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Journal of Clinical Epidemiology 54 (2001) 702–709

**Journal of  
Clinical  
Epidemiology**

## Risk factors for neuroblastoma at different stages of disease. Results from a population-based case-control study in Germany

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Received 10 December 1999; received in revised form 15 September 2000; accepted 21 September 2000

### Abstract

Neuroblastoma is one of the childhood cancers included in two recent population-based case-control studies in West Germany. Altogether, 183 children under the age of 8 with neuroblastoma diagnosed in 1988–1994 and 1785 control children sampled from population registration files participated. Information on potential risk factors was obtained from the children's parents by a self-administered questionnaire and subsequent telephone interview. We observed positive associations with the use of oral contraceptives or other sex hormones during pregnancy (particularly with male offspring), a shorter gestational duration, lower birth weight, and maternal alcohol consumption during pregnancy. While the association with maternal use of oral contraceptives or sex hormones was strong for stages I/II (odds ratio 4.5, 95% confidence interval 1.2–16.5), the associations with shorter gestation duration (odds ratio 3.4, 95% confidence interval 1.7–6.7) as well as maternal alcohol consumption during pregnancy (>7 glasses/week odds ratio 5.2, 95% confidence interval 1.3–20.6) were observed only for the unfavourable advanced stages. It is notable that the associations in our study were either observed only for the advanced stages of disease or only for the less advanced stages, but not for both subgroups. This adds to evidence for the hypothesis that neuroblastoma consists of at least two distinct disease entities, which differ in clinical stage at the time of diagnosis. © 2001 Elsevier Science Inc. All rights reserved.

*Keywords:* Case-control study; Child; Neuroblastoma; Risk factors

### 1. Introduction

Neuroblastoma is the third most common malignancy in childhood. An annual incidence rate of 1.3 per 100,000 children accounts for about 9.0% of newly diagnosed childhood cancer cases in Germany per year, and with an age-specific incidence rate of 7.5 per 100,000 it is the most common malignancy in infants [1]. Boys are affected somewhat more frequently than girls, with a sex ratio of 1.2:1. Data from the US and Denmark suggest a persistent increase in the incidence of neuroblastoma during the last five decades [2–4]. This trend is also apparent in Germany; however, since the German Childhood Cancer Registry (GCCR) started registration in 1980 it is difficult to assess to what extent this trend might be due to underreporting during the early years of registration [1]. An incidence peak of infant neuroblastoma in 1988 was first attributed to fallout from the Chernobyl accident, but a subsequent investigation by the GCCR found a shift towards lower clinical stages indicating in-

creased diagnostic awareness rather than contamination by caesium-137 in food or drinking water [5]. In recent years an increase in incidence is attributable to a neuroblastoma screening project in parts of Germany [1,6,7].

Neuroblastoma is characterized by a diversity of clinical behaviour, ranging from an aggressive, unremitting growth on the one hand, to spontaneous remission on the other hand [8,9]. Since prognosis and clinical behaviour of the disease depend to some extent on the stage of disease and the age at diagnosis, it has been hypothesized that neuroblastoma represents at least two distinct disease entities [10]. Advanced stages III and IV represent the biologically unfavourable disease types more frequently than the less advanced stages I and II. Therefore, it seems appropriate to investigate potential risk factors by stage of disease. This was done using data derived from two recent comprehensive German population-based case-control studies primarily focusing on risk factors for childhood leukaemia. A second case group of children with solid tumours was recruited, in the first place to investigate possible recall bias effects in the leukaemia group, but also to study risk factors for less frequent types of tumours. Altogether, 186 families of children with neuroblastoma participated in these two studies.

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## 2. Methods

### 2.1. Subjects

The study population consists of participants from two recent population-based case-control studies carried out with identical methods of exposure assessment. The first study, which started in 1993, was restricted to the Northwestern part of Germany (Lower Saxony) [11,12]. Children with neuroblastoma from the GCCR were eligible if they were diagnosed between July 1988 and June 1993 and if they lived within the boundaries of Lower Saxony at the date of diagnosis. A second larger study, which started only a short time after the first one, was also based on the GCCR data and included neuroblastoma cases if they were diagnosed between October 1992 and September 1994 and if they lived in the former Federal Republic of Germany (FRG; West Germany) at the date of diagnosis [13–17]. Patients who lived in Lower Saxony and were diagnosed between October 1992 and June 1993 were eligible for both studies; therefore, they were used twice in study-specific analyses but only once in the combined analyses presented in this article.

