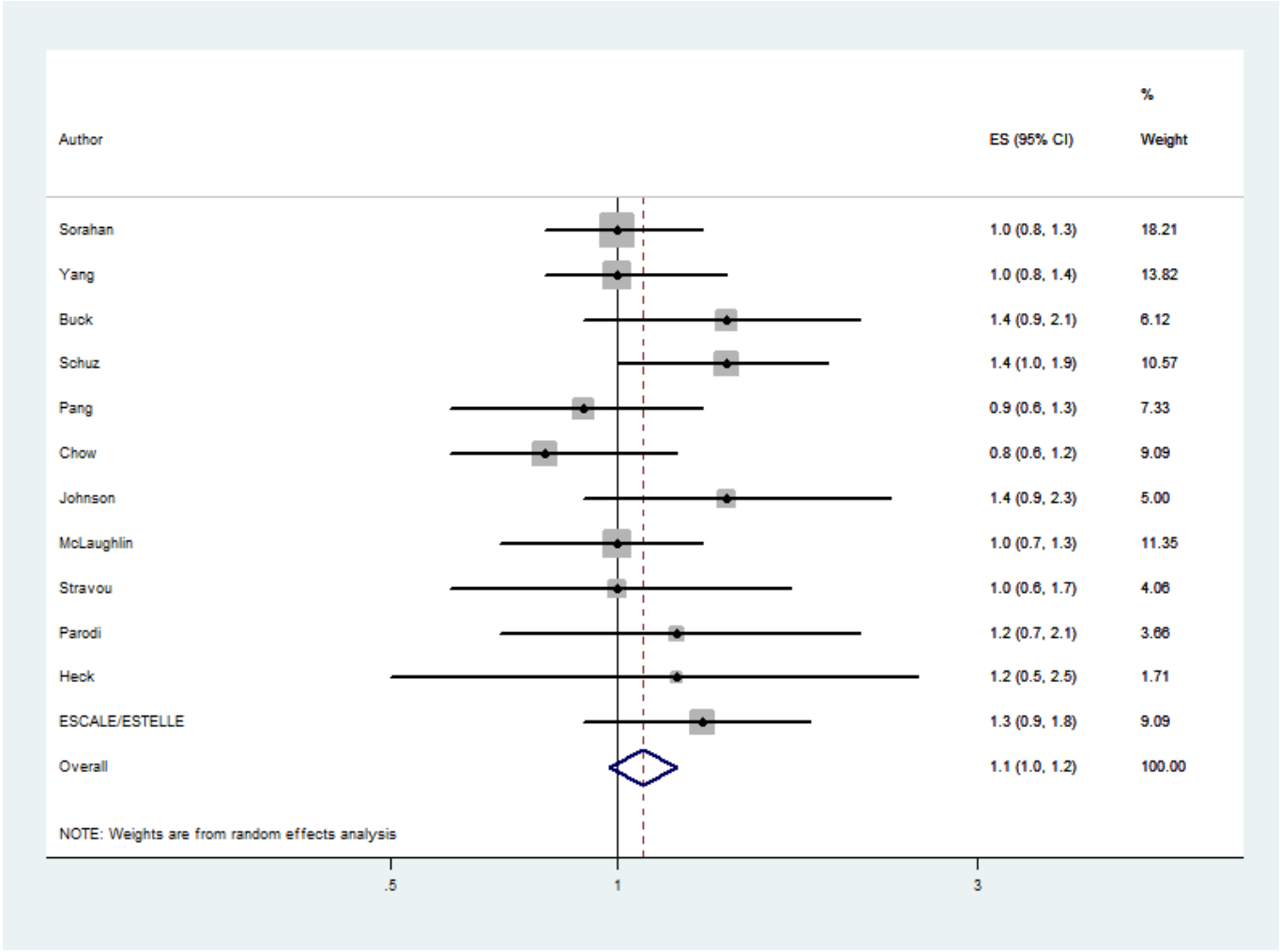
(a)



(b)


Author
ES (95% CI)
%
Weight
Kramer
1.3 (0.8, 2.1)
7.05
Schwartzbaum
1.9 (1.1, 3.2)
5.90
Sorahan
1.0 (0.8, 1.3)
20.01
Yang
1.0 (0.8, 1.4)
16.61
Buck
1.4 (0.9, 2.1)
8.80
Schuz
1.4 (1.0, 1.9)
13.65
Pang
0.9 (0.6, 1.3)
10.22
Parodi
1.2 (0.7, 2.1)
5.61
ESCALE/ESTELLE
1.3 (0.9, 1.8)
12.15
Overall
1.2 (1.0, 1.4)
100.00
NOTE: Weights are from random effects analysis
.5
1
3

(c)


Author
ES (95% CI)
%
Weight
Sorahan
1.0 (0.8, 1.3)
18.21
Yang
1.0 (0.8, 1.4)
13.82
Buck
1.4 (0.9, 2.1)
6.12
Schuz
1.4 (1.0, 1.9)
10.57
Pang
0.9 (0.6, 1.3)
7.33
Chow
0.8 (0.6, 1.2)
9.09
Johnson
1.4 (0.9, 2.3)
5.00
McLaughlin
1.0 (0.7, 1.3)
11.35
Stravou
1.0 (0.6, 1.7)
4.06
Parodi
1.2 (0.7, 2.1)
3.66
Heck
1.2 (0.5, 2.5)
1.71
ESCALE/ESTELLE
1.3 (0.9, 1.8)
9.09
Overall
1.1 (1.0, 1.2)
100.00
NOTE: Weights are from random effects analysis
.5
1
3