書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
仲井邦彦他	環境由来化学物質の胎児期曝露の影響		周産期学シンポジウム No.23	メジカルビュー社	Tokyo	2005	19-26
Murata K.et al.	Effects of perinatal methylmercury exposure on child neurodevelopment in japan in relation to maderiran study	Etoh K.	NIMD Forum 2003	NIMD	Minamata	2004	29-50

NIMD: National Institute for Minamata Disease

雑誌

発表者氏名	論文タイトル名	発表雑誌	巻名	ページ	出版年
Suzuki K.et al.	Effects of perinatal exposure to environmentally persistent organic pollutants and heavy metals on neurobehavioral development in Japanese children: III. maternal smoking confounds neonatal neurobehavioral status.	Organohalogen Compounds	65	218-221	2003
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Iwasaki Y.et al.	Estimation of daily mercury intake from seafood in Japanese women: Akita cross-sectional study.	Tohoku Journal of Experimental Medicine	200	67-73	2003
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Murata K.	Delayed brainstem auditory evoked potential latencies in 14-year-old children exposed to methylmercury.	Journal of Pediatrics	144	177-183	2004
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村田勝敬	妊婦は魚を食べない方がよいか	総合臨床	53	2750-2752	2004
佐藤　洋	魚介類に蓄積するメチル水銀と健康	モダンメディア別冊	50	143-149	2004
嶽石美和子 他	環境疫学における小児の神経生理機能の評価法	医学のあゆみ	212	243-246	2005
岩田豊人 他	環境有害因子に曝露された小児の神経運動機能の評価	医学のあゆみ	212	247-250	2005
鈴木恵太 他	新奇選好を応用した乳幼児の視覚認知検査	医学のあゆみ	212	253-257	2005
岡知子 他	Bayley 式乳幼児発達検査第 2 版の日本国内での実施の試み	医学のあゆみ	212	259-263	2005
村田勝敬 他	小児の神経発達から見た食の安全性	秋田県公衆衛生学雑誌	3	7-15	2005
Dakeishi M. et al.	Effects of hair treatment on hair mercury-The best biomaker of methylmercury Exposure?	Environmental Health andPreventive Medicine	10	208-212	2005
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Sakamoto M. et al.	Difference in methylmercury exposure to fetus and breast-feeding offspring : a mini-review.	Kor J Environ Health	31	179-186	2005
Iwata T. et al.	Factors affecting hand tremor and postural sway in children.	Environmental Health and Preventive Medicine	11	17-23	2006
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III. 研究成果の刊行物・別刷

環境由来化学物質の胎児期曝露の影響

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東北大学教育学研究科発達障害学[4] 秋田大学医学部環境保健学[5]
仲井 邦彦[1] 堺 武男[2] 岡村 州博[3]
細川 徹[4] 村田 勝敬[5] 佐藤 洋[1]

要旨

ダイオキシン類，PCBs，メチル水銀など環境由来の化学物質による周産期曝露に起因した健康影響が危惧されている。健康影響が最も危惧される集団は胎児と新生児であり，その健康リスクを評価するため，周産期における化学物質曝露をモニタリングするとともに，出生児の成長，特に認知行動面の発達を追跡する前向きコホート調査を計画し，599組の新生児－母親の登録を得て疫学調査を進めている。まだ児の発達と化学物質曝露の関係について解析途中であるが，母親毛髪総水銀，臍帯血および母体血甲状腺ホルモン関連指標の分析を終えるとともに，臍帯血ダイオキシン類およびPCBsについて高分解能ガスクロマトグラフィー質量分析装置（GC/MS）を用いた解析を実施中である。本コホート調査の概要を紹介するとともに，化学分析の状況についてまとめ，PCB曝露のレベルについて海外で行われたコホート調査の結果との比較を試みた。

はじめに

ダイオキシン類，PCBsおよびメチル水銀といった化学物質は，難分解性および脂溶性の特徴を有しており，そのため環境中に蓄積し食物連鎖による生物濃縮を受け，ヒトは主に魚介類を介して取り込むと考えられる。その曝露レベルは低いものの，発生，成長過程にある胎児や新生児は中枢神経系の成長過程にあり，成人に比較して，これら化学物質の曝露に対する感受性が高いと考えられる。

PCBもしくはメチル水銀に関しては，1980年代から1990年代にかけて海外でいくつかの出生コホート調査が行われている。調査が行われた実施地点を**図1**に示すとともに，PCBに関する報告について**表1**にその主な報告内容を整理した。

