Published by Oxford University Press on behalf of the International Epidemiological Association
International Journal of Epidemiology 2013;42:1702–1713
doi:10.1093/ije/dyt183
© The Author 2013; all rights reserved. Advance Access publication 24 October 2013

DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Prenatal paracetamol exposure and child neurodevelopment: a sibling-controlled cohort study

Ragnhild Eek Brandlistuen,[1,2,3]* Eivind Ystrom,[2] Irena Nulman,[3] Gideon Koren[3] and Hedvig Nordeng[1,2]

[1]School of Pharmacy, University of Oslo, Oslo, Norway, [2]Division of Mental Health, Norwegian Institute of Public Health, Oslo, Norway and [3]Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, Canada

*Corresponding author. Department of Pharmacy, School of Pharmacy, University of Oslo, PO Box 1068 Blindern, 0316 Oslo, Norway. E-mail: r.e.brandlistuen@farmasi.uio.no

Accepted 5 August 2013

Background Paracetamol is used extensively during pregnancy, but studies regarding the potential neurodevelopmental sequelae of foetal paracetamol exposure are lacking.

Method Between 1999 and 2008 all pregnant Norwegian women were eligible for recruitment into the prospective Norwegian Mother and Child Cohort Study. The mothers were asked to report on their use of paracetamol at gestational weeks 17 and 30 and at 6 months postpartum. We used data on 48 631 children whose mothers returned the 3-year follow-up questionnaire by May 2011. Within this sample were 2919 same-sex sibling pairs who were used to adjust for familial and genetic factors. We modelled psychomotor development (communication, fine and gross motor development), externalizing and internalizing behaviour problems, and temperament (emotionality, activity, sociability and shyness) based on prenatal paracetamol exposure using generalized linear regression, adjusting for a number of factors, including febrile illness, infections and co-medication use during pregnancy.

Results The sibling-control analysis revealed that children exposed to prenatal paracetamol for more than 28 days had poorer gross motor development [β 0.24, 95% confidence interval (CI) 0.12–0.51], communication (β 0.20, 95% CI 0.01–0.39), externalizing behaviour (β 0.28, 95% CI 0.15–0.42), internalizing behaviour (β 0.14, 95% CI 0.01–0.28), and higher activity levels (β 0.24, 95% CI 0.11–0.38). Children exposed prenatally to short-term use of paracetamol (1–27 days) also had poorer gross motor outcomes (β 0.10, 95% CI 0.02–0.19), but the effects were smaller than with long-term use. Ibuprofen exposure was not associated with neurodevelopmental outcomes.

Conclusion Children exposed to long-term use of paracetamol during pregnancy had substantially adverse developmental outcomes at 3 years of age.

Keywords Paracetamol, acetaminophen, ibuprofen, neurodevelopment, sibling design, Norwegian Mother and Child Cohort Study, MoBa

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

Introduction

Paracetamol is one of the most commonly used medications during pregnancy.[1] Despite this, studies of potential adverse effects on neurodevelopmental outcomes in children prenatally exposed to paracetamol are lacking. The only previous study addressing long-term cognitive outcomes after exposure to paracetamol suggested no association between the drug and the child's intelligence quotient (IQ) and attention in women receiving neonatal care in 1974–1975.[2] No recent neurodevelopmental follow-up of children prenatally exposed to paracetamol has been performed.

Previous studies focusing on risk of miscarriage, low birthweight or prematurity after paracetamol exposure typically show no association, but the studies often included a small number of exposed women resulting in low power to detect effects. One large cohort study reported associations between paracetamol exposure and preterm birth in women with pre-eclampsia.[3] In addition, childhood asthma has repeatedly been reported to be associated with prenatal paracetamol exposure.[4–6] A recent study reported increased risk of cryptorchidism after long-term use (more than 4 weeks) of paracetamol during pregnancy.[7] These reported risks of adverse outcomes after paracetamol exposure have raised concern about the safety of paracetamol use during pregnancy and enhanced the need to explore a broader range of outcomes to detect potential domains of child development that might be affected.

The causes of these risks have been the subject of much controversy—is the medication to blame or could the adverse outcomes be attributed to factors relating to the women taking paracetamol during pregnancy? Separating the effect of paracetamol from the factors leading to the need for paracetamol use during pregnancy is difficult. Paracetamol use during pregnancy might be associated with several familial or genetic factors such as IQ, socioeconomic status and neurodevelopmental problems. When unobserved and uncontrolled, these factors can lead to either an overestimation of adverse outcomes or an underestimation of adverse outcomes. For example, paracetamol intake during pregnancy is negatively associated with the personality trait of conscientiousness.[8] Conscientiousness is, with a heritability of 40–50%,[9] familial and associated with externalizing behaviour problems already at 3 years of age.[10] Thus, an effect observed after paracetamol exposure on externalizing behaviour problems in the child could be explained by genetic confounding. We attempted to address the problem of comparability by keeping the maternal factors as constant as possible by comparing the difference in developmental outcomes between siblings that were discordant on paracetamol exposure during pregnancy with the difference between siblings who were concordant on exposure. This sibling-control design is particularly suitable to separate the effect of familial and genetic confounding from the effect of the medication because siblings share familial environment and 50% of their genetic predisposition, but may differ on medication exposure during pregnancy. When comparing the sibling-controlled results with the results from the cohort in general, important information on the familial and genetic confounding can be gained. Using the Norwegian Mother and Child Cohort Study, we were able to identify a large number of sibling pairs and prospectively examined potential associations between prenatal exposure to paracetamol and psychomotor, behavioural and temperament outcomes in children after 3 years of follow-up.

Methods

Study population and data collection

This study is a subproject of the Norwegian Mother and Child Cohort Study (MoBa) conducted by the Norwegian Institute of Public Health. MoBa is a prospective pregnancy cohort that was previously described in detail.[11] Participants were recruited at the routine ultrasound examination at gestational week 17. During the period of recruitment between 1999 and December 2008, 108 841 pregnant women enrolled in the study, a participation rate of 38.7% of all pregnant women (http://www.fhi.no/moba-en). A total of 15 256 mothers with more than one child participated. Written informed consent was obtained from all participating women. The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate.

