表 1　出生時の体格に関連する要因

1）出生体重（調整済み R^2=0.251, p=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	−6611.218	9240.307		−.715	.474	−24745.564	11523.129
	母体出生年	1.940	4.636	.018	.418	.676	−7.158	11.038
	母体年齢	−.342	5.687	−.002	−.060	.952	−11.503	10.818
	非妊娠時BMI	20.436	4.025	.145	5.077	.000	12.536	28.336
	受動喫煙	2.361	22.302	.003	.106	.916	−41.408	46.130
	乳児性	−86.083	21.964	−.111	−3.919	.000	−129.188	−42.977
	在胎週数	140.274	8.558	.471	16.391	.000	123.479	157.069
	PCDDs+PCDFs +CoPCB(12)	−1.262	1.719	−.023	−.734	.463	−4.636	2.112

2）出生時身長（調整済み R^2=0.226, p=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	31.199	50.071		.623	.533	−67.067	129.464
	母体出生年	−.005	.025	−.009	−.206	.837	−.054	.044
	母体年齢	−.037	.031	−.049	−1.215	.225	−.098	.023
	非妊娠時BMI	.053	.022	.070	2.417	.016	.010	.095
	受動喫煙	.108	.121	.026	.895	.371	−.129	.346
	乳児性	−.559	.119	−.136	−4.699	.000	−.793	−.326
	在胎週数	.726	.047	.456	15.595	.000	.634	.817
	PCDDs+PCDFs +CoPCB(12)	.000	.009	−.001	−.033	.974	−.019	.018

3）出生時頭囲（調整済み R^2=0.140, p=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	−11.726	36.424		−.322	.748	−83.209	59.758
	母体出生年	.014	.018	.036	.784	.433	−.022	.050
	母体年齢	.040	.022	.077	1.804	.072	−.004	.084
	非妊娠時BMI	.034	.016	.067	2.170	.030	.003	.066
	受動喫煙	−.051	.088	−.018	−.581	.561	−.224	.122
	乳児性	−.386	.087	−.136	−4.453	.000	−.556	−.216
	在胎週数	.375	.034	.342	11.092	.000	.309	.442
	PCDDs+PCDFs +CoPCB(12)	.019	.007	.094	2.814	.005	.006	.032

表 2. 生後 1 か月時点の体格に関連する要因

1） 体重（調整済み R^2=0.528、P＝0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	2620.091	8019.796		.327	.744	-13118.859	18359.040
	母体出生年	-.947	4.015	-.007	-.236	.814	-8.826	6.932
	母体年齢	-4.390	5.287	-.023	-.830	.407	-14.765	5.986
	非妊娠時BMI	5.206	4.499	.027	1.157	.248	-3.623	14.035
	乳児性	-140.858	24.025	-.133	-5.863	.000	-188.009	-93.708
	在胎週数	26.042	10.381	.065	2.509	.012	5.669	46.414
	出生体重	.901	.036	.660	25.119	.000	.831	.972
	母乳回数	-8.107	4.908	-.038	-1.652	.099	-17.740	1.525
	PCDDs+PCDFs +CoPCB(12)	-6.505	1.882	-.088	-3.456	.001	-10.199	-2.811

2） 身長（調整済み R^2=0.300、P=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	39.199	38.558		1.017	.310	-36.475	114.874
	母体出生年	-.011	.019	-.021	-.566	.572	-.049	.027
	母体年齢	.008	.025	.010	.320	.749	-.042	.058
	非妊娠時BMI	-.001	.021	-.002	-.070	.944	-.043	.040
	乳児性	-.638	.116	-.146	-5.474	.000	-.866	-.409
	在胎週数	.285	.049	.173	5.766	.000	.188	.382
	出生時身長	.525	.032	.489	16.288	.000	.462	.589
	母乳回数	-.040	.024	-.044	-1.667	.096	-.086	.007
	PCDDs+PCDFs +CoPCB(12)	-.020	.009	-.068	-2.265	.024	-.038	-.003

3） 頭囲（調整済み R^2=0.270、P=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	11.473	26.908		.426	.670	-41.336	64.282
	母体出生年	.003	.013	.008	.199	.842	-.024	.029
	母体年齢	-.006	.018	-.012	-.347	.729	-.041	.029
	非妊娠時BMI	.041	.015	.079	2.781	.006	.012	.071
	乳児性	-.628	.081	-.221	-7.756	.000	-.786	-.469
	在胎週数	.219	.032	.204	6.774	.000	.156	.283
	出生時頭囲	.350	.031	.347	11.464	.000	.290	.410
	母乳回数	-.015	.017	-.026	-.925	.355	-.048	.017
	PCDDs+PCDFs +CoPCB(12)	-.004	.006	-.023	-.702	.483	-.017	.008

表 3　生後 1 歳時点の体格に関連する要因

1）体重（調整済み R^2=0.244, p=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	-6979.370	27311.183		-.256	.798	-60630.362	46671.622
	母体出生年	6.642	13.719	.026	.484	.628	-20.308	33.593
	母体年齢	36.265	16.992	.102	2.134	.033	2.885	69.644
	非妊娠時BMI	47.851	14.375	.126	3.329	.001	19.613	76.088
	乳児性	-445.012	74.244	-.230	-5.994	.000	-590.858	-299.165
	在胎週数	-27.495	31.979	-.036	-.860	.390	-90.316	35.326
	体重 (1Mo)	.737	.079	.390	9.314	.000	.582	.893
	母乳回数	-18.079	15.995	-.043	-1.130	.259	-49.501	13.342
	PCDDs+PCDFs +CoPCB(12)	6.584	5.829	.048	1.130	.259	-4.866	18.034

2）身長（調整済み R^2=0.205, p=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	-94.421	88.531		-1.067	.287	-268.350	79.507
	母体出生年	.073	.044	.091	1.652	.099	-.014	.161
	母体年齢	.058	.055	.052	1.045	.297	-.051	.166
	非妊娠時BMI	.026	.046	.022	.568	.570	-.065	.117
	乳児性	-1.145	.244	-.190	-4.703	.000	-1.624	-.667
	在胎週数	-.188	.105	-.078	-1.794	.073	-.394	.018
	身長 (1Mo)	.585	.063	.403	9.244	.000	.461	.709
	母乳回数	-.049	.053	-.037	-.934	.351	-.153	.054
	PCDDs+PCDFs +CoPCB(12)	.022	.019	.052	1.195	.233	-.014	.059

3）頭囲（R^2=0.223, p=0.000）

モデル		非標準化係数 B	非標準化係数 標準誤差	標準化係数 ベータ	t 値	有意確率	B の 95.0% 信頼区間 下限	B の 95.0% 信頼区間 上限
1	(定数)	-18.578	54.113		-.343	.732	-124.889	87.733
	母体出生年	.027	.027	.055	.974	.331	-.027	.080
	母体年齢	.026	.034	.039	.769	.442	-.041	.094
	非妊娠時BMI	.088	.027	.127	3.247	.001	.035	.142
	乳児性	-.764	.144	-.214	-5.306	.000	-1.047	-.481
	在胎週数	-.159	.060	-.110	-2.661	.008	-.277	-.042
	頭囲 (1Mo)	.471	.056	.357	8.489	.000	.362	.580
	母乳回数	-.064	.031	-.081	-2.067	.039	-.125	-.003
	PCDDs+PCDFs +CoPCB(12)	.026	.011	.101	2.348	.019	.004	.048