PCBの影響については，多くの報告で児の心理行動，認知面に対して何らかの影響があることを示唆する結果となっている[1]。全体的な傾向としては，母乳を介した曝露よりは，胎児期曝露の影響が大きいことが示唆される。例外はドイツで行われた疫学であり，臍帯血中PCBではなく母乳中PCBが児の認知面の発達の遅れと関連したことが報告されている[2]。授乳については，授乳そのものが児の発達を促す要因となっていることも調査から示されており，母乳を介した曝露のリスク評価は今後の課題となっている。

いずれにしても，胎児または新生児の時期は脳の発生，発達時期に相当し，環境の変化に対する感受性が高い。さらに，成人におけるこのような化学物質の主な摂取経路は食事であり，ダイオキシン類耐容１日摂取量（TDI）についてみれば多くの成人が基準以下とされている。しかしながら，児は母体に長年にわたって蓄積した化学物質を胎盤または母乳を通して短期間に受け取ることとな

図1　PCBもしくはメチル水銀による健康影響が調べられた海外の主な出生コホート調査

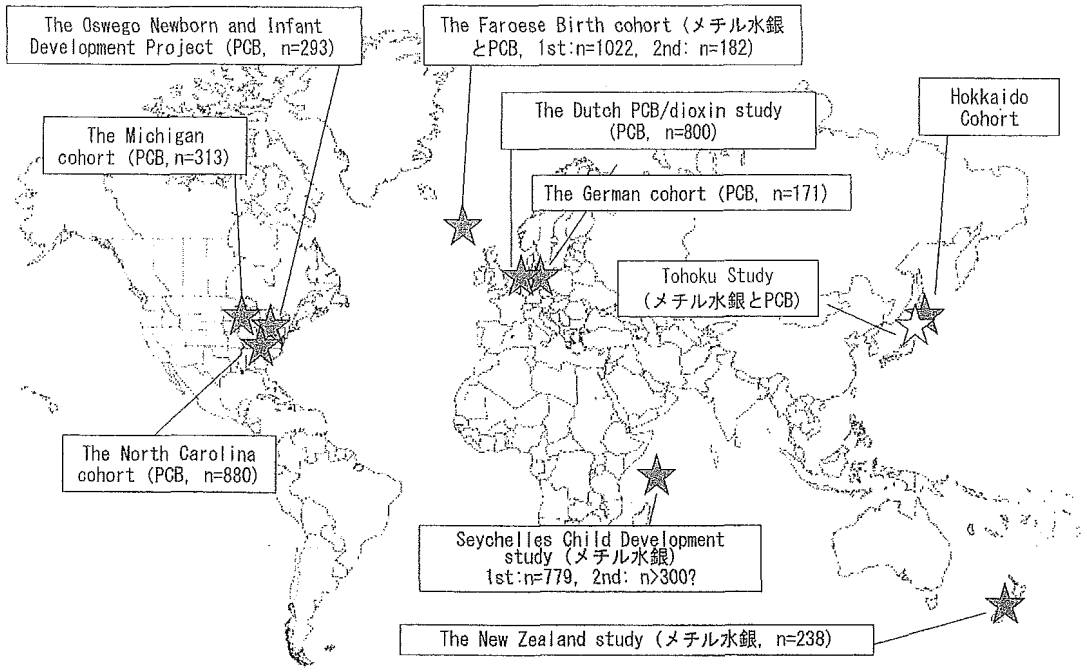

表1　海外におけるコホート調査の結果

Test	Major finding			Reference
	Fish intake	Prenatal exposure[1]	Postnatal exposure[1]	
Michigan 1980-1981				
NBAS (60 hr)	Motor immaturity, Poorer lability of states, Hypoactive reflexes	No relation		16
BSID (5, 7 mo)	No relation	No relation		17
FTII (5, 7 mo)	Less performance	Less performance	No relation	18
MS (4 yr)		Poorer scores in verbal and numerical memory	Weak relation	19
IQ test (11 yr)		Intellectual impairment	No relation	15
North Carolina, 1978-1982				
NBAS (72 hr)		Less muscle tone, Lower activity levels, Hyporeflexive[2]		20
BSID (2 yr)		Lower psychomotor scores[2]		21
MDS (2 yr)		No relation[2]		22
MS (3-5 yr)		No relation[2]		23
Oswego, NY, 1991-1994				
NBAS (48 hr)	Lower scores in habituation, autonomic and reflex	Lower scores in habituation, autonomic and reflex	No relation	24
FTII (6 and 12 mo)		Less performance	No relation	13
Performance test (4.5 yr)		Increase in errors of commission	No relation	25
Netherlands, 1990-1992				
PNE (10-21 d)		No relation	Less muscle tone, Reduced neurological optimality	26
BSID (3 mo)		Lower psychomotor scores	No relation	27
BSID (7 mo)		No relation	Lower psychomotor scores	27
Neurological (18 mo)		Lower optimality	No relation	28
Neurological (42 mo)		No relation	No relation	29
K-ABC (42 mo)		Intellectual impairment[3]	No relation	30