Data collection during pregnancy involved two questionnaires administered around gestational weeks 17 and 30, which included questions regarding sociodemographic characteristics, maternal health and medication use during the current pregnancy. In addition, the women completed a questionnaire 6 months after the birth, reporting on the remaining weeks of pregnancy after week 30. The cohort was linked to the Medical Birth Registry of Norway (MBRN)[12] by using the women's personal 11-digit identification numbers. The MBRN contains detailed medical information and diagnostics regarding the infant, originating from mandatory notification forms completed by midwives, obstetricians and paediatricians at delivery and during the hospital stay.[12] Up to 3 years of age, follow-up of children included questionnaires periodically sent to mothers for the entire sample. We used the quality-ensured Data Version 6 released by MoBa in 2012. A total of 48 631 children born before 2009, for whom the age 3 years questionnaire was returned by the mother by 4 May 2011 and processed for inclusion in Data Version 6, were included in this study. Within this sample were 2919 same-sex sibling pairs. A flow chart of study participants included in the sibling design is presented in Figure 1. If mothers participated with more than two children only the

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

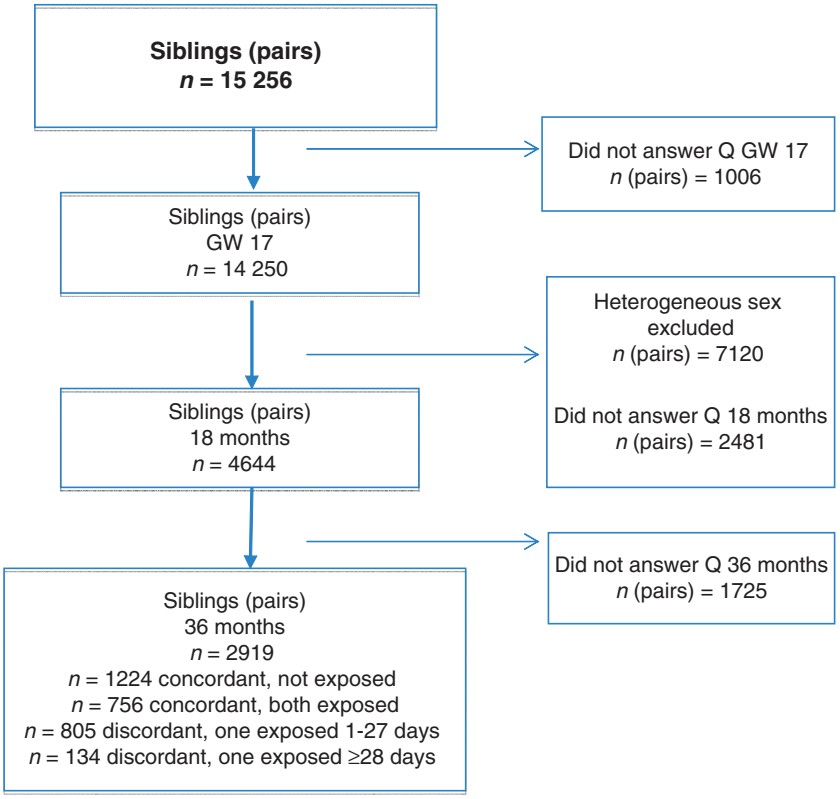

Figure 1 Flow chart of study participants in the sibling design. Q, questionnaire; GW, gestational week

first two siblings were kept in the study. All questionnaires used in MoBa can be found online at http://www.fhi.no/moba-en.

Measure of paracetamol use

Information on paracetamol use was available from two prenatal and one postnatal questionnaire. Several indications were specifically named to increase the reporting of paracetamol use (i.e., headache, fever, cold and back pain). For each indication, the woman could specify the following exposure windows: gestational weeks 0 to 4, 5 to 8, 9 to 12, and 13+ (until completion of the first questionnaire), 13 to 16, 17 to 20, 21 to 24, 25 to 28, and 29+ (until completion of the second questionnaire), and 30+ (until birth) in the 6 months postpartum questionnaire, and name the medication taken in an open textbox. When multiple medication use was reported and multiple time periods indicated, we assumed all drugs had been used in all time periods. Finally, the women reported the number of days they had used paracetamol at each time point. We classified and grouped drug exposure according to the Anatomical Therapeutic Chemical (ATC) Classification System developed by the World Health Organization.[13] Paracetamol exposure was defined as exposure to a drug belonging to ATC code N02BE01.

In order to differentiate between short-term and long-term, use we divided the children exposed to paracetamol *in utero* into two groups based on exposure duration during pregnancy: exposure for 1–27 days and exposure for 28 days or more. We classified the pairs of siblings as concordant when both siblings were equally exposed (both exposed or both unexposed) and discordant on exposure when the siblings differed on exposure.

Measure of ibuprofen

We examined use of ibuprofen during pregnancy as a secondary predictor. If prenatal use of paracetamol exhibits an association with neurodevelopmental outcomes and prenatal use of ibuprofen does not, it would suggest a specific effect of paracetamol less likely to be confounded by indication. Ibuprofen use was reported by the mother in the same manner as described for paracetamol. Ibuprofen exposure was defined as exposure to a medication belonging to ATC code M01AE01.

Measures of neurodevelopmental outcomes

Psychomotor development

Psychomotor development was assessed by items from the validated Norwegian version of the Ages and Stages Questionnaire (ASQ).[14,15] Mothers were asked to find time to observe the child and rate the extent to which the child typically exhibited mastery of different skills using the response categories: yes,

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

very often (1); yes, sometimes (2); not yet (3); and don't know (missing). Six items rated communication skills. Four items rated fine and gross motor impairment. Mean scores were calculated and standardized. To explore the reliability of the scales, we applied a 2-parameter item response theory (IRT) analysis.[16] We calculated the average factor loading to be 0.82 for the communication scale, indicating good reliability. Average factor loading for the fine motor items was 0.61, 0.75 for the gross motor items, indicating adequate reliability.

In addition, as a measure of a motor milestone achievement, the age when the child started walking unaided was assessed. Mothers reported the number of months of age at which the child could walk unaided, in the 18-month questionnaire. If the child had not yet started walking unaided at 18 months, we used information from the 36-months questionnaire when the question was repeated. Maternal reports of gross motor milestone attainment have been reported to be highly reliable.[17] Motor development is one of the more objective changes during infancy and is presumably less likely to be misinterpreted than are changes in less overt domains of development.

Behaviour

Externalizing and internalizing behaviours were measured by the Child Behaviour Checklist (CBCL/11/2-5/LDS).[18] The selected 20 items represented subscales of the Internalizing domain ('emotionally reactive', 'anxious/depressed' and 'somatic complaints') and subscales of the Externalizing domain ('attention problems' and 'aggressive behaviour'). Mothers reported the extent to which they agreed with the behaviour statements using the following 3-point Likert scale: 1 = not true; 2 = somewhat or sometimes true; 3 = very true or often true. Mean scores were calculated and standardized. Average factor loadings for the behaviour scales indicated adequate reliability for the externalizing and internalizing behaviour scales (0.58 and 0.52, respectively). The subset of items used in the MoBa study was found to be representative, with a correlation of 0.92 with the full scale.[19]

Temperament

Temperament was assessed by the Emotionality, Activity and Shyness Temperament Questionnaire (EAS).[20] The EAS measures the four temperament dimensions: emotionality (irritability/anger), activity (activity level), sociability (positive affect/including approach) and shyness (fear of strangers, social inhibition). Three out of five questions from each temperament dimension were selected for use in MoBa. Five response categories were available, from 'very typical' to 'not at all typical'. Means scores were calculated and standardized. The average factor loading for emotionality was 0.71, for activity 0.68, for sociability 0.58 and for shyness 0.69, indicating adequate reliability. The short-form versions of the EAS have been shown to be as reliable and precise as the original.[21]

Assessment of potential confounders

Differences across siblings in maternal risk factors were considered as covariates. Potential confounding factors related to maternal health before and during pregnancy included infections (respiratory, urinary tract/bladder, genital, diarrhoea/gastric flu), fever, back pain and headache or migraine. Concomitant use of nonsteroidal anti-inflammatory drugs (NSAIDs) (M01A and N02BA), triptans (N02CC), opioids (N02A), other analgesics (N02CA and N02CX), benzodiazepines (N05CD, N05BA), antidepressants (N06A), antipsychotics (N05A) and antiepileptic drugs (N03A) was recorded and grouped as co-medication. Mothers also reported on their psychological distress (anxiety and depression) using a validated short version of the Hopkins Symptom Checklist, the SCL-5.[22] A mean score greater than 2.0 on the SCL-5 at week 17 and/or 30 was defined as the presence of depression.[22] In the current sample, the SCL-5 had adequate internal consistencies with reliabilities of 0.80 and 0.83. Other potential confounding factors included maternal age at delivery, years between pregnancies, parity, smoking during pregnancy and alcohol use during pregnancy. The characteristics of potential confounding factors for the sibling pairs were categorized as presented in Table 1.

When assessing the overall cohort, the children could differ on additional confounders, such as maternal education and chronic diseases (asthma, allergy, rheumatism or hypothyroidism). Maternal and child characteristics for the cohort were categorized as presented in Table 2.

Statistical analysis

Statistical analyses were performed using SPSS software, version 20.0. Characteristics of the difference in discordance between the sibling pairs according to discordance in exposure to paracetamol (Table 1) were compared by two-sided chi-square tests of independence for categorical variables and analysis by Student's two-sided t-test for means of continuous variables. We estimated the size of familial confounding by calculating the intraclass sibling correlation (i.e. how similar the siblings are) for all outcomes. The intraclass correlation for a given outcome corresponds to the percentage of familial variance in the outcome adjusted for in the sibling analysis.

Maternal characteristics in the cohort of mothers for the group exposed <28 days and the group exposed $\geqslant 28$ days were compared with those of the unexposed group using two-sided chi-square tests (Table 2). We performed sibling-control analyses by subtracting the absolute value of the developmental outcome score for sibling 1 from the developmental outcome score for sibling 2. Comparing within-pair mean difference is often used in co-twin control

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

Table 1 Descriptive characteristics of same-sex sibling pairs by concordant and discordant paracetamol exposure[a]

	Concordant exposure *n* (pairs) = 1980	Discordant exposure (1-27 days) *n* (pairs) = 805	*P*-value[a]	Discordant exposure (⩾ 28 days) *n* (pairs) = 134	*P*-value[a]
Age at first delivery (mean, SD)	29.4 (3.9)	28.8 (3.9)	<0.001	28.7 (3.5)	0.02
Years between pregnancies (mean, SD)	2.0 (1.3)	2.5 (1.0)	<0.001	2.5 (1.1)	<0.001
Multiparity (*n*, %)[b]	55 (3.5)	37 (4.7)	0.38	5 (3.9)	0.68
Discordance in pregnancy for (*n*, %)					
Smoking[c]	47 (2.4)	35 (4.5)	0.06	2 (1.5)	0.35
Alcohol[d]	165 (8.3)	102 (12.7)	<0.001	19 (14.2)	0.02
Depression[e]	116 (5.9)	72 (8.9)	0.003	12 (9.0)	0.05
Co-medication	176 (8.9)	95 (11.8)	0.02	35 (26.1)	<0.001
Fever	338 (14.1)	251 (31.2)	0.001	52 (38.8)	<0.001
Infections	636 (32.1)	332 (41.2)	<0.001	40 (29.9)	0.29
Muscle pain	485 (24.5)	218 (27.1)	0.15	29 (21.6)	0.38
Headache or migraine	413 (20.9)	253 (41.2)	<0.001	38 (28.4)	0.001
Days of paracetamol use (median)[f]	0	2		37	
Fist pregnancy (mean, SD)	2.0 (18.1)	3.9 (4.7)		57.5 (48.3)	
Second pregnancy (mean, SD)	2.6 (23.3)	4.0 (4.6)		51.2 (27.6)	

[a]Two-sided chi-square test of independence for categorical variables, analysis by Student two-sided t-test for means.
[b]Three or more siblings in total.
[c]Smoking daily or sometimes during pregnancy.
[d]Use of one or more alcohol units reported during pregnancy.
[e]Mean score of >2 on the Hopkins Symptom Checklist (SCL-5) at week 17 and/or week 30.
[f]In the pregnancy where use was reported.

studies, and is akin to the dependent sample t-test.[23] We used generalized linear regression models with 95% profile likelihood CIs to determine the relationship between paracetamol exposure for siblings discordant on short-term exposure (<28 days) and siblings discordant on long-term exposure (⩾28 days) on nine different developmental outcomes, adjusting for confounding variables. Variables were included as confounders if they were associated with discordance in paracetamol use and the outcome variable, or if the effect of paracetamol changed by >1% when the variable was included in the model. Normal-based CIs are inaccurate when the sampling distribution of the estimate is not normal. Therefore we used a profile likelihood CI that is asymmetrical and more trustworthy than a standard error-based CI. For independent categorical variables, we computed dummies in which concordant siblings were given the value zero and discordant siblings were given the value one. Thereafter, the absolute difference between sibling 1 and sibling 2 was predicted by the dummies denominating sibling discordance. The beta represented the mean sibling difference in the developmental outcomes in a discordant pair.

The potential effect of sibling order was tested by comparing the effect of paracetamol use in first pregnancy but not second with the effect of use in second pregnancy and not the first. If the CIs were overlapping, suggesting no effect of sibling order, the discordant pairs of siblings were collapsed into one group. Finally, we tested whether any interaction effects existed between time of exposure to paracetamol according to trimester and the outcomes. Due to multiple explorative testing in this step of the analysis, we applied a *P*-value of 0.01.

As secondary analyses, we calculated the effects of discordant use of ibuprofen on the same developmental outcomes as for paracetamol, to explore the specific effect of paracetamol.

Finally, we performed cohort analyses using the same approach as the sibling analyses: generalized linear regression models with 95% profile likelihood CIs to determine the relationship between paracetamol exposure for <28 days and ⩾28 days on nine different developmental outcomes, adjusting for confounding variables in the entire cohort. Multivariable adjustment was made for potential confounders. As the scores used are the same metric as from the sibling analyses, the betas from the cohort analyses are comparable to the betas from the sibling-control analyses. A complete list of covariates included in the final adjusted models is provided in Tables 3 and 4.

To exemplify relative risks for disorders, we found the percentage of exposed children above a given threshold for disorder under a normal curve with a

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

Table 2 Maternal and child's characteristics based on use of paracetamol during pregnancy

	No use $n = 26\,213$ No. (%)	**< 28 days use** $n = 20\,587$ No. (%)	***P*-value[a]**	**⩾ 28 days use** $n = 1\,831$ No. (%)	***P*-value[a]**
Maternal characteristics					
Age, years					
<25	2330 (8.9)	1999 (9.7)	<0.001	137 (7.5)	<0.01
25–29	8597 (32.8)	7001 (34.0)		546 (29.8)	
30–34	10 408 (39.7)	8188 (39.8)		785 (42.9)	
35–39	4227 (16.1)	3019 (14.7)		323 (17.7)	
⩾40	599 (2.3)	360 (1.7)		37 (2.0)	
Missing data	52 (0.2)	20 (0.1)		3 (0.2)	
Education, years					
<13	7786 (29.7)	6435 (31.3)	<0.001	588 (32.1)	<0.01
13–16	10 862 (41.4)	8771 (42.6)		796 (43.5)	
⩾17	6330 (24.2)	4443 (21.6)		370 (20.2)	
Missing data	1235 (4.7)	939 (4.6)		77 (4.2)	
Parity					
0	13 167 (50.2)	9161 (44.5)	<0.001	672 (36.7)	<0.001
1	8417 (32.1)	7542 (36.6)		730 (39.9)	
⩾2	4577 (17.5)	3864 (18.8)		426 (23.3)	
Missing data	52 (0.2)	20 (0.1)		3 (0.2)	
Smoking					
Daily	619 (2.4)	672 (3.3)	<0.001	79 (4.3)	<0.001
Sometimes	1141 (4.4)	1082 (5.3)		116 (6.3)	
Never	22 895 (87.3)	17 693 (85.9)		1548 (84.5)	
Missing data	1558 (5.9)	1140 (5.5)		88 (4.8)	
Alcohol use[b]					
⩾once a week	892 (3.4)	737 (3.6)	<0.001	80 (4.4)	<0.001
1–3 times a month	7512 (28.7)	6584 (32.0)		612 (33.4)	
Never	13 227 (50.5)	9993 (48.5)		856 (46.8.8)	
Missing data	4582 (17.5)	3273 (15.9)		283 (15.5)	
Depression[c]					
Yes	1967 (7.5)	2044 (9.9)	<0.001	275 (15.0)	<0.001
No	24 094 (91.9)	18 446 (89.6)		1554 (84.9)	
Missing data	151 (0.6)	97 (0.5)		2 (0.1)	
Child's characteristics					
Sex					
Boys	13 555 (51.7)	10 404 (50.5)	0.01	890 (48.6)	0.01
Girls	12 606 (48.1)	10 163 (49.4)		938 (51.2)	
Missing data	52 (0.2)	20 (0.1)		3 (0.2)	
Serious malformation					
Yes	706 (2.7)	569 (2.8)	0.66	52 (2.8)	0.69
No	25 455 (97.1)	19 998 (97.1)		1776 (97.0)	
Missing data	52 (0.2)	20 (0.1)		3 (0.2)	

(continued)

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

Table 2 Continued

	No use $n = 26\,213$ No. (%)	**< 28 days use** $n = 20\,587$ No. (%)	***P*-value[a]**	**⩾ 28 days use** $n = 1\,831$ No. (%)	***P*-value[a]**
Gestational age (week)					
<37	1550 (5.9)	1177 (5.7)	0.36	127 (6.9)	0.08
⩾37	24 517 (93.5)	19 315 (93.8)		1687 (92.1)	
Missing data	146 (0.6)	95 (0.5)		17 (0.9)	
Birthweight (g)					
<2500	970 (3.7)	775 (3.8)	0.73	70 (3.8)	0.78
⩾2500	25 178 (96.1)	19 782 (96.1)		1757 (96.0)	
Missing data	65 (0.2)	30 (0.1)		4 (0.2)	

[a]Two-sided chi-square test of independence.
[b]Use of one or more alcohol units reported during this pregnancy.
[c]Mean score of >2 on the Hopkins Symptom Checklist (SCL-5) at week 17 and/or week 30.

Table 3 Multiple generalized regression models of the neurodevelopmental effects of paracetamol use during pregnancy in same-sex sibling pairs

	Concordant No. of pairs = 1980	**Discordant on use <28 days** No. of pairs = 805		**Concordant** No. of pairs = 1346	**Discordant on use ⩾28 days** No. of pairs = 134	
	Reference	Crude β (95% CI)	Adjusted[a] β (95% CI)	Reference	Crude β (95% CI)	Adjusted[a] β (95% CI)
Psychomotor problems						
Motor milestone	Ref	0.12 (0.04–0.20)*	0.10 (0.02–0.18)*	Ref	0.24 (0.06–0.42)*	0.26 (0.06–0.45)*
Gross motor	Ref	0.11 (0.02–0.20)*	0.10 (0.02–0.19)*	Ref	0.31 (0.12–0.50)*	0.24 (0.12–0.51)*
Fine motor	Ref	0.08 (0.01–0.15)*	0.07 (0.001–0.15)*	Ref	0.07 (-0.08–0.22)	0.05 (-0.12–0.21)
Communication	Ref	0.04 (-0.04–0.13)	0.05 (-0.04–0.14)	Ref	0.22 (0.03–0.41)*	0.20 (0.01–0.39)*
Behaviour problems						
Externalizing	Ref	0.08 (0.02–0.14)*	0.03 (-0.03–0.09)	Ref	0.24 (0.12–0.37)*	0.24 (0.12–0.37)*
Internalizing	Ref	0.03 (-0.03–0.10)	0.03 (-0.03–0.10)	Ref	0.14 (0.01–0.28)*	0.14 (0.01–0.28)*
Temperament problems						
Emotionality	Ref	0.08 (0.01–0.14)*	0.05 (-0.02–0.11)	Ref	0.10 (-0.04–0.24)	0.10 (-0.04–0.24)
Activity	Ref	0.004 (-0.06–0.07)	0.004 (-0.06–0.07)	Ref	0.23 (0.10–0.36)*	0.22 (0.11–0.36)*
Sociability	Ref	0.03 (-0.04–0.09)	0.03 (-0.04–0.09)	Ref	-0.01 (-0.15–0.13)	-0.01 (-0.15–0.13)
Shyness	Ref	0.04 (-0.02–0.11)	0.04 (-0.02–0.11)	Ref	0.01 (-0.14–0.15)	0.01 (-0.04–0.24)

[a]Motor milestone adjusted for discordance in co-medication, muscle pain, fever and headache/migraine; gross motor adjusted for discordance in muscle pain, alcohol use, and maternal depression, fine motor adjusted for discordance in co-medication, alcohol use, infections, and muscle pain; communication adjusted for discordance in maternal depression; externalizing behaviour adjusted for discordance in maternal depression and fever; internalizing behaviour adjusted for discordance in fever and co-medication; emotionality adjusted for discordance in co-medication; activity adjusted for discordance in maternal depression and headache/migraine.
*Significant effect as shown by the profile-likelihood-based confidence intervals (CIs).

mean corresponding to the beta found in the sibling analyses.

To avoid sample distortions caused by list-wise deletion of missing values, we used SPSS Missing Value Analysis (MVA), expectation maximization (EM) for imputation of missing values for respondents with valid data for at least half of the items on the measurement scales (ASQ, CBCL, EAS or SCL-5). The percentage of data imputed in the total sample was: 1.0% on the gross motor scale, 3.5% on the fine motor scale, 3.0% on the communication scale, 4.1% on the externalizing scale, 1.5% on the internalizing scale, 1.2% on the emotionality temperament scale, 0.8% on the activity scale, 1.6% on the sociability scale, 0.7% on the shyness scale and 3.6% on the maternal depression scale.

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

Table 4 Multiple generalized regression models of psychomotor, behavioural and temperament outcomes in 3-year-old children based on *in utero* paracetamol exposure: exposed vs non-exposed in the total MoBa cohort

	No exposure $n = 26\,213$ Reference β	**Exposure <28 days** $n = 20\,587$				**Exposure ⩾28 days** $n = 1\,831$			
		Crude β (95% CI)	*P*-value	Adjusted[a] β (95% CI)	*P*-value	Crude β (95% CI)	*P*-value	Adjusted[a] β (95% CI)	*P*-value
Psychomotor problems									
Motor milestone	0.00	0.03 (-0.02–0.07)	0.24	0.02 (-0.02–0.04)	0.10	0.10 (0.05–0.14)	<0.001	0.08 (0.03–0.13)	0.004
Gross motor	0.00	0.02 (0.01–0.04)	0.01	0.03 (0.01–0.05)	0.01	0.05 (0.001–0.10)	0.06	0.05 (0.002–0.11)	0.03
Fine motor	0.00	0.01 (-0.01–0.03)	0.31	0.01 (-0.02–0.03)	0.35	-0.002 (-0.05–0.05)	0.87	-0.02 (-0.07–0.03)	0.58
Communication	0.00	0.03 (0.01–0.05)	0.04	0.01 (-0.01–0.02)	0.22	0.08 (0.03–0.13)	0.005	0.08 (0.02–0.14)	0.02
Behaviour problems									
Externalizing	0.00	0.09 (0.07–0.10)	<0.001	0.05 (0.03–0.07)	<0.001	0.18 (0.13–0.23)	<0.001	0.13 (0.07–0.18)	<0.001
Internalizing	0.00	0.03 (0.01–0.04)	0.01	-0.001 (-0.02–0.02)	0.76	0.08 (0.03–0.13)	0.002	0.03 (-0.03–0.08)	0.42
Temperament problems									
Emotionality	0.00	0.06 (0.04–0.08)	<0.001	0.04 (0.02–0.06)	<0.001	0.12 (0.07–0.17)	<0.001	0.10 (0.04–0.15)	<0.001
Activity	0.00	0.01 (-0.02–0.02)	0.99	-0.01 (-0.03–0.01)	0.31	-0.002 (-0.05–0.05)	0.94	0.001 (-0.05–0.04)	0.76
Sociability	0.00	0.01 (-0.01–0.03)	0.41	-0.01 (-0.03–0.02)	0.60	-0.04 (-0.08–0.01)	0.13	-0.03 (-0.05–0.01)	0.65
Shyness	0.00	0.02 (-0.003–0.03)	0.10	0.01 (-0.01–0.03)	0.24	0.02 (-0.03–0.06)	0.53	0.01 (-0.04–0.06)	0.54

[a]Adjusted for maternal co-medication, fever, any allergy, asthma, rheumatism, respiratory infection, urinary tract or bladder infection, alcohol use, age, depression (SCL-5) and smoking.

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

Results

Study population

Out of 48 631 eligible children aged 3 years, 22 418 (46.1%) had been exposed to paracetamol during pregnancy, of whom 1831 (3.8%) had been exposed for ⩾28 days. Among the 2919 pairs of siblings with complete data on paracetamol exposure: 134 (4.6%) were discordant for exposure ⩾28 days; 805 (27.6%) were discordant for 1–27 days of exposure; and 1980 were concordant (1224 were both unexposed, 756 were both exposed). The most common indication reported for more than 28 days of paracetamol use was headache or migraine (63.4%), back pain and pelvic girdle pain were reported in 19.5%, fever in 19.5% and influenza or cold in 12.2%, often in combination. The characteristics of the sibling pairs who were concordant or discordant for paracetamol exposure are presented in Table 1. The characteristics of mothers and infants exposed to paracetamol and of mothers and infants who were not exposed to the drug are summarized in Table 2. There was strong evidence of shared familial confounding for the outcomes: the intraclass correlation (i.e. the similarity between the siblings) for the outcomes were 0.30 (communication), 0.27 (gross motor), 0.44 (fine motor), 0.35 (internalizing), 0.41 (externalizing), 0.34 (activity), 0.31 (emotionality), 0.34 (sociability) and 0.32 (shyness) (all $P < 0.001$).

Long-term prenatal paracetamol exposure

In the sibling-control analysis, prenatal paracetamol exposure for ⩾28 days was associated with poor gross motor functioning, delayed age starting to walk, poor communication skills, externalizing and internalizing behaviour problems and an active temperament. We found no difference between siblings discordant for first pregnancy exposure compared with second pregnancy exposure. The results from the sibling analysis are presented in Table 3 (standardized values). We found no interaction effect between trimester and exposure on any of the outcomes ($P > 0.01$). There was a tendency for the interaction term of paracetamol exposure and third trimester to be more highly associated for some of the outcomes. For example, the effect of communication was β 0.25 (95% CI 0.01–0.50), *P*-value 0.045 for exposure in the third trimester. Similar tendencies were shown for externalizing behaviour problems β 0.21 (95% CI -0.15–0.57), *P*-value 0.25, and activity β 0.23 (95% CI -0.16–0.62), *P*-value 0.25. In the cohort analysis, ⩾28 days of paracetamol exposure was associated with poor gross motor functioning, poor communication skills, externalizing behaviour and negative emotionality after adjusting for a number of covariates. The effects from the sibling-control analysis were stronger than those from the cohort analysis. The results from the cohort analysis are presented in Table 4 (standardized values).

Short-term prenatal paracetamol exposure

In the sibling-control analysis, <28 days of prenatal paracetamol exposure was associated with poor gross motor functioning. The results from the sibling analysis are presented in Table 3 (standardized values). In the cohort analysis, <28 days of prenatal paracetamol exposure was associated with a range of adverse developmental outcomes. However, after adjusting for important covariates, paracetamol exposure was related only to poor gross motor functioning, externalizing behaviour and negative emotionality. The results from the cohort analysis are presented in Table 4.

Ibuprofen exposure

To assess the specificity of our findings, we analysed the effects of ibuprofen on the same outcomes as for paracetamol in a sibling-control analysis. There were 155 pairs of same-sex siblings discordant on exposure to ibuprofen during pregnancy. The results from the crude regression analyses showed an association between ibuprofen and difference between siblings on motor milestone delay (the age at which they started walking) β 0.17 (95% CI 0.04–0.30), *P*-value 0.007. Adjusted for difference in paracetamol exposure, other medication exposure and premature birth (<week 37) the association for motor milestone delay was β 0.14 (95% CI -0.03–0.30), *P*-value 0.09. The results on the remaining developmental outcomes showed no associations with ibuprofen exposure crude: gross motor problems β 0.02 (95% CI -0.16–0.21), *P*-value 0.81; fine motor problems β 0.12 (95% CI -0.02–0.25), *P*-value 0.08; communication problems β 0.01 (95% CI -0.16–0.19), *P*-value 0.87; externalizing behaviour problems β 0.09 (95% CI -0.03–0.20), *P*-value 0.15); and internalizing behaviour problems β 0.04 (95% CI -0.03–0.20), *P*-value 0.49.

Discussion

Despite the extensive use of paracetamol during pregnancy, data on its potential effects on neurodevelopmental outcomes are lacking. In this study, paracetamol use for more than 28 days during pregnancy was associated with adverse outcomes for gross motor and communication development, behaviour and activity at 3 years of age. In contrast we found no association between ibuprofen on the same neurodevelopmental outcomes, which suggests a specific effect of paracetamol less likely to be confounded by indication.

To the best of our knowledge, no previous study has examined the relationship between neonatal paracetamol exposure and psychomotor, behaviour or temperamental outcomes. The only previous study addressing long-term cognitive outcomes after exposure to paracetamol suggested no association between the drug and the child's IQ and attention, in women receiving neonatal care in 1974–75.[2] However, studies focusing

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

on other child outcomes such as prematurity, cryptorchidism and asthma have observed adverse effects of paracetamol exposure.[3] These studies have been limited by comparing exposed children with unexposed children with potential familial confounding. When we compared differences between siblings, strong associations with paracetamol exposure for more than 28 days became apparent, suggesting an underestimation of the effects in the cohort analyses. As given by the significant sibling intraclass correlations, there was strong evidence for familial confounding on all outcomes. We effectively adjusted for 27% (gross motor) to 44% (fine motor) of the variance in the outcomes by using sibling-control. The differences in results from the current study and the previous study could be due to the different outcome instruments used and better statistical power to detect the differences observed in the current study. It could also be a result of the lack of sibling controls in the previous study, in line with our finding that the effect of paracetamol became stronger when comparing siblings than when comparing unrelated children in the cohort analyses. This could suggest that the factors comprising the familial effects had a positive impact on the outcomes, suppressing the estimates in the cohort analyses. An analogy to this would be if we were interested in the association between smoking and depression. Men are more likely to smoke, but less likely to have depression, compared with women. If you do not control for gender, you will observe a smaller association because your exposed group (smokers) have a lower risk of the outcome (depression) for reasons unrelated to smoking.

The mean difference in externalizing behaviour between the exposed and unexposed children in the sibling-control analysis was 0.28, which corresponds to a relative risk (RR) of 1.69 when assuming a 6% prevalence of behavioural problems in the normal population of preschool children.[24] Assuming the same prevalence of psychomotor problems, the observed difference of 0.24 corresponds to a RR of 1.67. With a prevalence of 4% for language disorders, a score of 0.20 corresponds to a RR of 1.51.[25] In clinical terms, these results suggest that exposure to paracetamol for more than 28 days during foetal life increases the risk of adverse psychomotor and behavioural outcomes by almost 70% and doubles the risk of language problems in 3-year-old children. For comparison, the effect of a well-established association between prenatal smoking and externalizing behaviour problems has been reported to be as small as 0.07 in a recent study using sibling design.[26] Thus, the size of the effect of extensive paracetamol use found in this study for developmental outcomes is surprisingly high. However, because clinical assessments with diagnostic tools were not available in this study, we could not determine the clinical importance of the difference observed. Future studies should seek to include clinical diagnoses of neurodevelopmental and behavioural diagnoses, to explore whether there is an increased risk of, for example, attention deficit hyperactivity disorder (ADHD) or language disorders after exposure to long-term paracetamol use during pregnancy.

We attempted to explore potential effects of timing of exposure by studying interaction effects of trimester of exposure. We did not identify any effects of timing, but the demonstrated trends of third-trimester effects observed suggest that there might be a stronger effect for third-trimester exposure that we did not have sufficient power to detect in this study. Although the central nervous system develops throughout pregnancy, the third trimester is a time of rapid brain growth and structural differentiation and this time might be especially sensitive to exposures affecting brain development. Although the cohort analysis of paracetamol use for 1–27 days found several effects on gross motor abilities, externalizing behaviour problems, and emotionality, the sibling-control analysis found effects on only poor gross motor development (corresponding to a RR of 1.21). This finding suggests that short-term use of paracetamol during pregnancy does not seem as harmful to the neurological development of the foetus. These findings are in line with previous studies, which found stronger effects after long-term exposure: 28 days for cryptorchidism,[7] most days/daily use for asthma[4] and high use in childhood for asthma.[27]

The risks of adverse outcomes after paracetamol exposure have been suggested to be mediated through oxidative stress. It has long been known that paracetamol crosses the placenta[28] but the pharmacokinetics of paracetamol in pregnancy are not well understood. In animals, it has been shown that the foetus (to a lesser degree than adults) is capable of generating the toxic metabolite N-acetyl-p-benzoquinone imine (NABQI) that is produced by paracetamol use.[29] In humans it has been suggested that even therapeutic doses of paracetamol may have important effects on oxidant/antioxidant balance.[5] It has also been shown that chronic ingestion of maximum therapeutic doses of acetaminophen can reduce serum antioxidant capacity in a few weeks.[30] An effective antioxidant system is important for the development of brain functions,[31] and thus this mechanism might apply to the potential adverse effects on brain development found in this study. However, this has not yet been studied in relation to paracetamol exposure and foetal brain development and is therefore only speculative. Future research will need to explore potential mechanism of how paracetamol might affect foetal brain development.

A major strength of this study was the large sample size, enabling sibling-control design. This design offers important advantages over cross-sectional studies exploring the effects of exposure during pregnancy by ruling out important confounders that are stable across pregnancies, such as maternal ADHD and IQ. In addition, all systematic measurement

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

errors relating to the mother are controlled for. Randomized controlled trials that involve the use of paracetamol are unethical in pregnant women. Because twins are always concordant in maternal medicine use during pregnancy, a sibling-controlled cohort is the most apt natural experiment for studying prenatal exposure to medication. Yet, confounding due to non-familial factors cannot be ruled out. Therefore, in the current study, indications of fever, infection and back pain, in addition to concomitant use of other medications during pregnancy, were adjusted for in the final analysis. The results were essentially unchanged. Importantly, the most common indications for long-term use of paracetamol were headache and migraine which were not identified to confound the results. Moreover, discordance in alcohol use and smoking did not explain the effect of long-term paracetamol use. The study was conducted in Norway, where paracetamol was sold in single-ingredient and not multi-ingredient preparations during the study time period, which rules out the possibility of confounding by other ingredients.

Some limitations do need to be considered. First, the relatively low participation rate in MoBa could potentially cause selection bias. The sibling-control design represents a more conservative step toward control for potential selection bias than conventional covariate adjustment because stable selection factors (e.g. socio-economic status) are completely adjusted for in the sibling-control design. However, with the lack of a randomly distributed exposure, we cannot rule out that selection factors varying between pregnancies might still confound the associations observed. Previous studies have demonstrated that non-participation in cohort studies has a small or no effect on the internal validity when reporting associations.[32] Unfortunately a similar study has not been performed for developmental outcomes, thus there remains a risk that our findings were confounded by selection bias. A second possible limitation of the study is assessment by self-report. Social desirability and the stigma attached to some of the measures, e.g. smoking during pregnancy, may result in underestimates. However, in a sub-study of the MoBa cohort, the self-reported smoking status during pregnancy had a sensitivity of 82% and specificity of 99%, in contrast to plasma cotinine concentrations, indicating that self-reported smoking is a valid measure in the MoBa cohort.[33] Third, we could not take dose into consideration because it was not reported, and we could not distinguish between continuous use for 28 days or more and long-term sporadic use across pregnancy because the number of mothers reporting continuous use was too small. In general the possibility of exploring different cutoffs in relation to use of paracetamol was limited in this study by the number of discordant siblings. Future studies should include these parameters. Finally, we cannot rule out the possibility that residual confounding such as unreported infections or illness could be causing the effects observed. More studies consistently showing effects of paracetamol exposure are needed to determine the probability of a cause effect.

In summary, paracetamol use during pregnancy was associated with adverse neurodevelopmental outcomes at 3 years of age. If replicated, these findings may suggest limiting long-term use of paracetamol during pregnancy.

Funding

This study was supported by grant no. 204493/H10 from the Norwegian Research Council. The Norwegian Mother and Child Cohort Study is supported by the Norwegian Ministry of Health, and the Ministry of Education and Research, and National Institutes of Health/National Institute of Environmental Health Sciences (contract no N01-ES 75558), National Institutes of Health/National Institute of Neurological Disorders and Stroke (grants UO1 NS 047537-01 and UO1 NS 047537-06A1), and the Norwegian Research Council/FUGE (The National Programme for Research in Functional Genomics in Norway) (grant no. 151918/S10).

Acknowledgements

We are grateful to all of the participating families in Norway who have taken part in this ongoing cohort study.

Conflict of interest: None declared.

KEY MESSAGES

- Long-term exposure to paracetamol during pregnancy was associated with adverse psychomotor, behavioural and temperamental outcomes at 3 years of age, after adjusting for familial and genetic confounding.
- Prenatal exposure to ibuprofen was not associated with adverse neurodevelopmental outcomes.
- If replicated, these findings may suggest limiting long-term use of paracetamol during pregnancy.

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024

References

[1] Werler MM, Mitchell AA, Hernandez-Diaz S, Honein MA. Use of over-the-counter medications during pregnancy. *Am J Obstet Gynecol* 2005;**193(3 Pt 1):**771–77.

[2] Streissguth AP, Treder RP, Barr HM *et al*. Aspirin and acetaminophen use by pregnant women and subsequent child IQ and attention decrements. *Teratology* 1987;**35:** 211–19.

[3] Rebordosa C, Kogevinas M, Bech BH, Sorensen HT, Olsen J. Use of acetaminophen during pregnancy and risk of adverse pregnancy outcomes. *Int J Epidemiol* 2009;**38:**706–14.

[4] Shaheen SO, Newson RB, Henderson AJ *et al*. Prenatal paracetamol exposure and risk of asthma and elevated immunoglobulin E in childhood. *Clin Exp Allergy* 2005; **35:**18–25.

[5] Shaheen SO, Newson RB, Ring SM, Rose-Zerilli MJ, Holloway JW, Henderson AJ. Prenatal and infant acetaminophen exposure, antioxidant gene polymorphisms, and childhood asthma. *J Allergy Clin Immunol* 2010;**126:** 1141–48, e1147.

[6] Shaheen SO, Newson RB, Davey Smith G, Henderson AJ. Prenatal paracetamol exposure and asthma: further evidence against confounding. *Int J Epidemiol* 2010;**39:** 790–94.

[7] Jensen MS, Rebordosa C, Thulstrup AM *et al*. Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism. *Epidemiology* 2010;**21:**779–85.

[8] Ystrom E, Vollrath ME, Nordeng H. Effects of personality on use of medications, alcohol, and cigarettes during pregnancy. *Eur J Clin Pharmacol* 2012;**68:**845–51.

[9] Bouchard TJ Jr, Loehlin JC. Genes, evolution, and personality. *Behav Genet* 2001;**31:**243–73.

[10] Caspi A. The child is father of the man: personality continuities from childhood to adulthood. *J Pers Soc Psychol* 2000;**78:**158–72.

[11] Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C. Cohort profile: The Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol* 2006;**35:** 1146–50.

[12] Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. *Acta Obstet Gynecol Scand* 2000;**79:**435–39.

[13] World Health Organization. Classifications. The anatomical therapeutic chemical classification system with defined daily doses (ATC/DDD). 2012. http://www.who.int/classifications/atcddd/en (21 November 2012, date last accessed).

[14] Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. *J Pediatr Psychol* 1997;**22:**313–28.

[15] Richter J, Janson H. A validation study of the Norwegian version of the Ages and Stages Questionnaires. *Acta Paediatr* 2007;**96:**748–52.

[16] Lord FM. *Applications of Item Response Theory to Practical Testing Problems*. Hillsdale, NJ: Lawrence Earlbaum Associates, 1980.

[17] Bodnarchuk JL, Eaton WO. Can parent reports be trusted? Validity of daily checklists of gross motor milestone attainment. *J Appl Dev Psychol* 2004;**25:**481–90.

[18] Achenbach TM, Ruffle TM. The Child Behavior Checklist and related forms for assessing behavioral/emotional problems and competencies. *Am Acad Pediatr* 2000;**21:** 265–71.

[19] Zachrisson HD, Dearing E, Lekhal R, Toppelberg CO. Little evidence that time in child care causes externalizing problems during early childhood in Norway. *Child Dev* 2013;**84:**1152–70.

[20] Buss AH, Plomin R. *Temperament: Early Developing Personality Traits*. Hillsdale, NJ: Erlbaum, 1984.

[21] Mathiesen KS, Tambs K. The EAS temperament questionnaire – factor structure, age trends, reliability, and stability in a Norwegian sample. *J Child Psychol Psychiatry* 1999; **40:**431–39.

[22] Strand BH, Dalgard OS, Tambs K, Rognerud M. Measuring the mental health status of the Norwegian population: a comparison of the instruments SCL-25, SCL-10, SCL-5 and MHI-5 (SF-36). *Nord J Psychiatry* 2003;**57:**113–18.

[23] Goldberg J FM. *Co-Twin Control Methods*. Chichester: John Wiley, 2005.

[24] Wichstrom L, Berg-Nielsen TS, Angold A, Egger HL, Solheim E, Sveen TH. Prevalence of psychiatric disorders in preschoolers. *J Child Psychol Psychiatry* 2012;**53:**695–705.

[25] Nelson HD, Nygren P, Walker M, Panoscha R. Screening for speech and language delay in preschool children: Systematic evidence review for the US preventive services task force. *Pediatrics* 2006;**117:**E298–E319.

[26] D'Onofrio BM, Van Hulle CA, Waldman ID *et al*. Smoking during pregnancy and offspring externalizing problems: An exploration of genetic and environmental confounds. *Dev Psychopathol* 2008;**20:**139–64.

[27] Beasley R, Clayton T, Crane J *et al*. Association between paracetamol use in infancy and childhood, and risk of asthma, rhinoconjunctivitis, and eczema in children aged 6-7 years: analysis from Phase Three of the ISAAC programme. *Lancet* 2008;**372:**1039–48.

[28] Levy G, Garrettson LK, Soda DM. Letter: Evidence of placental transfer of acetaminophen. *Pediatrics* 1975;**55:**895.

[29] Rollins DE, von Bahr C, Glaumann H, Moldeus P, Rane A. Acetaminophen: potentially toxic metabolite formed by human fetal and adult liver microsomes and isolated fetal liver cells. *Science* 1979;**205:**1414–16.

[30] Nuttall SL, Khan JN, Thorpe GH, Langford N, Kendall MJ. The impact of therapeutic doses of paracetamol on serum total antioxidant capacity. *J Clin Pharm Ther* 2003;**28:**289–94.

[31] Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* 2000;**62:**649–71.

[32] Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? *Epidemiology* 2006;**17:**413–18.

[33] Kvalvik LG, Nilsen RM, Skjaerven R *et al*. Self-reported smoking status and plasma cotinine concentrations among pregnant women in the Norwegian Mother and Child Cohort Study. *Pediatr Res* 2012;**72:**101–07.

Downloaded from https://academic.oup.com/ije/article/42/6/1702/739709 by guest on 24 April 2024