厚生労働科学研究（食品の安全確保推進研究事業）
母乳のダイオキシン類汚染の実態調査と乳幼児の発達への影響に関する研究
分担研究報告書

周産期のダイオキシン類推定暴露量と3～5歳児の行動問題との関連

研究分担者　河野由美　自治医科大学小児科 学内教授

研究要旨
母乳中ダイオキシン量から周産期の暴露量を推定し、行動スクリーニング尺度「子どもの強さと困難さアンケート（SDQ)」を用いて3歳～5歳児の行動問題との関係を検討した。出生前の暴露指標は母乳中総ダイオキシン類量とし、生後1年間の暴露指標として母乳中ダイオキシン類濃度、平均脂肪濃度と母乳摂取量から推定摂取量(EDE)を算出した。対象は初産母体の3～5歳児44名(男23名、女21名)。平均EDEは男児15.9、女児16.2 ngTEQ/kg/year、生後1年間の1日平均摂取量は男児44、女児45 pgTEQ/kg/dayであった。母体年齢、母体の喫煙歴、児の年齢、出生体重を共変量とした重回帰分析で、母乳中総ダイオキシン類濃度あるいはEDE と、SDQの行動問題のスコア total Difficulties score(TDS)とに有意な関連を認めなかった。

A．研究目的

ダイオキシン類などの内分泌攪乱物質が生体に多様な影響を与えることが指摘され、中でも小児においては胎児期の暴露の影響、母乳栄養による摂取の影響が懸念される。平成26年度の研究で、過去に母乳中ダイオキシン類濃度を測定された母体の学童(6歳～13歳)を対象として、①1か月時の母乳中総ダイオキシン類量、②生後1年間の母乳からのダイオキシン推定摂取量(Estimated dioxin exposure、EDE)と、行動スクリーニング尺度「子どもの強さと困難さアンケート」(Strengths and Difficulties Questionnaires :SDQ)のスコアに有意な関連がないことを示した。平成27年度は、3歳～5歳児を対象として、同様の検討を行った。

B．研究方法

1)　対象

産後1か月時の母乳中PCDD、PCDF、CoPCB(12種)の濃度が測定され、0～12ヵ月までの哺乳方法（母乳、混合、人工栄養の別）から母乳からのダイオキシン類の摂取量が推定可能な2006年～2008年出生の3歳～5歳児を対象とした。保護者にSDQの質問紙を88名の保護者に郵送し、44名から回答が得られた。

1)　ダイオキシン類推定摂取量(EDE)

対象の母体の母乳中のPCDDs+PCDFs+CoPCB12の総和濃度、母乳中平均脂肪濃度、生後1年間の母乳率（すべて母乳の場合＝1）から、「日本人の食事摂取基準」に基づく乳児期の哺乳量を用いて、生後1年間のEDE(ngTEQ/kg/year)を求めた。ダイオキシン類濃度の毒性等価係数(TEQ)は2005 WHO consensus TEFを用いた。

2)　SDQ

SDQは、児の年齢に相当した日本語版を保護者に郵送し回収した。質問項目は、情緒、行為、多動性、仲間関係、向社会性の5分野からなり、情緒、行為、多動性、仲間関係の4分野のサブスコアの合計でTDS(total Difficulties score)を算出した。

3)　統計学的方法

母乳中の総ダイオキシン類濃度、EDE、 SDQスコアの男女別平均値、SD、中央値、4分位を求めた。母体年齢、母体の喫煙歴、児の年齢、出生体重を共変量とした重回帰分析により、母乳中の総ダイオキシン類濃度あるいはEDEとTDSの関連を解析した。統計解析にはSPSS ver. 19を用いた。

C．研究結果

1)　解析対象

産後1か月時の母乳中PCDD、PCDF、CoPCB(12種)の濃度が測定され、SDQのすべての項目に回答した初産母体の男児23名、女児21名を解析対象とした。

2)　暴露指標（表1）

①　母乳中の総ダイオキシン類濃度

出生前暴露の指標となる母乳中の総ダイオキシン類濃度には、明らかな男女差は認めなかった。母乳栄養率は0ヶ月から12ヶ月まですべての期間で女児の方が高率であった。

②　ダイオキシン推定摂取量(EDE)

2005 WHO TEFを用いた生後1年間の母乳からの平均EDEは男児15.9、女児16.2 ngTEQ/kg/year、生後1年間の1日平均摂取量は男児44、女児45 pgTEQ/kg/dayであった。

3)　SDQ

SDQのTDSおよび5分野のサブスコアの平均値(SD)を表2に示した。多動・不注意、行為問題、仲間関係のサブスコアはやや男児が高値だったが、TDSも含め、男女間に有意差を認めなかった。4, 5歳児のみにすると、多動・不注意、行為問題のサブスコアは全体より低下したが、男女差はやはり認めなかった。

4)　SDQのスコアと総ダイオキシン類濃度、ダイオキシン推定摂取量との相関

SDQのスコアと①母乳中の総ダイオキシン類濃度、②EDEとの相関について、Spearmannの相関係数で検討した。TDSと①、②との関係について図1，図2に示した。いずれも男女ともに有意な相関は認めなかった。サブスコアのうち男児で仲間関係と①に弱い負の相関($\rho = -0.44$、p=0.04)を認めた。男児の他のスコア、女児でのすべてのサブスコアとは有意な相関は認めなかった。②は、男女ともにすべてのスコアと有意な相関は認めなかった。

5)　重回帰分析

①母乳中の総ダイオキシン類濃度とSDQのTDSとの関連（表3）

母の年齢、母の喫煙歴、SDQ実施時の児の年齢、出生体重を共変量とした男女別の重回帰分析の結果、男女ともに、母乳中総ダイオキシン類濃度とTDSに有意な関連を認めなかった。

②EDEとSDQのTDSとの関連（表4）

男女ともに、EDEとTDSに有意な関連を認めなかった。

D．考察

1)推定暴露量

出生前の胎児暴露は、臍帯血や母体血液中のダイオキシン類濃度から推定される。母乳中濃度は母体内のダイオキシン類の蓄積を反映することから、血液サンプルを用いない推定方法として母乳中濃度を用いた報告もある。本研究では出生前の暴露として、母乳中の総ダイオキシン類濃度を、出生後1年間の暴露として、乳児期の哺乳量、母乳比率と母乳中の総ダイオキシン類濃度からEDEを算出した。2006年～2008年出生の3歳～5歳児のEDEは、男児では1998～2005年出生と同等であり、女児では平均値で 3.4 pgTEQ/kg/day低下していた。生後1年間の1日平均摂取量は、WHOの提唱する成人の耐用1日摂取量4 pg TEQ/kg/dayの約11倍で変化はなかった。

2)推定暴露量と SDQ スコアとの関連からみた行動発達への影響

SDQの標準化において、TDSは性別および年

齢と関連することが報告されている。本研究の3歳～5歳児のスコアは、TDSの平均値(標準偏差)は男児10.9(6.1)、女児10.3(4.1)で、日本の4歳～5歳児の平均値7.9 (4.8)、7.0(4.5)と比較し高値の傾向が見られた。サブスコアでは、多動・不注意、行為問題、仲間関係のスコアが特に男児で高い傾向を認めた。SDQ調査票の回収率が50%であり、回収の有無によるバイアス、すなわち児の行動により不安をもつ保護者が回答した可能性が考えられた。

児の行動発達への影響が示唆されている、母の年齢、母の喫煙歴、SDQ 実施時の児の年齢、出生体重を共変量とし、男女別に重回帰分析により暴露量の影響を検討した。①母乳中総ダイオキシン類量、②母乳からのダイオキシン推定摂取量(EDE)のどちらもTDSとの有意な関連は認めなかった。

E. 結論

母乳栄養により乳児は成人の耐用摂取量を越えるダイオキシン類に暴露されていると推定されるが、3歳～5歳児においてもSDQで評価した行動発達と周産期のダイオキシン類推定暴露とに有意な関連は認めなかった。

F. 研究発表

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G. 知的財産権の出願・登録状況

なし

表1 Dioxin level in the breast milk at 1 month and estimated dioxin exposure through breastfeeding

	Boys (n=23)	Girls (n=21)	
	mean (SD)	mean (SD)	p
Dioxin level in breast milk			
PCDDs (pg TEQ/g fat)	5.3 (2.1)	4.8 (2.5)	0.16
PCDFs (pg TEQ/g fat)	2.7 (1.1)	2.5 (1.2)	0.40
Co PCBs (pg TEQ/g fat)	5.0 (2.4)	4.5 (2.1)	0.40
total dioxins (pg TEQ/g fat)	13.1 (5.3)	11.9 (5.5)	0.23
Fat in breast milk (g/100 ml)	3.6 (1.2)	3.6 (1.4)	0.99
Total volume of breast milk intake (ml/kg/year)	28510 (6636)	34520 (5556)	0.01
EDE (ng TEQ/kg/year)	15.9 (9.1)	16.2 (7.5)	0.70
DDI (pg TEQ/kg/day)	43.6 (25.0)	44.5 (20.5)	0.70

TEQ: toxic equivalence by the World Health Organization in 2005, EDE: estimated dioxin

表 2 Total difficulties scores (TDS) and five subscale scores of the Social Difficulties Questionnaire compared by gender

	total			4-5y		
	Boys N=23	Girls N=21		Boys N=16	Girls N=17	
	mean (SD)	mean (SD)	P	mean (SD)	mean (SD)	P
TDS	10.9 (6.1)	10.3 (4.1)	0.77	9.8 (5.6)	10.5 (4.0)	0.71
Emotional symptoms	1.7 (1.7)	1.9 (1.7)	0.56	1.7 (1.8)	1.9 (1.7)	0.67
Conduct problems	3.0 (2.1)	2.7 (1.6)	0.77	2.6 (1.9)	2.8 (1.8)	0.71
Hyperactive/inattention	4.2 (2.5)	4.1 (1.9)	0.90	3.6 (2.4)	4.1 (2.0)	0.29
Peer problems	2.0 (1.5)	1.6 (1.4)	0.24	1.9 (1.5)	1.8 (1.5)	0.68
Prosocial behavior	6.2 (2.6)	6.6 (2.2)	0.53	6.7 (2.4)	6.5 (2.3)	0.82

p: p values by Mann-Whitney U test, TDS: total difficulties score

表 3 Linear multiple regression analysis to examine association between total dioxin level in breast milk and TDS of SDQ

	Boys				Girls			
	B	(95% C.I.)	beta	p	B	(95% C.I.)	beta	p
Log_{10}(total dioxins)	1.10	(-18.5 - 20.7)	0.30	0.91	-0.76	(-14.9 - 13.4)	-0.03	0.91
Age at SDQ	-1.52	(-5.41 - 2.37)	-0.21	0.42	1.09	(-2.57 - 4.75)	0.21	0.53
Maternal age	-0.65	(-2.03 - 0.72)	-0.27	0.31	-0.11	(-1.06 - 0.84)	-0.08	0.81
Birth weight	0.00	(-0.01 - 0.01)	0.16	0.95	0.004	(-0.005 -0.013)	0.30	0.37
Any history of smoking habit	1.28	(-4.83 - 7.39)	0.11	0.66	0.31	(-4.47 - 5.09)	0.38	0.89

表 4 Linear multiple regression analysis to examine association between EDE through breastfeeding and TDS of SDQ

	Boys				Girls			
	B	(95% C.I.)	beta	p	B	(95% C.I.)	beta	p
Log_{10} EDE	7.20	(-31.3- 45.7)	0.25	0.67	9.38	(-3.00 - 21.8)	0.56	0.12
Age at SDQ	-3.89	(-11.4 - 3.65)	-0.46	0.26	0.21	(-5.24 - 5.65)	0.04	0.93
Maternal age	-0.93	(-4.96 - 3.10)	-0.31	0.60	-0.21	(-1.45 - 1.04)	-0.14	0.71
Birth weight	0.00	(-0.01 - 0.11)	0.01	0.97	0.00	(-0.008 - 0.015)	0.31	0.47
Any history of smoking habit	4.58	(-10.4 - 19.6)	0.35	0.49	-4.16	(-10.2 - 1.86)	-0.57	0.15

図1 Correlation between total difficulties score(TDS) of the SDQ and total dioxin level of breastmilk in boys and in girls.

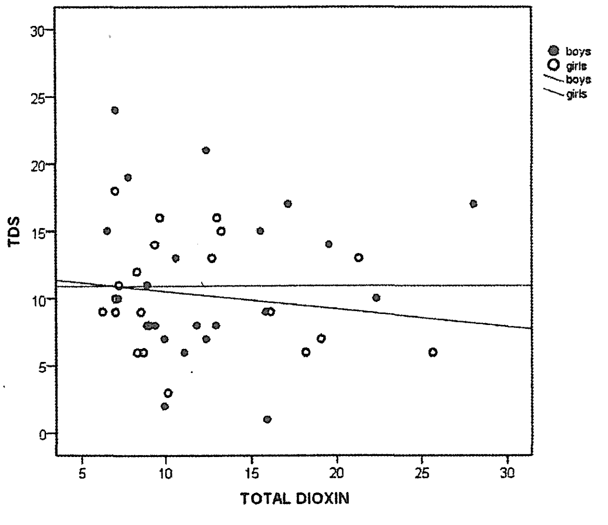
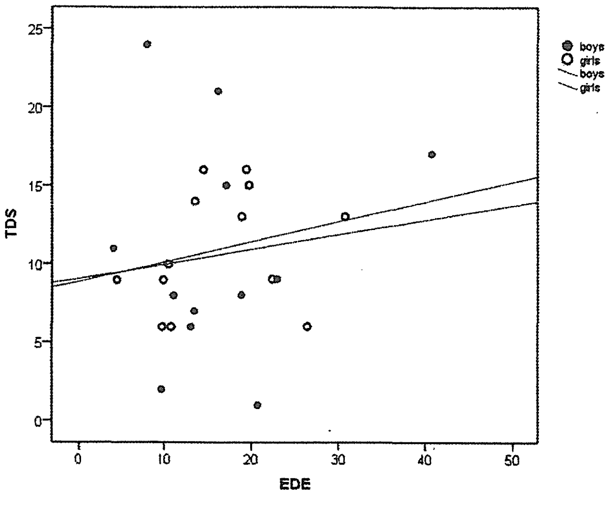


図2 Correlation between total difficulties score(TDS) of the SDQ and EDE by breastfeeding in boys and in girls.



厚生労働科学研究費補助金（食品の安全確保推進研究事業）
分担研究報告書

母乳のダイオキシン類汚染の実態調査と乳幼児の発達への影響に関する研究
分担課題名：乳幼児の健康影響調査（免疫機能等）

分担研究者　松井永子　岐阜大学大学院医学系研究科　小児病態学　非常勤講師

研究要旨
新生児、乳児の栄養として不可欠な母乳には、脂肪分が多く含まれているため、母乳に蓄積したダイオキシン類などの脂溶性の汚染物も脂肪に溶けて母乳中に高濃度に分泌される。このため、母乳哺育児のダイオキシン摂取量は多くなる可能性がある。本研究では、母乳から摂取したダイオキシン類がアレルギー疾患発症にどのような影響を与えるかについて検討を行った。結果として、母乳中のダイオキシン類濃度とアレルギー疾患発症との間には明らかな関係はみられなかった。

A.研究目的
母乳中のダイオキシン類の摂取が乳児に与える影響は直ちに問題となる程度ではないが、今後も継続して母乳の安全性に関する検討を行う必要があると考えられている。本研究の目的は、ダイオキシン摂取推計値とアレルギー疾患発症との関連を検討し、ダイオキシン類がアレルギー疾患発症におよぼす影響について検討することである。

B.研究方法
1997年より地域を定めて産後1か月の母乳の提供を受け、母乳中のダイオキシン類などの濃度を測定している。得られたダイオキシン類濃度とアレルギー疾患発症との関連について検討した。
（倫理面への配慮）
研究対象者には本研究の内容、方法および予想される結果について十分に説明し十分な理解（インフォームドコンセント）を得たうえで調査が行われた。また、倫理面でも、結果による不利益は全く生じないか、または配慮がなされることから問題ないと判断された。

C.研究結果
2012 年に行った追跡アンケート調査の中から血清 IgE の値と母乳中のダイオキシン類濃度との関連について、年齢ごとに検討した。検討結果を図 1-図 5 に示す。

図 1

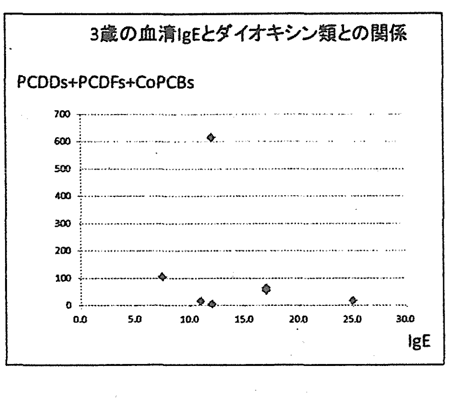


図2

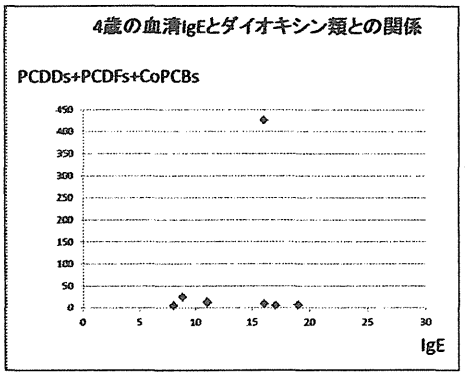


図3

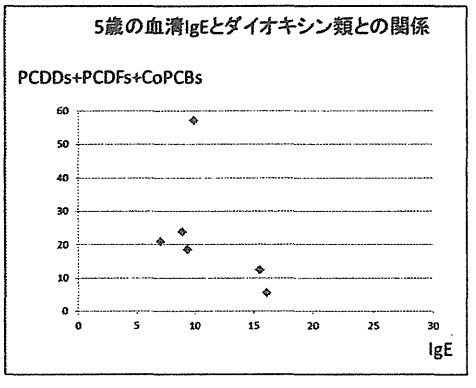


図4

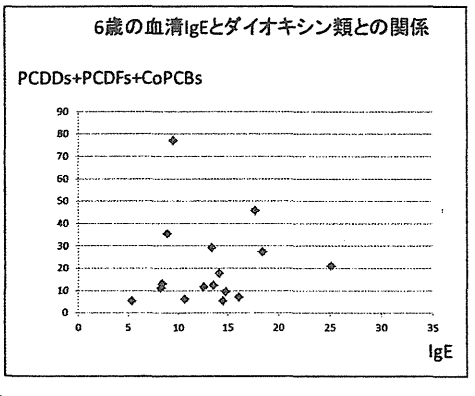
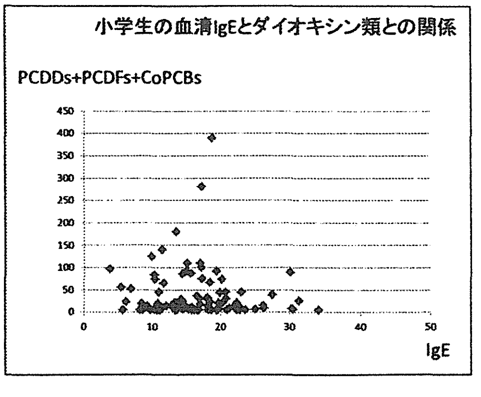


図5



いずれの年齢においても、血清 IgE と母乳中のダイオキシン濃度との間に有意な関連はみられなかった。

D.考察

母乳中のダイオキシン類濃度とアレルギー疾患に関連する血清 IgE の間に有意な関連がみられなかったことから、ダイオキシン類がアレルギー発症に関連しているとは現時点ではいえない。さらに今後の検討が必要である。

E.結論

本邦の乳児が摂取する母乳中のダイオキシン類の濃度は、近年低下傾向が著しく、地域による差の小さくなっているが、乳児への栄養食品という観点および環境汚染の評価の視点で、母乳中のダイオキシン類濃度は今後も継続して測定していくことが重要であり、アレルギー疾患発症に及ぼす影響についてもさらに経年的に観察が必要であると思われる。

F.健康危険情報

なし

G.研究発表

①Sasai H, Shimozawa N, Asano T, Kawamoto N, Yamamoto T, Kimura T, Kawamoto M, Matsui E, Fukao T. Successive MRI Finding of Reversible Cerebral White Matter Lesions in a Patient with Cystathionine be-ta Synthase Deficiency. Tohoku J Exp Med. 237,323-327(2015).

②松井永子：アレルギー疾患治療の有効性評価 小児科 QOLからの評価 アレルギー･免疫 22, 38-44 (2015)

H.知的財産権の出願、登録状況

１、特許出願　なし

２、実用新案登録　なし

３、その他　なし

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表
(2015年4月1日～2016年3月31日迄)

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Ⅳ．研究成果の刊行物・別冊

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Perinatal dioxin exposure and psychosocial and behavioral development in school-aged children

Yumi Kono [a,*], Akira Oka [b], Hiroshi Tada [c], Kazuo Itabashi [d], Eiko Matsui [e], Yosikazu Nakamura [f]

[a] Department of Pediatrics, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan
[b] Department of Pediatrics, Tokyo University, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[c] Department of Neonatology, Toho University School of Medicine, 5-21-16 Omorinishi, Ota-ku, Tokyo 143-8540, Japan
[d] Department of Pediatrics, Showa University of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
[e] Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu-shi, Gifu 501-1193, Japan
[f] Department of Public Health, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan

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ABSTRACT

Objective: The aim of this study was to elucidate the association between psychosocial and behavioral problems in children at school age and dioxin level in breast milk or estimated dioxin exposure (EDE) through breastfeeding in the general Japanese population.

Methods: Dioxin level of breast milk at 1 month of age and breastfeeding ratio through the first year of life were used to calculate the EDE of infants born in 1998–2005 in Japan. The Japanese Social Difficulties Questionnaire (SDQ) for the assessment of children's behavior was sent by mail to mothers whose breast milk underwent the dioxin survey, at the time when their infants were aged 6–13 years.

Results: The study subjects were 175 pairs of mothers and their first infants (79 boys, 96 girls). The mean total dioxin levels of breast milk were 18.3 and 19.8 (pgTEQ/g fat) and EDEs were 16.4 and 19.6 (ngTEQ/kg/year) in boys and girls, respectively. In linear multiple regression analyses after adjusting for age at SDQ, maternal age, birth weight and maternal smoking habit, dioxin level in breast milk was not significantly related to the total difficulties score (TDS) of SDQ in boys, B = 2.29 (95% CI −7.60–12.18), or in girls, B = −1.04 (95% CI −9.24–7.15). EDE correlated to the TDS in neither boys, B = −0.99 (95% CI −4.14–2.15), nor girls, B = 1.08 (95% CI −2.69–4.85).

Conclusion: No evidence was found of a correlation between perinatal dioxin exposure and behavioral and psychosocial problems of children measured by SDQ. These results support the benefits of recommending breastfeeding.

1. Introduction

Environmental contamination of organohalogen compounds, such as dioxins and polychlorinated biphenyls (PCBs), is of concern, especially exposure in utero and transfer through breastfeeding to infants. Because the developing brain is more vulnerable to these compounds than the adult brain, they could affect the neurodevelopment of infants and cause cognitive, psychological or psychiatric problems thereafter [1–3]. Most of the previous studies dealt with prenatal PCB and/or dioxin exposure, but few of them focused on postnatal intake through breastfeeding and the influence of perinatal exposure on neurodevelopment is unclear. In a dioxin-contaminated area in Southern Vietnam, Tai et al. reported that the levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)- and polychlorinated dibenzo-*p*-dioxin/furan (PCDD/PCDF)-toxic equivalents in breast milk and the estimated infant daily dioxin intake (DDI) via breastfeeding were inversely correlated to neurodevelopmental scores in 4-month-old infants [4]. The Duisburg Birth Cohort Study group showed that either maternal blood or milk levels of PCDD/PCDF and PCB at environmental background levels did not associate with the mental and motor developmental scores of the Bayley Scales of Infant Development of children at 24 months [5].

We previously reported on the dioxin levels of breast milk in the general population in Japan [6]. It would be of particular importance to investigate whether dioxin exposure at these background levels could affect the neurodevelopment and behavioral development of children in the long term [7]. The aim of this study was thus to elucidate whether the levels of prenatal and early postnatal dioxin exposure are associated with behavioral problems of school-aged children in our

Abbreviations: DDI, daily dioxin intake; EDE, estimated dioxin exposure; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; TCDD, tetrachlorinated dibenzo-*p*-dioxins; TDI, tolerable daily intake; TDS, total difficulties score; TEQ, toxic equivalence; SDQ, Strengths and Difficulties Questionnaire.

* Corresponding author at: Department of Pediatrics, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi-ken 329-0498, Japan. Tel.: +81 285587366.
E-mail address: ykono@jichi.ac.jp (Y. Kono).

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study population. We used dioxin concentration in breast milk itself as an indicator of prenatal exposure [5,8]. For early postnatal exposure, we calculated the estimated dioxin exposure (EDE) through breastfeeding using the dioxin levels and the ratio of breastfeeding throughout the first year of life [9]. The Strengths and Difficulties Questionnaire (SDQ), the Japanese parent version [10], was used to assess behavioral and psychosocial problems; it has been widely used to screen for child developmental disabilities, and psychological and psychiatric conditions or disorders [11].

2. Materials and methods

2.1. Human milk survey for dioxins

A human milk survey has been conducted in several prefectures and cities in Japan since 1997. The details of this survey carried out in various areas have been described in previous reports [6,12]. Healthy pregnant women aged between 20 and 39 years were recruited and signed informed consent was obtained from all of the participants. Approximately 30 ml of breast milk was collected manually from each participant at the time of a 1-month check-up for a baby. Polychlorinated dibenzo-*p*-dioxins (PCDDs, seven isomers), PCDFs (10 isomers) and coplanar PCBs (12 isomers), which are known as dioxin-like PCBs, were measured using gas chromatography and mass spectrometry, and were quantified by lipid-based calculations [6,12]. The levels of dioxins were described as the toxic equivalence (TEQ) values per gram of fat in breast milk by using toxic equivalent factors of 2,3,7,8-TCDD, which was previously documented in the 2005 World Health Organization (WHO) re-evaluated toxic equivalency [13]. The study protocol was approved by the medical ethics committee of Jichi Medical University, Tochigi, Japan, and all of the participating facilities.

2.2. Study subjects

In this study, the subjects were mother–child pairs in which birth occurred between 1998 and 2005. Because dioxin levels in breast milk are known to be decreased by a previous history of breastfeeding, primiparous mothers and their infants were analyzed in this study [14,15]. Information was collected using the first questionnaire at the time of consent from the mother, which included mother's age, weight, height and smoking habits, and using the second questionnaire at a 1-month check-up for the child, which included parity, gestational age, birth weight and gender. Breastfeeding status at each month until 12 months of age was obtained using the third questionnaire at a 1-year check-up for the children. In addition, blood samples were collected at 1 year of age from children born in 2003 to 2005 whose mothers' consent had been obtained. Concentrations of PCDDs and PCDF levels in the blood were measured by the same method used for the breast milk samples [6].

2.3. Estimation of dioxin exposure (EDE) through breastfeeding

The following equation was used to estimate the postnatal dioxin exposure by breastfeeding for the first year of life: EDE through breastfeeding, ng TEQ/kg/year = V {total volume of breast milk intake for the first year (ml/kg/year)} × L {% lipid content of breast milk/100 (g)} × Y {total dioxin levels (pg TEQ/g fat) × 10^{-3}} [9, 15]. We made the following assumptions for EDE. Firstly, the ratio of breastfeeding (R0–R11) was calculated monthly from 0 to 11 months according to the type of feeding recorded using the questionnaire, which was equal to 1 when feeding only on breast milk, 0.75 when breastfeeding exceeded formula feeding, 0.5 when breastfeeding and formula feeding were equal, 0.25 when formula feeding exceeded breastfeeding and 0 when feeding only on feeding formula. Secondly, average volume of daily milk intake and average body weight at 0 to 2 months, at 3 to 5 months, at 6 to 8 months and at 9 to 11 months by gender were obtained according to the Dietary Reference Intakes for Japanese 2010 [16]. The following formulas were used: $V = [780\ \text{ml} \times (R0 + R1 + R2)/4.9(\text{kg}) + 780\ \text{ml} \times (R3 + R4 + R5)/7.4(\text{kg}) + 600\ \text{ml} \times (R6 + R7 + R8)/8.5(\text{kg}) + 450\ \text{ml} \times (R9 + R10 + R11)/9.1(\text{kg})] \times 365/12$ in boys and $V = [780\ \text{ml} \times (R0 + R1 + R2)/4.6(\text{kg}) + 780\ \text{ml} \times (R3 + R4 + R5)/6.8(\text{kg}) + 600\ \text{ml} \times (R6 + R7 + R8)/7.8(\text{kg}) + 450\ \text{ml} \times (R9 + R10 + R11)/8.5(\text{kg})] \times 365/12$ in girls. Thirdly, because the lipid concentration in breast milk varies individually and over the period of lactation, we used the mean lipid contents in the breast milk of the study subjects in the calculation. The average daily dioxin intake (DDI, pg TEQ/kg/day) for the first year was calculated as follows: (EDE through breastfeeding × 10^3)/365.

2.4. SDQ score measurement

Japanese forms of SDQ were sent via mail to parents of the subjects whose mothers' milk underwent dioxin survey [10]. Children of the subjects were aged between 6 and 13 years at evaluation. Parents were asked to complete the extended Japanese version of SDQ, which comprised 25 items on specific strengths and difficulties, with an overall rating of whether their child had behavioral or psychological problems. The four SDQ subscales (emotional symptoms, conduct problems, hyperactive/inattention and peer problems) representing problem scores were obtained and the total difficulties score (TDS) rating of 0 to 40 was obtained, by adding the 4 subscale scores according to the instructions of SDQ [11]. Another subscale of prosocial behavior assesses the positive aspects of child behavior.

2.5. Statistical analyses

Because a gender difference has been reported in the milk intake volume during the first year of life [16], and gender and age effects have been shown in the Japanese version of SDQ [10,17], the study subjects were stratified into subgroups by gender and by age (6–10 years, 11–13 years). We employed non-parametric methods for analyses since the dioxin level in breast milk and EDE were not distributed normally. Spearman's rank correlations were used to explore the association between total dioxin level in breast milk or EDE by breastfeeding and the five subscale scores of SDQ.

Linear multiple regression analyses were performed to examine the association between TDS and the total dioxin level in breast milk or EDE through breastfeeding after $\log_{10}$ transformation. Adjustment for covariates, including maternal age, birth weight, any history of maternal cigarette smoking and age at SDQ assessment in boys and in girls, was performed. These covariates were chosen from the questionnaires as they have been reported to be related to the TDS of SDQ or the behavior of children [10,11,17]. In addition, to verify the correlation between dioxin exposure and the TDS of SDQ, the medians of TDS were compared between the children with high EDE in the top 10 percentiles and those with low EDE in the bottom 10 percentiles in boys and in girls. Results were considered significant at $p < 0.05$. All analyses were performed with SPSS 19.0 (IBM Japan, Tokyo, Japan).

3. Results

The study subjects consisted of 175 pairs of primiparous mothers and their children who replied and completed the 25 SDQ items in the 316 pairs to whom SDQ was sent. The residences of the study subjects extended across 20 prefectures in Japan. They were neither high- nor low-risk areas for dioxin pollution. The characteristics of the mother and child (79 boys and 96 girls) pairs and breastfeeding status are shown in Table 1. As represented by the breastfeeding ratios, breastfeeding was dominant until 8 months and equal to

Table 1
Characteristics of the mother and infant pairs and breastfeeding status by gender.

	Boys	Girls
	n = 79	n = 96
Mothers		
Age (years), mean ± SD	29.8 ± 2.5	29.5 ± 2.7
Weight (kg), mean ± SD	50.6 ± 6.3	51.5 ± 6.2
Height (cm), mean ± SD	156.8 ± 5.4	158.0 ± 5.3
Any history of smoking habit, n(%)	17(22)	22(23)
Smoking habit during pregnancy, n(%)	0(0.0)	2(2.1)
Infants		
Gestational age (weeks), mean ± SD	39.6 ± 1.2	39.9 ± 1.3
Birth weight (g), mean ± SD	3067 ± 340	3001 ± 348
Birth length (cm), mean ± SD	49.3 ± 2.0	49.0 ± 1.7
Birth head circumference (cm), mean ± SD	33.1 ± 1.5	32.8 ± 1.2
Breastfeeding ratio		
0–2 months, mean ± SD	0.73 ± 0.27	0.81 ± 0.24
3–5 months, mean ± SD	0.61 ± 0.41	0.69 ± 0.39
6–8 months, mean ± SD	0.52 ± 0.46	0.59 ± 0.44
9–11 months, mean ± SD	0.43 ± 0.46	0.43 ± 0.45
Age of infants at SDQ		
6–10 years, n(%)	31(39)	40(42)
11–13 years, n(%)	48(61)	56(58)

SDQ: Social Difficulties Questionnaire, SD: standard deviation.
Breastfeeding ratio: 1 when feeding only on breast milk, 0.75 when breastfeeding exceeded formula feeding, 0.5 when breastfeeding and formula feeding were equal, 0.25 when formula feeding exceeded breastfeeding and 0 when feeding only on feeding formula.

formula feeding thereafter in both gender groups. At the time of SDQ assessment, 48 (61%) boys and 56 (58%) girls were 11 to 13 years old.

Means, medians and interquartile range of dioxin concentrations measured in the breast milk at 1 month are presented in Table 2. There were no significant differences in the levels of PCDDs, PCDFs, dioxin-like PCBs and total dioxin level in the milk between the groups of boys and girls. The mean dioxin exposure through breastfeeding was estimated to be 16.4 ng TEQ/kg/year for boys and 19.6 ng TEQ/kg/year for girls. From these EDE levels, the mean DDI for the first year of life was calculated at 44.9 pg TEQ/kg/day in boys and 53.7 pg TEQ/kg/day in girls. The blood dioxin levels were measured in eight children at 1 year to evaluate whether our estimation was relevant. The EDE was highly associated with the blood dioxin levels in these children (Supplemental Fig. 1, Spearman's $\rho = 0.905$, $p = 0.002$).

The means of TDS of SDQ were 8.7 in boys and 7.4 in girls. Although the means of TDS and three problem subscale scores were higher in boys than in girls, a significant difference was found only in hyperactive/inattention score (Supplemental Table 1). The prosocial behavior score, which is a positive behavior subscale, was higher in girls than in boys, but the difference was also not significant. Comparing the groups aged 6–10 and 11–13, the means of TDS and subscales of conduct problem and hyperactive/inattention scores were significantly higher in the group aged 6–10.

There was no significant association between TDS of SDQ and total dioxin level in boys: Spearman's $\rho = -0.22$, $p = 0.05$, or in the group aged 6–10: Spearman's $\rho = -0.12$, $p = 0.33$, and aged 11–13: Spearman's $\rho = -0.16$, $p = 0.11$. A weak negative correlation was found in girls, with Spearman's $\rho = -0.24$, $p = 0.02$. Among the five subscales of SDQ, conduct problem scores and hyperactive/inattention scores were negatively correlated, meaning that a higher dioxin level in breast milk was related to fewer behavioral problems in girls. There was no significant association between other subscales and total dioxin level (Supplemental Table 2).

No significant association was found between TDS and EDE through breastfeeding in boys: Spearman's $\rho = -0.18$, $p = 0.11$, or in girls: Spearman's $\rho = -0.03$, $p = 0.79$, or in the groups aged 6–10: Spearman's $\rho = -0.20$, $p = 0.11$, and aged 11–13: Spearman's $\rho = -0.08$, $p = 0.45$. In addition, no correlation between any subscales of SDQ and EDE through breastfeeding was found in boys and in girls. A weak negative correlation was found between conduct problem scores and EDE in the group aged 6–10, with Spearman's $\rho = -0.26$, $p = 0.03$ (Supplemental Table 3).

In linear multiple regression models to examine the association between total dioxin level in breast milk and TDS of SDQ, total dioxins did not affect the TDS significantly in boys, B = 2.29 (95% CI −7.60–12.18, $p = 0.46$), or in girls, B = −1.04 (95% CI −9.24–7.15, $p = 0.80$). Younger age at SDQ assessment both in boys and girls and any history of maternal cigarette smoking only in boys increased TDS (Table 3). Similar results were obtained in the models in which EDE through breastfeeding instead of the dioxin level was included. The EDE was not a significant factor for the TDS of SDQ either in boys, B = −0.99 (95% CI −4.14– 2.15, $p = 0.53$), or girls, B = 1.08 (95% CI −2.69–4.85, $p = 0.57$) (Table 4). The medians (interquartile range) of TDS of SDQ of the children with high EDE in the top 10 percentiles were 5 (3–10) in boys and 5 (3–15) in girls, while those of the children with low EDE in the bottom 10 percentiles were 8 (6–12) in boys and 7 (6–12) in girls. There were no significant differences between the medians of TDS or subscale scores between the high-EDE group and the low-EDE group in either boys or girls (Supplemental Table 4).

4. Discussion

To the best of our knowledge, the present study provides the first evidence that either estimated prenatal or lactational dioxin exposure did not correlate with behavior assessed using the Japanese parent-rated SDQ of children living in Japan. Dioxin concentration in the breast

Table 2
Dioxin level in the breast milk at 1 month and estimated dioxin exposure through breastfeeding.

	Boys (n = 79)		Girls (n = 96)	
	Mean (SD)	Median (interquartile range)	Mean (SD)	Median (interquartile range)
Dioxin level in breast milk				
PCDDs (pg TEQ/g fat)	8.6 (3.1)	8.3 (6.6–10.3)	9.2 (3.7)	8.6 (6.3–11.3)
PCDFs (pg TEQ/g fat)	3.2 (1.5)	3.0 (2.4–3.6)	3.4 (1.4)	3.1 (2.4–4.2)
Co PCBs (pg TEQ/g fat)	6.5 (2.5)	6.2 (4.7–7.9)	7.2 (2.8)	6.6 (5.3–9.3)
Total dioxins (pg TEQ/g fat)	18.3 (6.2)	17.8 (13.9–21.7)	19.8 (7.1)	18.6 (14.0–25.3)
Fat in breast milk (g/100 ml)	4.1 (1.4)	4.1 (3.1–5.1)	3.9 (1.3)	3.8 (3.0–4.7)
Total volume of breast milk intake (ml/kg/year)	21716 (11499)	22691 (10894–33886)	25895 (11734)	30511 (15078–36747)
EDE (ng TEQ/kg/year)	16.4 (10.7)	14.0 (8.0–23.0)	19.6 (10.8)	18.8 (11.4–26.3)
DDI (pg TEQ/kg/day)	44.9 (29.3)	38.5 (22.0–62.9)	53.7 (29.6)	51.4 (31.2–72.0)

PCDDs: polychlorinated dibenzo-p-dioxins (sum of 2,3,7,8-tetra CDD, 1,2,3,7,8-penta CDD, 1,2,3,4,7,8-hexa CDD, 1,2,3,6,7,8-hexa CDD, 1,2,3,7,8,9-hexa CDD, 1,2,3,4,6,7,8-hepta CDD and octa CDD).
PCDFs: polychlorinated dibenzofurans (sum of 2,3,7,8-tetra CDF, 1,2,3,7,8-penta CDF, 2,3,4,7,8-penta CDF, 1,2,3,4,7,8-hexa CDF, 1,2,3,6,7,8-hexa CDF, 1,2,3,7,8,9-hexa CDF, 2,3,4,6,7,8-hexa CDF, 1,2,3,4,6,7,8-hepta CDF, 1,2,3,4,7,8,9-hepta CDF, octa CDF).
Co PCBs: coplanar polychlorinated biphenyls (sum of PCB 77, 81, 126, 169, 105, 114, 118, 123', 156, 157, 167 and 189).
TEQ: toxic equivalence by the World Health Organization in 2005, EDE: estimated dioxin exposure.

Table 3
Linear multiple regression analysis to examine association between total dioxin level in breast milk and TDS of SDQ.

	Boys				Girls			
	B	(95% CI)	beta	p	B	(95% CI)	beta	p
Log_{10} (total dioxins)	2.29	(−7.60 - 12.18)	0.06	0.46	−1.04	(−9.24 - 7.15)	−0.03	0.80
Age at SDQ	−0.69	(−1.27 - −0.12)	−0.30	0.02	−0.43	(−0.91 - 0.05)	−0.20	0.08
Maternal age	−0.36	(−0.83 - 0.11)	−0.16	0.13	0.31	(−0.11 - 0.74)	0.15	0.15
Birth weight	−0.002	(−0.006 - 0.001)	−0.14	0.21	−0.001	(−0.004 - 0.003)	−0.04	0.73
Any history of smoking habit	3.28	(0.26 - 6.30)	0.24	0.03	0.68	(−2.08 - 3.44)	0.05	0.63

B: partial regression coefficient.
beta: standardized partial regression coefficient.
TDS: total difficulties score.

milk at 1 month after delivery was considered as an indicator of prenatal exposure and lactational exposure was estimated according to the dioxin levels of breast milk and the ratio of breastfeeding to formula feeding during the first year.

The average DDI of the infants during the first year of life calculated from the EDE in this study was 10 to 14 times higher than the tolerable daily intake (TDI) (i.e. 4 pg TEQ/kg/day) for the general population, as defined by the WHO or by the Japanese Ministry of Health, Labour and Welfare [13,18]. This result is compatible with previous reports stating that DDI in breastfed infants is 4 to 40 times higher than the TDI because of the high transfer via the mother's milk [14,15]. The EDE level in our study was similar or slightly higher than the estimated dioxin exposure levels in infants by breastfeeding recently reported in Japan and other countries [14,19–21]. Dioxin level in breast milk is known to decrease after several months, reflecting a decrease in the burden on the mother by breastfeeding [15]. However, this decrease may not be linear during the lactation period and may differ from mother to mother [15,21]. We did not, therefore, take the decrease in months into consideration and the EDE level in this study might have been overestimated. On the other hand, actual PCDD level in breast milk at 1 month was comparable to the levels in other studies [14,19–21]. Thus, we analyzed the associations of both dioxin level in breast milk and EDE level to TDS of SDQ. No linear dose–effect association between either dioxin level itself or EDE and TDS was observed, meaning that an increase of dioxin intake from breast milk was not associated with behavioral problems of children. We found no significant differences in the TDS or subscale scores of SDQ between the cases with a high EDE level and those with a low EDE level. These results suggested that the perinatal dioxin exposure in the range in this study population did not affect the behavioral and psychosocial development of the children.

SDQ is a screening instrument to assess positive and negative aspects of children's behavior; it can be filled in by parents or teachers, and was originally published in English [11]. The Japanese parent-rated SDQ was validated by Matsuishi et al. and normative data of Japanese school-aged children were reported very recently [10,17]. The means of TDS and four subscale scores relating to problematic behavior in this study were similar to the validation study and slightly higher than the scores reporting normative data [17]. However, the distribution patterns of the four subscale scores and the effects of gender and age on the scores, namely, higher hyperactivity/inattention scores or TDS for boys than for girls, and TDS and scores for emotional symptoms and hyperactivity/inattention tending to be lower in older children, were similar to those in these studies and studies in other countries [10,17, 22]. Thus, we could assume that the scores of the parent-rated SDQ obtained in this study were reasonable.

It has been revealed that perinatal dioxin exposure may affect neurobehavioral development; however, the identified effects appear to be contradictory and subtle [1–3,5,23]. In animal models, exposure *in utero* and via lactation to a low dose of TCDD disrupted the functions of memory and emotion in male mouse offspring [24]. The pups reaching adulthood showed behavioral inflexibility, compulsive repetitive behavior and lowered competitive dominance [25]. Maternal TCDD exposure delayed motor development assessed by righting response from an inclined position of the offspring at an early stage, especially in male rats [26]. The effects on learning behaviors are, however, controversial; TCDD-exposed rats made more errors in the early phase of the learning process in terms of spatial discrimination/reversal learning, but they improved in terms of performance in the visual discrimination/reversal learning task compared with the control group [27].

In human studies, because environmental exposure levels are much lower than those in animal models, the effect of perinatal exposure on neurodevelopment and behavior in children remains unclear. Although it is difficult to distinguish the effect between prenatal and postnatal exposure, more significant correlations were found for prenatal exposure, especially that to PCBs. In children from Michigan and North Carolina whose mothers had consumed fish presumed to be contaminated with PCBs, levels of prenatal PCB exposure were associated with adverse outcomes such as greater impulsivity, poorer concentration and poorer working memory only in children who had not been breastfed [28]. Sioen et al. studied prenatal exposure to environmental contaminants of lead, cadmium, PCBs and dioxin-like compounds measured in cord blood and behavioral problems at 7–8 years using SDQ [29]. Although higher lead exposure was associated with a higher risk for hyperactivity in both boys and girls, higher prenatal exposure to dioxin-like compounds was found to be associated with a lower score for hyperactivity. Recently, the Duisburg Birth Cohort Study showed similar results; PCDD/PCDF and PCB in maternal blood negatively associated with hyperactive behavior of school-aged children, while the effect of the amount of PCDD/PCDF and PCB ingested with breast milk was not

Table 4
Linear multiple regression analysis to examine association between EDE through breastfeeding and TDS of SDQ.

	Boys				Girls			
	B	(95% C.I.)	beta	p	B	(95% C.I.)	beta	p
Log_{10} EDE	−0.99	(−4.14 - 2.15)	−0.07	0.53	1.08	(−2.69 - 4.85)	0.06	0.57
Age at SDQ	−0.61	(−1.11 - −0.11)	−0.27	0.02	−0.47	(−0.91 - −0.03)	−0.22	0.04
Maternal age	−0.34	(−0.81 - 0.12)	−0.16	0.15	0.31	(−0.11 - 0.73)	0.15	0.15
Birth weight	−0.002	(−0.005 - 0.002)	−0.12	0.29	0.000	(−0.004 - 0.003)	−0.03	0.78
Any history of smoking habit	3.10	(0.21 - 5.99)	0.23	0.04	0.92	(−1.80 - 3.64)	0.07	0.50

B: partial regression coefficient.
beta: standardized partial regression coefficient.
EDE: estimated dioxin exposure, TDS: total difficulties score.

significant [30]. In contrast to the assumed unfavorable effects of dioxins and dioxin-like compounds on neurological or developmental measurements, we have to consider that a number of benefits of breastfeeding for short- and long-term health as well as optimal brain development in children have been noted [31].

One of the limitations of this study is the potential bias due to a lack of SDQ data via mailing questionnaires, and 55% of the mailed subjects completed SDQ. The mothers who responded to SDQ questionnaires could be more conscious of the subject matter or have concerns over their children's behavior, as shown by the slightly higher TDS in our study than the Japanese normative data [17]. However, the dioxin level in breast milk and EDE in the subjects ranged widely and linear multiple regression analysis was performed, which supported the absence of a significant correlation between dioxin exposure and the behavior of the children. Because SDQ is a tool for screening children's psychological and psychiatric conditions, to clarify the influence on psychological and psychiatric development of children in the longer term, longitudinal follow-up and diagnostic evaluations are needed. The second limitation is that we could not include socio-demographic covariates, for example, education level of the parents, maternal alcohol consumption and economic status, which are known as cofounders for children's behavior [29,30]. Only maternal age and maternal smoking habit were available from the questionnaires and were included in the analyses. In addition, simultaneous exposure to other environmental contaminants like heavy metals, such as lead, cadmium, mercury, or pesticides, which may affect neurodevelopmental disorders in children, was not measured in this study [29,32].

In summary, we investigated the correlation between dioxin level in breast milk, dioxin exposure via breastfeeding and behavioral and psychosocial problems using SDQ in pairs of mothers and children in the general Japanese population. There was no significant association between TDS of SDQ and dioxin level in breast milk or EDE. This supports the benefits of breastfeeding and provides evidence that breastfeeding should be recommended, even in the presence of measureable amounts of dioxins in the current environment.

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Conflict of interest statement

None.

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