In both studies, control children were selected from complete files of local offices for registration of residents. While the sampling procedure was identical for both studies, they differed regarding the matching criteria. In the first study, we sampled two controls for each child with leukaemia, one from the community where the diseased child lived (local control) and another one from a randomly selected community in Lower Saxony by a population-weighted sampling procedure (state control). Further matching criteria were gender and date of birth within 1 year. No controls were drawn for the group of cases with solid tumours. In the second study, for each child with leukaemia, non-Hodgkin's lymphoma, or solid tumour one control matched for gender, date of birth within 1 year, and community was selected. Consequently, for the neuroblastoma cases, individually matched controls were available only for the second study.

### 2.2. Exposure assessment

We collected information on potential risk factors both by questionnaire and by a subsequent telephone interview. The questions were based on a structured questionnaire developed by the US Children's Cancer Group [18]. Questionnaires were mailed by the physician responsible for the cancer treatment (cases) or by the study center at the GCCR (controls) and were to be returned to the study center. In addition, we performed telephone interviews for validation and completion of the questionnaire. The interviews were done by trained personnel. Questionnaire, interview procedures, and interviewers were the same in both studies. With few exceptions, both parents were interviewed. We checked for discrepancies between questionnaire and telephone interview to ensure a high data quality and therefore some parents were contacted again. If the participating family had no phone, we had to rely solely on the information from the questionnaire. For each

control the date of diagnosis of the corresponding case was defined as a reference date. Assessment of exposures was done only for exposures that occurred before this reference date. The questions addressed factors related to pregnancy and birth, exposure to ionizing radiation, pesticides, electromagnetic fields, and wood preservatives, and factors related to the child's immune system. Showcards with different types of power lines were sent to parents who said they lived close to a power line. This information was used to classify power lines into high-voltage (123 to 420 kV), medium-voltage (10 to 60 kV) and low-voltage power lines (380 V).

### 2.3. Analyses

Odds ratios (OR) and respective 95% confidence intervals (CI) for the pooled analyses of the two studies were derived from a conditional logistic regression model using frequency matching [*m:n* matching (cases:controls)] [19,20]. This model, involving an a posteriori stratification for gender, age (age groups of 1 year) and year of birth, was performed with additional adjustments for degree of urbanization (urban, mixed, rural) and socioeconomic status (SES; high, other). SES was estimated by monthly family net income and parental education. All controls were included, even if they were selected as individual controls for other diagnoses or if the individual match partner had not participated, so this model uses the maximum amount of available data. To examine if pooling the studies introduced any bias, we also applied a second conditional logistic regression model with each case matched to its corresponding control (1:1 matching; only available for the second study). Furthermore, ORs were calculated separately for both studies and, restricted to the first study, separately for the local and state controls. In this article, we report only ORs calculated using the first approach, since this regression model produced the most precise risk estimates and any differences to the alternative approach and the study-specific analyses could be explained by random variability. All regression models were using PROC PHREG of the SAS Software Release 6.12 [21].

## 3. Results

Out of 241 eligible children having a neuroblastoma 10 families were not contacted, mainly for psychological reasons. The response rates were 83.1% among cases (192 of 231) and 71.0% among controls (2537 of 3575), respectively. More than two thirds of all nonparticipants were refusals. Other reasons for nonparticipation were lost to follow-up and insufficient knowledge of the German language. Six case families as well as 79 control families responded but were not included in the analyses because of violations of the study inclusion criteria. Only 3 of 186 children with neuroblastoma were older than 7 years of age at date of diagnosis. Due to their small number, they were excluded from the analysis. Hence, 183 cases and 1785 controls aged 7 years or younger were available for analysis.

Demographic characteristics of cases and controls are shown in Table 1. Stage of disease was classified according to the International Neuroblastoma Staging System (INSS) [22]. In Germany, this staging system was introduced in 1991, so 9 of 181 cases (5.0%; stages 1, 3, and 4 three times each) were classified according to the older staging system developed by Evans. Stage of disease was known for 181 cases (97.3%); 17 of these children had a stage IVs neuroblastoma. Because of the small numbers of the stage-specific subgroups, we combined stages I and II (hereafter called “low stage”) as well as stages III and IV (hereafter called “high stage”) into two disease groups. No stage-specific analyses were performed for neuroblastoma stage IVs. As expected, Table 1 shows a clear association between stage of disease and age at diagnosis.

Table 2 gives the results for factors that are related to pregnancy and birth. Neuroblastoma was strongly associated with a shorter gestational period and a birth weight of less than 2.5 kg. Both factors were correlated (Spearman correlation coefficient  $r = .36$ ), and multivariate analysis showed that the association with a shorter duration of gestation was somewhat stronger than the association with low birth weight. After restricting the analysis to children with a birthweight of  $\geq 2.5$  kg, we still observed a strong association with a shorter gestational period [OR 2.80, 95% CI 0.96–8.16 (all stages); OR 3.76, 95% CI 1.20–11.80 (high stage)]. However, risk estimates for lower birth weight were also elevated for children with a full-term duration of gesta-

tion [OR 2.05, 95% CI 0.63–6.65 (all stages); OR 2.17, 95% CI 0.60–7.87 (high stage)].

A 2.5-fold increase in risk was observed for maternal smoking during pregnancy ( $> 20$  cigarettes per day), but this was based on sparse data and statistically not significant (Table 2). Regarding maternal smoking before pregnancy, maternal active or passive exposure to cigarette smoke during pregnancy, and also paternal smoking during pregnancy, all ORs were close to unity and no pattern indicating a dose–response relationship was observed (data not shown). Maternal alcohol consumption of more than 7 glasses per week during pregnancy was associated with high-stage neuroblastoma, but no elevation in risk was observed for lower alcohol consumption and, again, numbers were small.

Table 2 shows a strong association of neuroblastoma risk with maternal use of oral contraceptives or other sex hormones during pregnancy, which was particularly pronounced for low-stage neuroblastoma. It was noticeable that five of the six exposed cases were males; thus, gender-specific ORs were 2.64 for boys and 0.67 for girls (all stages), but the numbers were small. Sixteen mothers of cases and 70 mothers of controls did not specify their hormone use during pregnancy, so no information was available as to whether they used sex hormones or others (e.g., thyroid hormones). The association with maternal use of unspecified hormones was similar to that with sex hormones (Table 2). The OR regarding solely the use of oral contraceptives during the index pregnancy was 5.74 (95% CI 1.46–22.6) based on 4 cases and 26 controls (all stages).

Table 1  
Distributions of age, gender, degree of urbanization, and socioeconomic status

	Controls		Neuroblastoma		Neuroblastoma							
					Stage I		Stage II		Stage III		Stage IV	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Age (years)												
0	229	12.8	90	49.2	27	77.1	12	57.1	19	48.7	12	18.2
1–4	1,030	57.7	80	43.7	8	22.9	7	33.3	16	41.0	47	71.2
5–7 <sup>a</sup>	526	29.5	13	7.1	0	0.0	2	9.5	4	10.3	7	10.6
Gender												
Male	988	55.4	100	54.6	24	68.6	11	52.4	19	48.7	34	51.5
Female	797	44.6	83	45.4	11	31.4	10	47.6	20	51.3	32	48.5
SES												
Other	1,272	71.3	139	76.0	26	74.3	17	81.0	28	78.8	52	64.7
High	513	28.7	44	24.0	9	25.7	4	19.0	11	21.2	14	35.3
Urbanization												
Urban	679	38.0	71	38.8	12	34.3	11	52.4	14	35.9	27	40.9
Mixed	602	33.7	68	37.2	15	42.9	5	23.8	16	41.0	22	33.3
Rural	504	28.2	44	24.0	8	22.9	5	23.8	9	23.1	17	25.8
Study population <sup>b</sup>												
West Germany study	1459	81.7	153	83.6	28	80.0	18	85.7	31	79.5	60	90.9
Lower Saxony study	301	16.9	26	14.2	6	17.1	2	9.5	6	15.4	6	9.1
Both studies	25	1.4	4	2.2	1	2.9	1	4.8	2	5.1	0	0.0
Total <sup>c</sup>	1,785	100.0	183	100.0	35	100.0	21	100.0	39	100.0	66	100.0

<sup>a</sup>Only children aged 7 or less were included in the analyses.

<sup>b</sup>Due to partially overlapping study regions and diagnostic periods, some participants were eligible for both studies (but were counted only once in the pooled analysis).

<sup>c</sup>The sum of the right three columns (neuroblastoma by stage of disease) is less than the total of all neuroblastoma cases because 17 cases had neuroblastoma stage IVs (not shown) and for 5 cases the stage was not known.

Table 2  
Association of neuroblastoma with factors related to pregnancy and birth

	Neuroblastoma			Neuroblastoma			Neuroblastoma			
	Controls	(all stages)		(stages I/II)			(stages III/IV)			
	<i>n</i>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>
Maternal age at time of delivery										
Less than 20 years	29	4	1.21	0.37–4.00	1	1.10	0.12–9.74	2	1.20	0.27–5.31
20 to 34 years (reference)	1,599	163	1.00	—	48	1.00	—	97	1.00	—
35 years or more	155	15	0.90	0.50–1.63	6	1.22	0.48–3.13	6	0.63	0.27–1.50
Duration of gestation										
<37 weeks	73	15	2.46	1.30–4.64	1	0.51	0.06–4.02	12	3.40	1.72–6.72
37–42 weeks (reference)	1,677	163	1.00	—	53	1.00	—	92	1.00	—
>42 weeks	9	0	—	—	0	—	—	0	—	—
Birth weight										
<2500 g	60	14	2.41	1.24–4.70	2	0.96	0.21–4.38	11	3.13	1.52–6.43
2500–4000 g (reference)	1,527	144	1.00	—	48	1.00	—	80	1.00	—
>4000 g	192	25	1.35	0.83–2.19	6	1.02	0.41–2.57	14	1.38	0.75–2.53
First born										
No (reference)	930	89	1.00	—	22	1.00	—	56	1.00	—
Yes	848	92	1.14	0.82–1.59	32	1.70	0.93–3.12	49	0.95	0.63–1.43
Preceding fetal losses										
No (reference)	1,376	135	1.00	—	42	1.00	—	77	1.00	—
Yes	406	47	1.30	0.89–1.90	14	1.56	0.80–3.01	27	1.16	0.73–1.86
Maternal smoking during pregnancy										
No (reference)	1,396	133	1.00	—	41	1.00	—	77	1.00	—
1–10 cigarettes/day	312	41	1.39	0.93–2.09	11	1.20	0.57–2.51	23	1.29	0.78–2.14
11–20 cigarettes/day	53	5	0.96	0.35–2.61	2	1.02	0.21–5.08	3	0.98	0.29–3.33
>20 cigarettes/day	9	3	2.57	0.61–10.8	1	2.88	0.26–31.9	2	3.00	0.60–15.0
Maternal alcohol consumption during pregnancy										
No alcohol (reference)	1,335	140	1.00	—	43	1.00	—	79	1.00	—
1–7 glasses/week	414	38	0.84	0.56–1.26	12	0.90	0.45–1.80	23	0.88	0.53–1.45
>7 glasses/week	15	3	3.04	0.75–12.2	0	—	—	3	5.23	1.33–20.6
Hormonal treatment because of infertility										
No (reference)	1,619	157	1.00	—	47	1.00	—	92	1.00	—
Yes	81	9	1.05	0.49–2.25	2	0.94	0.21–4.34	6	1.02	0.42–2.52
Oral contraceptives or sex hormones during pregnancy										
No (reference)	1,665	157	1.00	—	44	1.00	—	92	1.00	—
Unspecified <sup>c</sup>	70	16	2.23	1.20–4.14	7	4.29	1.64–11.2	8	1.80	0.82–3.93
Yes	32	6	1.80	0.68–4.78	4	4.52	1.24–16.5	2	1.25	0.29–5.47

<sup>a</sup>Odds ratio from a frequency matched conditional logistic regression analyses stratified for gender, age, and birth year, and adjusted for SES and degree of urbanization.

<sup>b</sup>95% confidence interval.

<sup>c</sup>Subjects for which an intake of hormones during pregnancy was not specified (sex hormones cf. other hormones, e.g., thyroid hormones).

Neuroblastoma risk was higher for children who had a previous tonsillectomy or appendectomy (Table 3). However, three cases had an appendectomy within 1 month before diagnosis, indicating that the appendectomy may have been performed due to unspecific symptoms of the neuroblastoma. Omitting these three cases from the analysis produced an OR of 1.89 (95% CI 0.78–4.56). Children reported as having allergies had a decreased neuroblastoma risk (Table 3).

Table 4 presents the results for different environmental exposures. No consistent pattern can be seen for the use of pesticides. A seven-fold risk increase for high-stage neuroblastoma was observed with maternal occupational exposure to pesticides, all these cases were diagnosed with stage IV neuroblastoma. Self-reported paternal occupational exposure to pesticides, on the other hand, was statistically significantly associated with low-stage neuroblastoma. Finally,

no association was seen for the child's exposure to insecticides and pesticides used in gardens or on farms. The risk of neuroblastoma with the child's exposure to wood preservatives was elevated with a lower confidence limit of 0.96. No association was seen with exposure to ionizing radiation; neuroblastoma risk with X-ray examinations of the child (Table 4) or with maternal X-ray examinations in different periods during pregnancy (data not shown) were close to unity.

Regarding nonionizing radiation, no case lived close to a high-voltage transmission line (123–420 kV;  $\leq$  50 m). However, six of 164 case families reported that they had lived near to a medium-voltage transmission line (10–60 kV;  $\leq$  25 m), revealing a moderately elevated OR of 1.70 (95% CI 0.65–4.43), which was particularly pronounced for low-stage (OR 4.39, 95% CI 1.06–18.2) but not for high-stage neuroblastoma.

Table 3  
Association of neuroblastoma with factors related to the child's immune system

	Controls	Neuroblastoma (all stages)			Neuroblastoma (stages I/II)			Neuroblastoma (stages III/IV)		
	<i>n</i>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>
Duration of breast feeding										
≤1 month (reference)	659	73	1.00	—	22	1.00	—	41	1.00	—
2–6 months	755	75	0.95	0.66–1.37	26	1.04	0.56–1.93	40	0.89	0.56–1.42
>6 months	358	32	1.12	0.69–1.81	8	1.15	0.47–2.83	23	1.18	0.67–2.09
Infectious diseases										
≤1 infection	904	140	1.28	0.82–2.00	49	1.68	0.59–4.77	72	1.26	0.76–2.09
>1 infection (reference)	867	41	1.00	—	6	1.00	—	32	1.00	—
Tonsillectomy and/or appendectomy										
Neither (reference)	1,560	158	1.00	—	50	1.00	—	89	1.00	—
Yes	146	11	2.75	1.29–5.86	1	1.26	0.12–12.8	10	3.02	1.38–6.63
Allergy of the child										
No (reference)	1,594	178	1.00	—	54	1.00	—	102	1.00	—
Yes	187	3	0.20	0.06–0.65	0	—	—	3	0.30	0.09–0.96
Allergy of the mother										
No (reference)	1,346	140	1.00	—	43	1.00	—	79	1.00	—
Yes	435	41	0.88	0.60–1.30	11	0.80	0.39–1.62	26	0.94	0.59–1.51

<sup>a</sup>Odds ratio from a frequency matched conditional logistic regression analyses stratified for gender, age, and birth year, and adjusted for SES and degree of urbanization.

<sup>b</sup>95% confidence interval.

#### 4. Discussion

One strength of our German study is that it was population based. All cases were identified by an almost complete nationwide cancer registry [23]. Controls were drawn at random from complete files of population registries within the same population base that produced the cases. Since in Germany registration is compulsory for all residents, these files provide an excellent sampling frame for epidemiological studies. However, selection bias can not completely be ruled out. One reason is that the population files are not available historically, leading to a lower residential mobility among the controls, particularly in the first study. Another reason is that cases and controls differed with respect to SES. There is some evidence that control families of higher SES were more likely to participate than those of average SES; therefore, no risk analysis was done regarding SES, although earlier studies observed that neuroblastoma may be associated with lower SES [3,10,24]. There are also some risk factors that are related to social factors, so we adjusted for SES in all analyses. However, residual confounding by SES cannot be excluded.

The two studies differ regarding the periods of diagnosis and the matching of the controls (Lower Saxony: two controls for each case with leukaemia, FRG: one control for each case with leukaemia, non-Hodgkin lymphoma, or solid tumour). Otherwise the designs were identical, the same questionnaires were used in both studies, and identical interviews were performed by the same interviewers who were trained regularly. By comparing cases and controls using a post hoc frequency matched approach [20], the strategies of analyses of both studies became identical, enabling the pooling of the data sets. Additionally, we carried out 1:1 matched analyses for the FRG study to identify potential

differences in the risk estimates between the two models. Major differences would indicate that individual districts have an impact on the prevalence of a risk factor, in which case frequency matching methods might be inadequate. However, the differences between the two approaches were generally marginal.

Recall bias is a problem in studies relying on information from self-administered questionnaires and interviews, particularly for exposures that are not easy to remember. The format of the interview was standardized in detail and some items on the questionnaire were probed during the telephone interview, while interviewers were not blinded to case-control status. Looking at the patterns of risk estimates derived for different diagnostic groups gives us the opportunity to evaluate effects of recall bias, but, nevertheless, recall bias could have had some impact on some of our results. While nondifferential misclassification leads to an underestimation of an effect, overestimation might occur if the perception of exposure is more sensitive among parents of children with cancer.

The large number of comparisons could result in some statistically significant findings that might have occurred by chance. However, we did not adjust for multiple testing. Instead, we discuss each individual finding taking into account its internal consistency as well as its consistency with previous studies. Since data on risk factors for neuroblastoma by stage of disease are rare, we considered multiple analyses to be justified.

Little's [25] comprehensive review of the epidemiology of childhood cancer classified risk factors for neuroblastoma according their consistency of association with the disease (p. 348); from that only maternal use of sex hormones before or during the index pregnancy has been associated with neuroblastoma to some degree of consistency. Factors eval-

Table 4  
Association of neuroblastoma with environmental exposures

	Controls <i>n</i>	Neuroblastoma (all stages)			Neuroblastoma (stages I/II)			Neuroblastoma (stages III/IV)		
		<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>	<i>n</i>	OR <sup>a</sup>	CI <sup>b</sup>
Use of household insecticides <sup>c</sup>										
No (reference)	1,500	145	1.00	—	45	1.00	—	84	1.00	—
Once per year	68	6	1.23	0.50–3.01	2	1.71	0.35–8.26	4	1.22	0.42–3.53
More than once per year	102	14	1.77	0.92–3.38	3	1.17	0.33–4.15	9	1.90	0.89–4.07
Indoor extermination of insects by pest controller <sup>c</sup>										
No (reference)	1,678	165	1.00	—	50	1.00	—	96	1.00	—
At least once	28	3	1.85	0.49–6.94	0	—	—	3	2.54	0.70–9.21
Use of pesticides <sup>c</sup>										
No (reference) <sup>d</sup>	1,377	142	1.00	—	43	1.00	—	82	1.00	—
In garden	186	13	0.86	0.46–1.61	4	1.16	0.37–3.62	9	0.90	0.44–1.87
On farms	59	4	1.20	0.39–3.70	3	3.46	0.80–15.0	1	0.49	0.06–3.71
Paternal occupational exposure to pesticides										
No (reference)	1,699	168	1.00	—	48	1.00	—	99	1.00	—
Ever <sup>e</sup>	72	11	1.75	0.83–3.67	7	4.18	1.49–11.7	4	1.25	0.43–3.64
Maternal occupational exposure to pesticides										
No (reference)	1,766	179	1.00	—	56	1.00	—	101	1.00	—
Ever <sup>e</sup>	12	3	5.16	1.14–23.4	0	—	—	12	7.04	1.59–31.2
Use of wood preservatives <sup>c</sup>										
No (reference)	1,036	98	1.00	—	33	1.00	—	46	1.00	—
Yes	509	54	1.41	0.96–2.07	12	1.17	0.56–2.45	26	1.51	0.95–2.39
Maternal use of electric blankets during pregnancy										
No (reference)	1,599	160	1.00	—	47	1.00	—	95	1.00	—
Yes	88	5	0.54	0.21–1.41	1	0.43	0.06–3.38	3	0.58	0.18–1.92
X-ray examinations of the child <sup>f</sup>										
None (reference)	1,353	159	1.00	—	53	1.00	—	87	1.00	—
At least one	416	18	0.71	0.41–1.22	3	0.60	0.17–2.06	14	0.72	0.39–1.33

<sup>a</sup>Odds ratio from a frequency matched conditional logistic regression analyses stratified for gender, age, and birth year, and adjusted for SES and degree of urbanization.

<sup>b</sup>95% confidence interval.

<sup>c</sup>After birth.

<sup>d</sup>Neither in garden nor on farms.

<sup>e</sup>Includes the year before pregnancy, during pregnancy and/or after birth.

<sup>f</sup>Excluding X-ray examinations 1 year before date of diagnosis/reference date (0.5 year for children diagnosed in their first year of life).

uated in our studies, for which the evidence is inconsistent or unconfirmed, were parental smoking habits, fetal loss, maternal age at time of delivery, birth order, birth weight, parental exposure to pesticides, and maternal alcohol consumption during pregnancy.

In our studies we observed positive associations with maternal use of oral contraceptives or sex hormones during pregnancy, a shorter gestational period, lower birth weight, maternal alcohol consumption during pregnancy, tonsillectomy or appendectomy, parental self-reported occupational exposure to pesticides, use of wood preservatives, and proximity to medium-voltage transmission lines, while a protective effect of allergies of the child was apparent. However, as will be discussed below, we regard only the associations with maternal use of oral contraceptives or sex hormones during pregnancy, a shorter gestational duration, and maternal alcohol consumption during pregnancy as plausible. The other associations lack consistency and might have occurred by chance or might be due to bias. We found little evidence that neuroblastoma might be associated with parental smoking habits, fetal loss, maternal age at time of delivery, and

birth order; all these factors for which previous investigations found inconsistent results (reviewed in [25]). A recent epidemiological investigation confirmed a lack of neuroblastoma in children with Down syndrome [26]. None of our neuroblastoma cases had Down syndrome, but due to the number of cases we did not expect one.

Two earlier studies reported an association between neuroblastoma and the use of sex hormones [27,28], while a third one did not [29], but this last study used controls with other types of childhood cancer and there is some evidence for nonparticipation bias [25]. A more recent case-control study observed no association for the use of oral contraceptives before or during pregnancy [30], but a suggestive pattern was found for gender of the offspring, with an increased risk for males but not females after exposure to oral contraceptives. This finding was confirmed by our study with an elevated risk for male offspring. The positive association found in our study is unlikely to be due to recall bias, since out of the group of solid tumours neuroblastoma was the only disease for which an association was apparent (data not shown). Furthermore, mothers are well aware when they

stopped using oral contraceptives in relation to their pregnancy. Unfortunately, a large number of mothers did not specify which hormones they used during pregnancy. Of those who specified hormonal intake during pregnancy, there were more mothers who used thyroid hormones or insulin compared to those who received sex hormones. However, we think that this pattern was reversed among those of unspecified hormonal intake for two reasons. Firstly, the duration of thyroid hormone or insulin medication is usually quite long, while sex hormones are used for a considerably shorter time period. Therefore, it might have been easier to remember the use of thyroid hormones or insulin and many mothers might still have used them during the interview period. Secondly, it might have been due to the way we put that question; we suggested thyroid hormones, cortisone, insulin, estrogen and gestagen as examples for hormone medications. Therefore, mothers who knew they used sex hormones, but could not remember whether these were estrogens or gestagens, were likely to state the use of unspecified hormones.

No consistent pattern of association between birth weight and neuroblastoma is apparent so far [25]. We observed an only slightly increased risk with high birth weight but a statistically significantly increased risk for lower birth weight. However, the association with lower birth weight was weaker after taking duration of gestation into account. The finding for a short duration of gestation is not in accordance with two previous studies where a risk close to unity [31] and even a decreased risk [32] had been observed. Nevertheless, we think our observation is of some importance, since the association was found only for high-stage neuroblastoma while for low-stage neuroblastoma the risk estimate was considerably below unity. Future studies should make efforts to better distinguish between the effects of prematurity and intrauterine growth retardation.

Two epidemiologic studies with contradicting results investigated the role of alcohol in the aetiology of neuroblastoma [27,29], both acknowledging possible selection bias. In our studies we observed an increased risk for maternal alcohol consumption for high-stage neuroblastoma, but because of the small numbers in this high-exposure category this finding is of limited value.

No clear association between neuroblastoma and exposure to pesticides emerged in our studies. A strong association was observed only for that exposure measure which is most likely to be biased by differential recall, namely self-reported occupational exposure to pesticides [17]. Positive associations (most of them nonsignificant) with different diagnostic groups have also been observed for several other occupation-related exposures from our questionnaire (e.g., wood dusts, metal dusts, different chemicals, and fumes), indicating that parents of diseased children are more aware of past occupational contacts with these substances (data not shown). However, to some extent our observation affirmed a finding from an earlier study of the GCCR where a four-fold increase in risk was seen with parental exposure to pes-

ticides [5], but neuroblastoma has not consistently been associated with paternal employment in agriculture [32–34].

Some wood preservatives contain substances that were carcinogenic in laboratory experiments (e.g., formaldehyde) [35]. The use of wood preservatives is not easy to recall, since tenants might not even know if the owner of their dwelling used wood preservatives or not. Therefore, our weak positive association is likely to be biased by differential recall, because parents of diseased children might have cared more about wood preservatives than parents of non-diseased children.

Distance to power lines is poor proxy for exposure to magnetic fields considering the German system for energy supply [36,37], and the association of low-stage neuroblastoma with residential proximity to medium-voltage power lines is likely to be a chance finding. This is strengthened by our observation that no case but 24 controls (1.4%) lived close to a high-voltage power line; the OR regarding an exposure measure combining the two types of power lines was close to unity (data not shown).

Regarding factors related to the child's immune system, epidemiologic studies have focused on childhood leukaemia [25]. Our finding for children who had tonsillectomy or appendectomy might rather be a secondary effect of the disease than evidence of a risk, because three cases had an appendectomy only a few weeks before diagnosis. Two of those cases were already suspected to have cancer before the appendectomy, while for the third case the neuroblastoma was a chance finding after the operation. The protective effect of allergies might partly be explained by differential recall bias, if we assume that parents of cases could better distinguish between allergies occurring before or after the reference date (date of diagnosis).

The considerable diversity in the clinical behaviour of neuroblastoma has been interpreted as evidence of biological diversity, which has been confirmed by molecular genetic studies [38]. This indicates analyses by subtypes of neuroblastoma. We derived information on the stage of disease from the GCCR for almost all cases, so we preferred stage of disease to age at diagnosis in our analyses; however, because of the strong correlation between these two factors the tendencies of the results are similar. Biological markers like N-myc amplification were only available for a small number of cases in the present study, so they could not be included in the statistical analyses. All factors that were associated with neuroblastoma in our study were either associated with low-stage or high-stage neuroblastoma, but not with both groups. This may indicate differences in the etiology of neuroblastoma; however, there is also a possibility of chance findings. We recommend that future studies on neuroblastoma should take into account both the stage of disease and cytogenetic and molecular biological characteristics, in order to enable us to not only to improve our understanding of the etiology of neuroblastoma but also to identify risk factors that might be responsible for its diverse clinical behaviour.

## Acknowledgments

This paper is partially based on the doctoral thesis by Uwe Kaletsch. The studies were supported by the German Federal Ministry for the Environment, Reactor Safety and Nature Preservation (West Germany) and the Ministry of Social Affairs of Lower Saxony.

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