Neuropsychological (9 yr)		Longer respone time	Week relation	31
Auditory P300 (9 yr)		Longer P300 latencies	No relation	32
Dusseldorf, 1993-1995				
BSID (7 mo)		No relation	Lower mental scores	33
FTII (7 mo)		No relation	No relation	33
BSID (30 mo)		No relation	Lower mental and psychomotor scores	2
K-ABC (42 mo)		No relation	Intellectual impairment	2
Faroe Islands, 1994-1995				
PNE (2 wk)		No relation[3]	No relation	34

1 Cord blood PCB level for prenatal exposure and maternal milk PCB level for postnatal exposure. 2 Prenatal PCB exposure was estimated based on the maternal milk PCB level obtained at birth. 3 Maternal blood PCB level.
Neurological and cognitive tests are abbreviated as follows: Neonatal Behavioral Assessment Scale (NBAS), Bayley Scales of Infant Development (BSID), Fagan Test of Infant Intelligence (FTII), McCarthy Scales (MS), Mental Development Scales (MDS), the Prechtl Neurological Examination (PNE), Kaufman Assessment Battery for Children (K-ABC).

り，例えば新生児が母乳を通して摂取する量はTDIの40～100倍にも達するとも試算されている。周産期，特に胎児期における化学物質曝露の健康リスクの評価が求められている。

わが国では，ダイオキシン類，PCB，メチル水銀などの化学物質は主に魚介類の摂取によって取り込まれると考えられているが，一方で魚介類は栄養学的に優れた栄養素を含んでいる。特に不飽和脂肪酸は新生児の中枢神経系の発達に必須と考えられれている。例えば，海外の疫学調査の中でSeychelles共和国で行われたコホート調査では，化学物質曝露の負の影響は見出されていないが[3]，このSeychelles共和国は多様な魚を摂取する食習慣を有しており，日本における魚摂取の状況に近い。Seychelles共和国ではPCBsによる魚の汚染はきわめて低いとされているため，わが国の状況との単純な比較は難しいものの，多様な魚を多食する食習慣を有する集団では化学物質の健康リスクも異なる可能性がある。疫学調査を進めるうえでは，化学物質曝露の健康リスクのみならず，魚摂取の意義を総合的に評価する研究が必要となっている[4]。

コホート調査の概要

1 登録

我々が東北地方で進めているコホート調査（Tohoku Study of Child Development；TSCD）の概要を図2に示した。プロトコールの詳細は文献を参照されたい[5]。2001年1月から2003年9月にわたり，仙台市内の複数の医療機関にて事前に調査の概要を説明し，インフォームドコンセントを実施し文書による同意を得た。低体重，早期産，除外疾患などを考慮し最終的に599名の新生児－母親のペアを登録した。出生した児の発達を追跡するため，東北大学医学系研究科内のコラボスペースに調査のための部屋を設置，音，温湿度環境に加え，児の安全性や居住性に配慮した環境にて発達検査を進めている。なお，調査に先立ち東北大学医学系研究科倫理委員会に研究計画の申請を行い許可を得ている。

2 児の成長の追跡

児の成長を追跡するための神経行動学的な手法に関して，生後3日目にブラゼルトン新生児行動評価（NBAS）を，生後7カ月で新版K式発達検査，

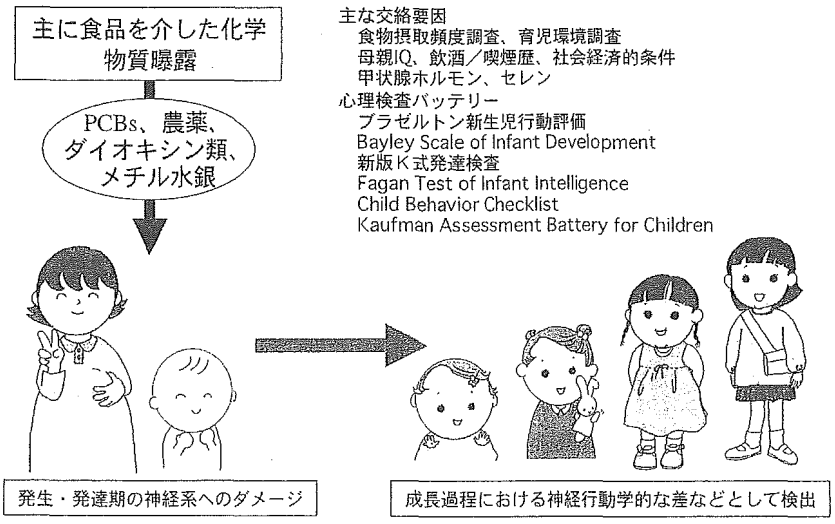

図2　コホート調査の概要
周産期における化学物質曝露と把握しつつ，出生児の成長を，認知行動面の発達を中心に追跡する。周産期に受けた神経系へのダメージが，児の成長過程で表れると危惧される。

Bayley Scales of Infant Development（BSID）およびFagan Test of Infant Intelligence（FTII）を，生後18カ月で新版K式発達検査およびBSIDを，生後30カ月でChild Behavior Checklist for 2-3 years（CBCL）を，生後42カ月でKaufman Assessment Battery for Children（K-ABC）を進めている。これらの追跡調査への出席率はおおむね82～88%で推移している。

検査バッテリーについては，新版K式発達検査はわが国における標準的な発達検査であるが，海外では**表1**に示すようにBSIDがよく用いられており[6]，TSCDの研究成果の国際比較を想定して新版K式発達検査とBSIDの併用による方法を採用した。BSIDは国内で標準化されておらず，1993年に第2版に改定された後は国内での使用例も見当たらない。そのため，Rochester大学の小児発達研究グループ（Davidson教授）との共同作業によりプロトコールの和訳と信頼性評価を実施した[7]。また，FTIIは乳幼児のもつ新奇選好を応用した視覚認知検査であり，将来の知的能力と高い相関をもつとされている[8]。海外の調査でもよく用いられている検査項目であり（**表1**），我々の調査でも日本の乳幼児で新奇選好が認められている[9]。なお，生後42カ月では児の神経運動機能の評価を試みるため，デンマークで開発されたCATSYS2000[10]の中から，身体重心動揺およびふるえ検査を試みている。

a. 交絡要因

児の成長と化学物質曝露を関連づけるうえで，母親の食事調査（半定量式食物摂取頻度調査），社会経済的要因（Hollingshed four facors version），育児環境調査，母親IQ（Raven's Standard Matrices）により実施している。

b. 化学分析

生体試料の化学分析について，母親毛髪総水銀ならびに臍帯血および母体血甲状腺ホルモン関連指標（TSH，総および遊離T3/T4）については全例で分析を終了した。総水銀分析は還元気化法により，甲状腺ホルモン関連指標は電気化学発光免疫測定法により分析を行った。

表2　文献比較の対象としたコホート調査とその化学分析の方法

Study	Method				No of	Lipid	Comment	Reference
	Extraction	Clean-up	GC	Detection	congeners identified	determination		
North Carolina, 1978-1982	Liquiud	Florisil	Packed	ECD	-	Not identified	Webb-McCall method	35
Michigan 1980-1981	Liquiud	Florisil	Packed	ECD	-	Not identified	Webb-McCall method	36
Netherlands, 1990-1992	Liquiud	Florisil	High Resolution	ECD	4	Gravimetric (milk)	Milk: 17 PCDD/F congeners, 3 planer and 23 non-planar PCBs, Plasma: Sum of 118, 138, 153, and 180	28
Oswego, NY, 1991-1994	Liquiud	Florisil	High Resolution	ECD	68	Gravimetric	a) Sum of 68 congeners b) Sum of highly chlorinated congeners	13
Dusseldorf, 1993-1995	Solid -Liquiud	Florisil	High Resolution	ECD	3	Photometric	Sum of 138, 153, and 180	2
Faroe Islands, 1994-1995	Solid -Liquiud	Florisil	High Resolution	ECD	6	Photometric (milk)	1.65 x Sum of 138, 153 and 180	34
Nonavik, Quebec, 1996-2000	Liquiud	Florisil	High Resolution	ECD	14	Gravimetric (milk) Enzymatic (serum)		37
Osaka, 1998	Liquiud	Florisil	Packed	ECD	-	Gravimetric	Japanese official procedure	11
Chiba and Yamanashi, 2002-2003	Liquiud	Silica gel	High Resolution	MS	All	Enzymatic (serum)		12
Tohoku, 2001-2003	Liquiud	Silica gel	High Resolution	MS	All	Gravimetric	Whole blood was used.	5

文献37、11および12は児の発達を追跡するコホート調査ではないが、比較のため記載した。
ダイオキシン類を分析したコホート調査は、オランダの疫学調査で母乳での分析が行われたのみであり、PCBについてまとめた。

有機塩素系化学物質のうち，ダイオキシン類はレポータージーンアッセイであるCALUX AssayおよびGC/MSによる方法とし，またPCBs全異性体分析もGC/MSによる方法とした。海外におけるコホート調査では，表2にそれぞれの調査で用いられた分析法を整理したが，生体試料中のPCBの分析はいずれもECDによる検出であり全異性体分析は行われていない。また，ダイオキシン類の分析に関しては，オランダの疫学調査にて母乳中濃度が測定されているのみである。本調査では，臍帯血を用いたダイオキシン類およびPCB全異性体分析を実施しているが，このような精密分析は初めての試みとなる。

まだ分析途中であるものの，高感度解析法の採用により，臍帯血でもほとんどの試料で2,3,7,8-TCDDを筆頭に多様なダイオキシン類が検出されている。中間報告になるが，臍帯血全血中のダイオキシン類濃度の中央値は，0.022 pg-TEQ/g-wet (0.005～0.13) であり，総PCBは115 pg/g-wet (36～670)，脂肪含量は0.27% (0.18～0.72) となっている。過去のコホート調査では血清もしくは血漿の値が示されているが，血球画分にはダイオキシン類およびPCBはほとんどど含まれないともされており，臍帯血のヘマトクリットを50%と仮定すると，血漿中の化学物質の濃度は全血での値の約2倍となる。

海外のコホート調査との比較 —PCB曝露に着目して

過去の海外のコホート調査では，PCBの分析結果が報告されているため，PCBについて文献的な比較を試みた。表3に臍帯血の分析結果を，さらに我々はまだ母乳の分析結果を得ていないものの，表4に母乳の分析の比較の結果を示した。国内における曝露レベルの参考値とするため，国内分についてはコホート調査以外からも引用した[11,12]。

数値は中央値での比較を優先し，臍帯血では表記単位はng/mlとした。文献上で脂肪重量当たりの数値が記載されている場合には，我々の調査で得られた脂肪含量0.27%を用いて換算した数値も記載した。臍帯血PCBについて異性体情報が記載されていた場合には，生体試料中の存在比率が最も高いIUPAC#153の値を記載するとともに，New

表3　臍帯血中PCBレベルに関する文献比較

ΣPCBに加え，#153および高度塩素化PCB（塩素数7-9）についても算出可能なものは記した

Study	No.	Chemical	Geometric mean		Comment
North Carolina, 1978-1982	744	Σ PCB	<4.27	ng/ml	
Michigan 1980-1981	293	Σ PCB	2.7	ng/ml	
Netherlands, 1990-1992	373	Σ PCB	0.38	ng/ml	
	373	153	0.15	ng/ml	
Oswego, NY, 1991-1994	293	Σ PCB	0.52	ng/g-wet	
	293	Σ 7-9 Cl PCB	0.05	ng/g-wet	
Dusseldorf, 1993-1995	141	Σ PCB	0.39	ng/ml	
Nonavik, Quebec, 1996-2000	98	Σ PCB	0.76[a]	ng/ml	279.9 ng/g-lipid (70.8-1420.1)
	98	153	0.23[a]	ng/ml	86.9 ng/g-lipid (13.4-550.9)
Chiba and Yamanashi, 2002-2003	20	Σ PCB	0.14[b]	ng/g-wet	63.8b ng/g-lipid (31-110)
Tohoku, 2001-2003	42	Σ PCB	0.23[b,c]	ng/ml	全血で 0.115ng/ml (0.035-0.67)
	42	153	0.05[b,c]	ng/ml	全血で 0.026 ng/ml (0.007-0.140)
	42	Σ 7-9 Cl PCB	0.06[b,c]	ng/ml	全血で 0.031 ng/ml (0.008-0.211)

[a]脂肪率027%と仮定して計算した。[b]Median。[c]全血での濃度をHt50と仮定して血漿値に換算した。

York州Oswegoでの調査からは塩素数7～9個の高度塩素化PCBが児の発達との関連性が高いと報告されていることから[13]，高度塩素化PCBについても並記した。なお，母体血PCBの比較はすでに論文でも報告されており[14]，今回は記載しなかった。

臍帯血中の総PCBについてみると，我々の結果を含め国内の曝露レベルは海外に比較して低値となっている。総PCBについては各調査で積算の方法が若干異なるものの，IUPAC#153のみに着目しても同様な結果であった。しかしながら，高度塩素化PCBに着目すると，我々の結果はOswegoの曝露レベルに匹敵した。すなわち，Oswegoの総PCB値が高いのは，塩素数1-3の低度塩素化PCBの割合が多いためであり[13]，これは分析方法論上のクリーンアップや検出装置の特性に起因するものとも考えられた。

次に，母乳中PCBのレベルについて比較すると，国内の曝露レベルはOswego調査に匹敵するか，もしくは全体として低くなる傾向にあった。その一方で，Faroe諸島における曝露が高いことが明らかであり，1980～1981年に実施されたMichiganにおける調査とほぼ同じレベルの曝露であることが示唆された。Faroe諸島においてはメチル水銀による健康影響についても調査が進められているが，PCBsとメチル水銀の複合曝露による健康影響が強く懸念され，Faroe諸島におけるPCBの胎児期曝露のリスク評価が課題とも考えられた。なお，Michiganにおける調査では，量的なPCB曝露は母乳を介した寄与が大きいものの，出生後の曝露の影響は見出せなかった[15]。理由として，児の脳の感受性が胎児期に高いこと，また授乳行為そのものが児の発達を促す効果が期待されるため，と述べられている。

おわりに

化学物質による周産期曝露の健康リスクの解析を進めるうえで，児の発達を追跡すること，交絡要因を的確に把握すること，そして適切な曝露指標を得ることが重要と考えられる。TSCDはその解析途上であり，結論を得るにはまだ時間がかかるものと思われるが，近い将来，ダイオキシン類，PCB，メチル水銀曝露と児の健康リス

表4　母乳中PCBレベルに関する文献比較

Study	No.	Chemical	Geometric mean		Range	Comment
North Carolina, 1978-1982	617	ΣPCB	1530	ng/g-lipid		Milk at 6 weeks postpartum
Michigan 1980-1981	124	ΣPCB	829.7	ng/g-lipid		Milk at 0.5-4.5 months postpartum
Netherlands, 1990-1992	194	ΣPCB	404.8	ng/g-lipid		Milk at 2 weeks postpartum
	194	#153	174.7	ng/g-lipid		
Oswego, NY, 1991-1994	86	ΣPCB	153	ng/g-lipid		Milk at 1-3 months postpartum
Dusseldorf, 1993-1995	126	ΣPCB	404	ng/g-lipid		Milk at 2-4 weeks postpartum
Faroe Islands, 1994-1995	168	ΣPCB	1520	ng/g-lipid	70-18500	Milk at 3-4 days postpartum
Nonavik, Quebec, 1996-2000	116	ΣPCB	385.6	ng/g-lipid	75.7-1915.8	Milk at 1 month postpartum
	116	#153	131.6	ng/g-lipid	21.7-727.9	
Osaka, 1998	49	ΣPCB	200[a]	ng/g-lipid		Milk at 2-4 weeks postpartum

オランダの疫学調査では母乳中ダイオキシン類の分析が行われており、総TEQ（PCDD/Fs + co-PCBs) 62 pg-TEQ/g-lipid.
[a]Arithmetic mean.

クについて関連性を明らかにできるものと期待される。これまで海外で報告されてきた調査事例を参考にしつつ，今後とも調査研究を進めていきたい。

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Effects of Prenatal Methylmercury Exposure on Child Neurodevelopment in Japan in Relation to Madeiran Study

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Abstract

Objective: A cross-sectional study was carried out to clarify neurodevelopmental effects of methylmercury exposure in Japanese children. ***Methods:*** The subjects were 327 mothers and their 7-year-old children in Japan. Mercury levels of hair samples obtained from mothers and children, and methylmercury levels in umbilical cord were determined. Neurobehavioral/neurophysiological tests including the brainstem auditory evoked potential (BAEP) were performed in the children. