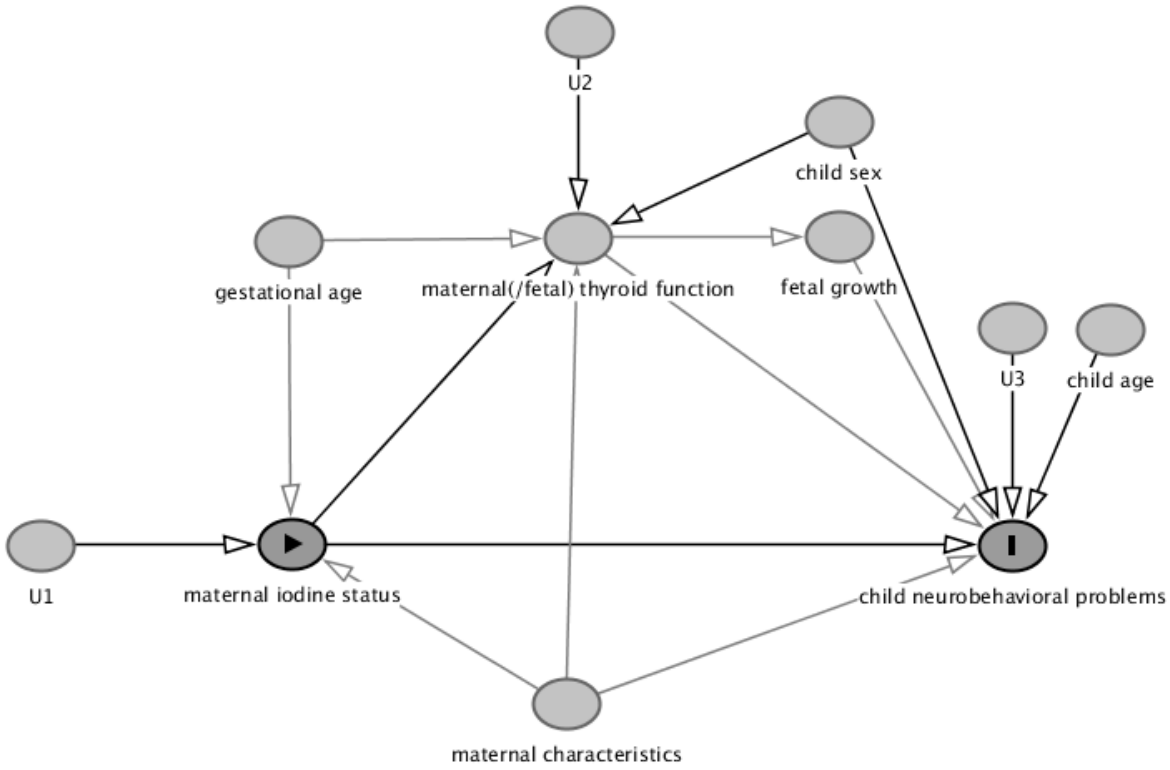
Supplementary material

Supplement to: Deborah Levie et al., Maternal iodine status during pregnancy is not consistently associated with attention-deficit hyperactivity disorder or autistic traits in the child.



Supplemental Figure 1 Directed Acyclic Graph showing the association between maternal iodine status and child neurobehavioral problems. Abbreviations: U, unmeasured.

Supplemental Table 1 Distribution of the child and maternal characteristics among the final study population and the mother-child pairs that were lost to follow up [1].

	Generation R			INMA			ALSPAC		
	This study (n=1634)	**Not enrolled in current study** (n=8115)	***P* value**	**This study** (n=1293)	**Not enrolled in current study** (n=857)	***P* value**	**This study** (n=2619)	**Not enrolled in current study** (n=12113)	***P* value**
Educational level [2]									
Low	102 (6.5)	843 (12.3)	<0.001	272 (21.1)	291 (35.2)	<0.001	480 (18.7)	3276 (33.0)	<0.001
Middle	627 (39.7)	3250 (47.4)		531 (41.2)	331 (40.0)		1614 (62.8)	5514 (55.6)	
High	85 (53.9)	2762 (40.3)		486 (37.7)	205 (24.8)		476 (18.5)	1133 (11.4)	
Maternal ethnicity									
Majority [3]	927 (56.8)	3575 (48.8)	<0.001	1207 (93.5)	708 (85.6)	<0.001	2527 (98.6)	9548 (97.0)	<0.001
Minority [4]	706 (43.2)	3749 (51.1)		84 (6.5)	119 (14.4)		35 (1.4)	291 (3.0)	
Maternal age, y	30.8 (4.6)	29.7 (5.5)	<0.001	31.6 (3.9)	30.7 (4.8)	<0.001	28.7 (4.4)	27.0 (5.1)	<0.001
Parity									
0	980 (60.0)	4127 (51.6)	<0.001	726 (56.2)	442 (53.5)	0.15	1211 (47.6)	4662 (44.1)	0.003
1	469 (28.7)	2326 (29.1)		480 (37.2)	313 (37.9)		864 (34.0)	3724 (35.2)	
≥2	185 (11.3)	1541 (19.3)		85 (6.6)	72 (8.7)		470 (18.5)	2193 (20.7)	
Smoking									
Never	1142 (76.6)	4825 (72.7)	<0.001	892 (69.9)	456 (63.1)	0.007	2182 (84.4)	7698 (71.5)	<0.001
At the beginning of pregnancy	149 (10.0)	542 (8.2)		169 (13.2)	115 (15.9)		95 (3.7)	712 (6.6)	
Continued	199 (13.4)	1266 (19.1)		215 (16.9)	152 (21.0)		309 (12.0)	2363 (21.9)	
Pre-pregnancy BMI, kg/m^2	22.6 (20.8, 25.1)	22.7 (20.7, 25.6)	0.59	22.5 (20.8, 25.0)	22.6 (20.6, 25.4)	0.96	22.2 (20.5, 24.4)	22.1 (20.5, 24.4)	0.31
Female sex	819 (50.1)	3990 (49.2)	0.49	643 (49.8)	337 (46.6)	0.17	1328 (50.7)	5498 (47.7)	0.006

[1] Values are number (percentages) for categorical variables and medians (IQR) for continuous non-normal-distributed variables. Values are based on unimputed data. *P* values were calculated using the Chi-square test for categorical variables and the Kruskal-Wallis test for continuous non-normal-distributed variables.

[2] Generation R: low = no education or primary; middle = secondary phase 1 and 2; high = higher phase 1 and 2; INMA: low = no education, unfinished primary, or primary; middle = secondary; high = university degree; ALSPAC: low = no qualification, certificate of secondary education, or vocational; middle = O level or A level; high = a degree.

[3] majority defined as Dutch (in Generation R), Spanish (in INMA) or White (in ALSPAC).

[4] minority defined as Indonesian, Cape Verdean, Moroccan, Dutch Antilles, Suriname, Turkish, Asian, other non-Western, other Western (in Generation R), Latin-American, European, others (in INMA), and non-White (in ALSPAC).

Abbreviations: BMI, body mass index; IQR, interquartile range.

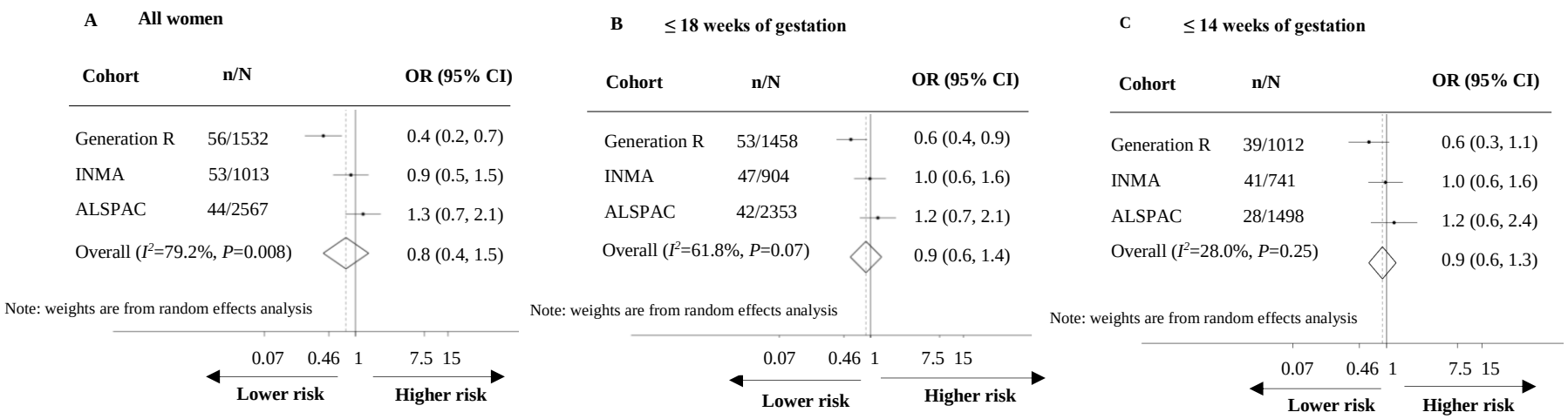
Supplemental Table 2 Maternal iodine status in women who gave a single urine sample and those who gave more than one [1].

	Generation R				
	women with one measure (n=344, 21.1%)	**women with repeated measures** (n=1290, 78.9%) **first** (n=1290)	**second** (n=1290)		***P* value**
UI/Creat, µg/g	214 (137, 316)	212 (143, 307)	217 (152, 307)		0.88
UI/Creat <150 µg/g, n (%)	104 (30.2)	352 (27.9)	310 (24.0)		0.28
UIC, µg/L	158 (87, 271)	156 (89, 261)	148 (86, 252)		0.50
UIC <150 µg/L, n (%)	166 (48.3)	618 (47.9)	659 (51.1)		0.91
Gestational age, wk	12.9 (11.9, 14.5)	13.2 (12.1, 14.6)	20.5 (19.9, 21.1)		0.19
	INMA [2]				
	women with one UI/Creat measure (n=364, 28.2%)	**women with repeated UI/Creat measures** (n=929, 71.8%) **first** (n=929)	**second** (n=929)		***P* value**
UI/Creat, µg/g	163 (98, 260)	152 (96, 258)	177 (113, 302)		0.33
UI/Creat <150 µg/g, n (%)	172 (47.2)	455 (49.0)	367 (39.5)		0.54
Gestational age, wk	13.0 (12.3, 32.1)	13.0 (12.4,13.9)	33.0 (32.0, 34.4)		<0.001
	women with one UIC measure (n=312, 23.4 %)	**women with repeated UIC measures** (n=1019, 76.6%) **first** (n=1019)	**second** (n=1019)		***P* value**
UIC, µg/L	129 (79, 204)	128 (75, 217)	143 (89, 249)		0.88
UIC <150 µg/L, n (%)	131 (42.0)	428 (42.0)	532 (52.2)		0.99
Gestational age, wk	12.6 (12.0, 13.0)	13.0 (12.4, 13.9)	33.0 (32.0, 34.4)		<0.001
	ALSPAC [2,3]				
	women with one UI/Creat measure (n=2207, 84.3%)	**women with repeated UI/Creat measures** (n=412, 15.7%) **first** (n=412)	**second** (n=412)	**third** (n=179)	***P* value**
UI/Creat, µg/g	128 (85 ,208)	101 (73, 149)	175 (106, 270)	161 (109, 239)	<0.001
UI/Creat <150 µg/g, n (%)	1304 (59.1)	311 (75.5)	170 (41.3)	78 (43.6)	<0.001
Gestational age, wk	13 (9, 17)	8.0 (6.0, 11.0)	17.0 (16.0, 19.0)	34.0 (33.0, 35.0)	<0.001
	women with one UIC measure (n=2277, 83.9%)	**women with repeated UIC measures** (n=437, 16.1%) **first** (n=437)	**second** (n=437)	**third** (n=197)	***P* value**
UIC, µg/L	96 (56, 157)	98 (63, 147)	104 (56, 171)	135 (79, 214)	0.70
UIC <150 µg/L, n (%)	1644 (72.2)	331 (75.7)	302 (69.1)	108 (55.1)	0.13
Gestational age, wk	13 (9, 17)	8.0 (6.0, 11.0)	17.0 (16.0, 18.0)	34.0 (34.0, 35.0)	<0.001

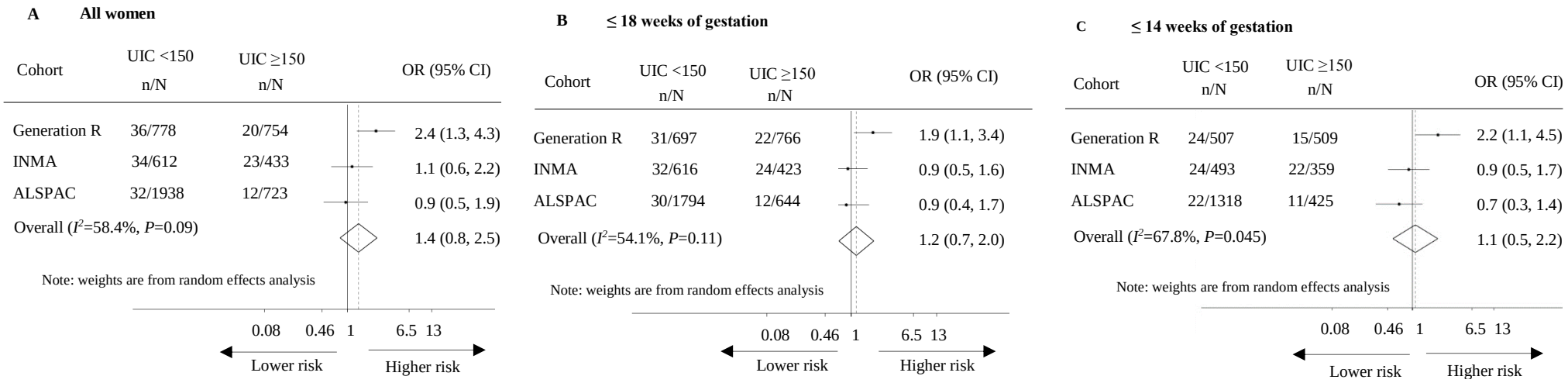
[1] Values represent median (IQR), unless indicated otherwise. *P* values were calculated using the Chi-square test for categorical variables and the Kruskal-Wallis test for continuous non-normal-distributed variables and indicate whether there are statistical significant differences between the first measures in the group of women who gave one urine sample and those who gave more than one urine sample.

[2] In INMA and ALSPAC the number of women with data on at least one UIC and one UI/Creat measure and child outcome data is not equal due to missing data on creatinine in INMA or contamination of urine samples in ALSPAC (difference: INMA, N=38; ALSPAC, N=95).

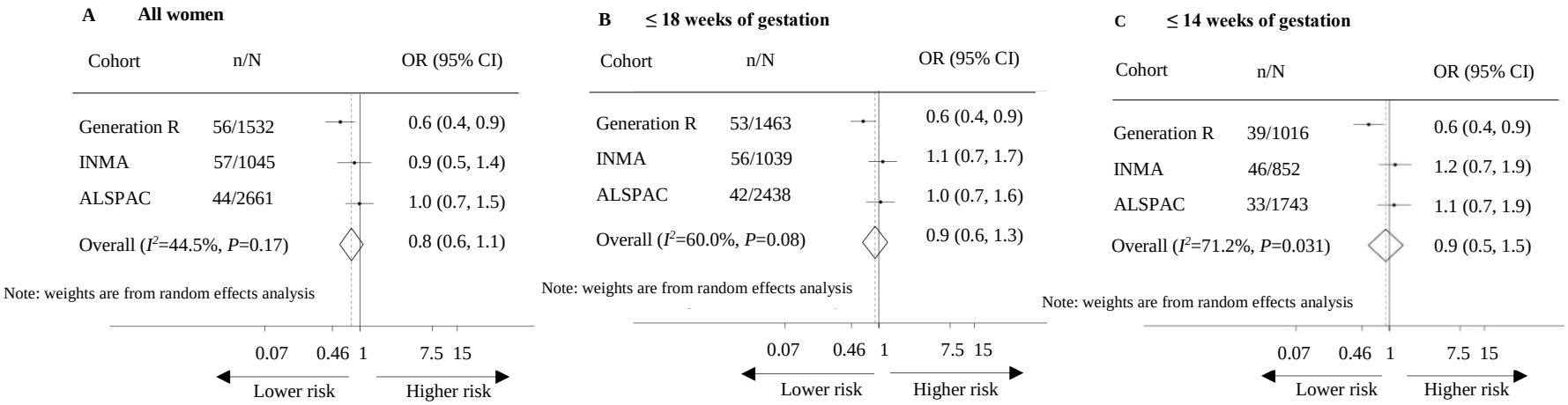
[3] The subgroup of women with four UI/Creat or UIC measures (n=1) was too small for meaningful descriptives and therefore omitted.



Supplemental Figure 2 Association between a one unit increase in the natural logarithm of maternal UI/Creat (µg/g) and child ADHD. Associations depicted as odds ratio (dot) with 95% confidence interval per cohort and overall associations as estimated by random-effects meta-analysis (diamond) in a) all mother-child pairs, b) in those with at least one measure of UI/Creat ≤18 weeks of gestation, and c) in those with at least one measure of UI/Creat ≤14 weeks of gestation. Analyses adjusted for maternal age, parity, pre-pregnancy body mass index, smoking during pregnancy, ethnicity/country of birth, maternal educational level, gestational age at urine sampling, child sex, child age, and sub-cohort in INMA. n=children with ADHD, N=children without ADHD.



Supplemental Figure 3 Association of maternal UIC < 150 µg/L with child ADHD. Associations depicted as odds ratio (dot) with 95% confidence interval per cohort and overall associations as estimated by random-effects meta-analysis (diamond) in a) all mother-child pairs, b) in those with at least one measure of UIC ≤ 18 weeks of gestation, and c) in those with at least one measure of UIC ≤ 14 weeks of gestation. Analyses adjusted for maternal age, parity, pre-pregnancy body mass index, smoking during pregnancy, ethnicity/country of birth, maternal educational level, gestational age at urine sampling, child sex, child age, and sub-cohort in INMA. n=children with ADHD, N=children without ADHD.



Supplemental Figure 4 Association between a one unit increase in the natural logarithm of maternal UIC (µg/L) and child ADHD. Associations depicted as odds ratio (dot) with 95% confidence interval per cohort and overall associations as estimated by random-effects meta-analysis (diamond) in a) all mother-child pairs, b) in those with at least one measure of UIC ≤ 18 weeks of gestation, and c) in those with at least one measure of UIC ≤ 14 weeks of gestation. Analyses adjusted for maternal age, parity, pre-pregnancy body mass index, smoking during pregnancy, ethnicity/country of birth, maternal educational level, gestational age at urine sampling, child sex, child age, and sub-cohort in INMA. n=children with ADHD, N=children without ADHD.

Supplemental Table 3 Continuous analysis of the association of UI/Creat <150 µg/g during pregnancy with ADHD symptoms and autistic traits

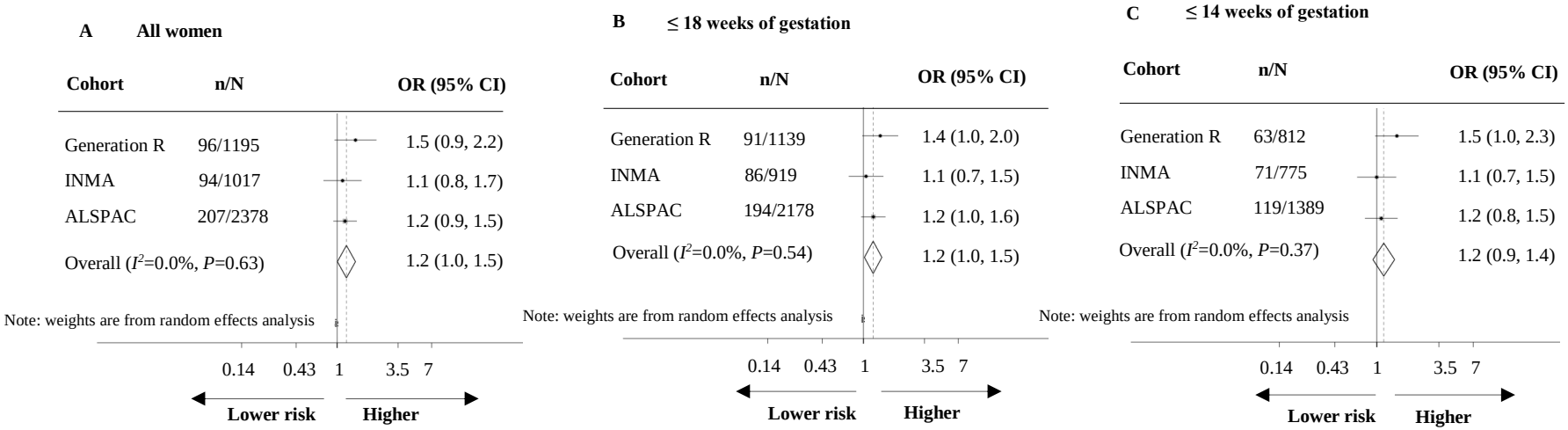
			ADHD symptoms	
Subgroup	**Cohort**	**N (% UI/Creat < 150 µg/g)**	**IRR (95% CI)**	***P* value**
all women	Generation R	1586 (23.1)	1.02 (0.92, 1.12)	0.72
	INMA	1056 (44.7)	1.11 (0.94, 1.31)	0.23
	ALSPAC	2592 (58.6)	0.99 (0.87, 1.130	0.86
≤18 weeks	Generation R	1509 (27.8)	1.09 (0.99, 1.20)	0.08
	INMA	943 (49.4)	0.98 (0.83, 1.16)	0.84
	ALSPAC	2377 (61.0)	0.99 (0.86, 1.14)	0.87
≤14 weeks	Generation R	1049 (27.7)	1.05 (0.93, 1.18)	0.37
	INMA	774 (47.0)	1.00 (0.84, 1.19)	0.98
	ALSPAC	1510 (69.7)	0.98 (0.82, 1.18)	0.85
			Autistic traits	
Subgroup	**Cohort**	**N (% UI/Creat < 150 µg/g)**	**IRR (95% CI)**	***P* value**
all women	Generation R	1291 (22.8)	0.94 (0.71, 1.24)	0.64
	INMA	1111 (40.1)	0.99 (0.93, 1.06)	0.83
	ALSPAC	2585 (58.7)	0.99 (0.88, 1.10)	0.79
≤18 weeks	Generation R	1230 (27.3)	0.96 (0.73, 1.26)	0.77
	INMA	1004 (47.1)	1.02 (0.96, 1.09)	0.51
	ALSPAC	2372 (60.8)	0.97 (0.86, 1.09)	0.63
≤14 weeks	Generation R	875 (27.3)	0.88 (0.63, 1.23)	0.47
	INMA	845 (46.2)	1.04 (0.97, 1.12)	0.29
	ALSPAC	1508 (69.5)	0.97 (0.84, 1.13)	0.72

Reported incidence rate ratio (IRR) and 95% confidence intervals represent the change in symptoms in the offspring born to women with UI/Creat < 150 µg/g versus those born to women with UI/Creat ≥ 150 µg/g in terms of a percentage increase or decrease, with the precise percentage determined by the amount the IRR is either above or below 1. Analyses were performed using negative binomial regression models and adjusted for gestational age at urine sampling, maternal education, maternal ethnicity/country of birth, age, parity, smoking during pregnancy, pre-pregnancy body mass index, child sex, child age, and sub-cohort in INMA.

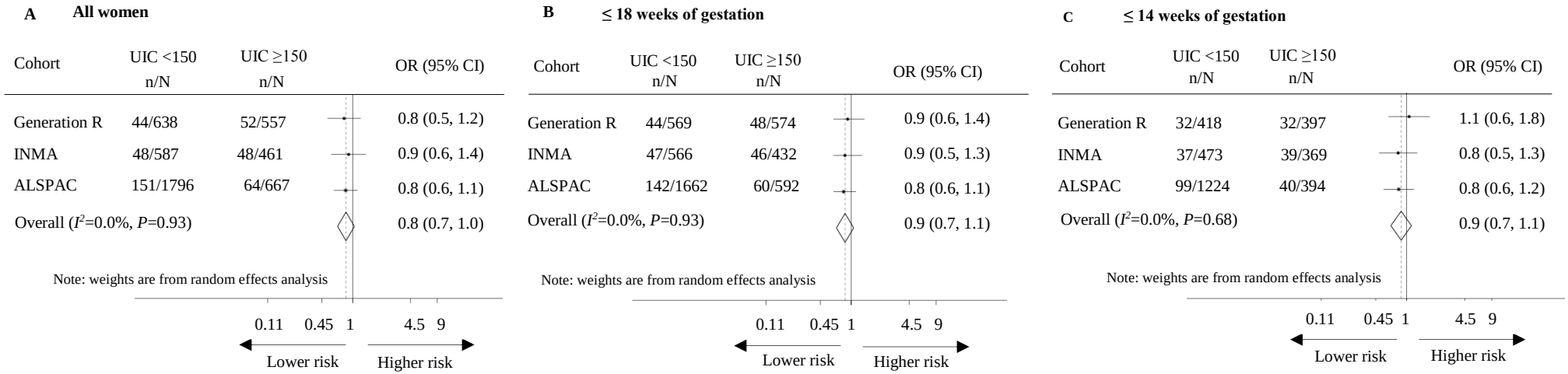
Supplemental Table 4 Association between a one unit increase in the natural logarithm of maternal UI/Creat (µg/g) and ADHD symptoms and autistic traits

			ADHD symptoms	
Subgroup	**Cohort**	**N**	**IRR (95% CI)**	***P* value**
all women	Generation R	1586	0.93 (0.86, 1.02)	0.13
	INMA	1056	1.02 (0.89, 1.17)	0.75
	ALSPAC	2592	1.05 (0.94, 1.16)	0.41
≤18 weeks	Generation R	1509	0.93 (0.86, 1.00)	0.05
	INMA	943	1.05 (0.93, 1.18)	0.46
	ALSPAC	2377	1.06 (0.95, 1.19)	0.32
≤14 weeks	Generation R	1049	0.95 (0.86, 1.04)	0.23
	INMA	774	1.03 (0.91, 1.17)	0.60
	ALSPAC	1510	1.06 (0.92, 1.22)	0.39
			Autistic traits	
Subgroup	**Cohort**	**N**	**IRR (95% CI)**	***P* value**
all women	Generation R	1291	1.06 (0.83, 1.34)	0.64
	INMA	1111	1.02 (0.97, 1.08)	0.39
	ALSPAC	2585	1.04 (0.95, 1.13)	0.38
≤18 weeks	Generation R	1230	1.05 (0.86, 1.29)	0.64
	INMA	1004	1.00 (0.96, 1.05)	0.90
	ALSPAC	2372	1.05 (0.96, 1.16)	0.26
≤14 weeks	Generation R	875	1.04 (0.82, 1.33)	0.73
	INMA	845	0.99 (0.94, 1.04)	0.60
	ALSPAC	1508	1.03 (0.92, 1.16)	0.57

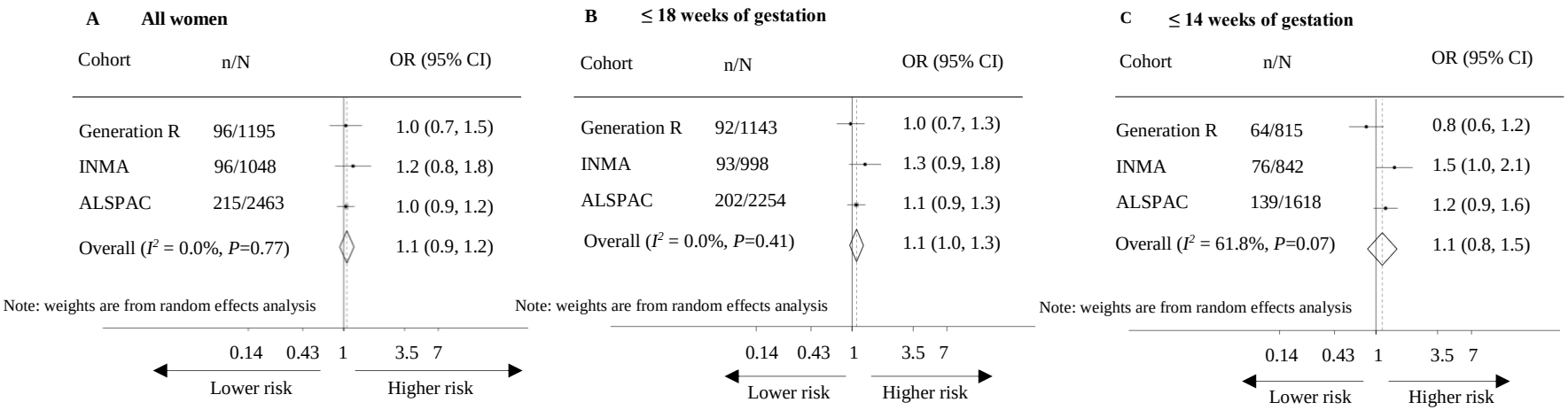
Reported incidence rate ratio (IRR) and 95% confidence intervals represent the change in symptoms per Ln of UI/Creat in terms of a percentage increase or decrease, with the precise percentage determined by the amount the IRR is either above or below 1. Analyses were performed using negative binomial regression models and adjusted for gestational age at urine sampling, maternal education, maternal ethnicity/country of birth, age, parity, smoking during pregnancy, pre-pregnancy body mass index, child sex, child age, and sub-cohort in INMA.



Supplemental Figure 5 Association between a one unit increase in the natural logarithm of maternal UI/Creat (µg/g) and a high child autistic trait score ≥ 93rd percentile. Associations depicted as odds ratio (dot) with 95% confidence interval per cohort and overall associations as estimated by random-effects meta-analysis (diamond) in a) all mother-child pairs, b) in those with at least one measure of UI/Creat ≤18 weeks of gestation, and c) in those with at least one measure of UI/Creat ≤14 weeks of gestation. Analyses adjusted for maternal age, parity, pre-pregnancy body mass index, smoking during pregnancy, ethnicity/country of birth, maternal educational level, gestational age at urine sampling, child sex, child age, and sub-cohort in INMA. n=children with a score ≥ 93rd percentile, N=children with a score < 93rd percentile.



Supplemental Figure 6 Association of maternal UIC < 150 µg/L with a high child autistic trait score ≥ 93rd percentile. Associations depicted as odds ratio (dot) with 95% confidence interval per cohort and overall associations as estimated by random-effects meta-analysis (diamond) in a) all mother-child pairs, b) in those with at least one measure of UIC ≤ 18 weeks of gestation, and c) in those with at least one measure of UIC ≤ 14 weeks of gestation. Analyses adjusted for maternal age, parity, pre-pregnancy body mass index, smoking during pregnancy, ethnicity/country of birth, maternal educational level, gestational age at urine sampling, child sex, child age, and sub-cohort in INMA. n=children with a score ≥ 93rd percentile, N=children with a score < 93rd percentile.



Supplemental Figure 7 Association between a one unit increase in the natural logarithm of maternal UIC (µg/L) and a high child autistic trait score ≥ 93rd percentile. Associations depicted as odds ratio (dot) with 95% confidence interval per cohort and overall associations as estimated by random-effects meta-analysis (diamond) in a) all mother-child pairs, b) in those with at least one measure of UIC ≤ 18 weeks of gestation, and c) in those with at least one measure of UIC ≤ 14 weeks of gestation. Analyses adjusted for maternal age, parity, pre-pregnancy body mass index, smoking during pregnancy, ethnicity/country of birth, maternal educational level, gestational age at urine sampling, child sex, child age, and sub-cohort in INMA. n=children with a score ≥ 93rd percentile, N=children with a score < 93rd percentile.

Supplemental Table 5 Maternal thyroid function among women with UI/Creat <150 µg/g and ≥150 µg/g.

	Generation R			INMA			ALSPAC		
	<150 µg/g	**≥ 150 µg/g**	***P* value**	**<150 µg/g**	**≥ 150 µg/g**	***P* value**	**<150 µg/g**	**≥ 150 µg/g**	***P* value**
TSH, mIU/L	1.28 (0.76-1.89)	1.36 (0.82-2.04)	0.17	1.27 (0.85-1.79)	1.22 (0.83-1.81)	0.88	0.98 (0.64-1.40)	0.99 (0.65-1.41)	0.35
FT4, pmol/L	14.8 (13.2-16.5)	14.5 (12.9-16.4)	0.12	10.6 (9.7-11.6)	10.5 (9.6-11.4)	0.12	16.2 (15.0-17.7)	16.1 (14.8-17.5)	0.71
TPOAb positivity, %	4.5	5.7	0.35	NA	NA	NA	12.4	13.4	0.67

Values represent medians (interquartile range), unless indicated otherwise. *P* values were calculated using the Chi-square test for categorical variables and the Kruskal-Wallis test for continuous non-normal-distributed variables. Abbrevation: NA, not available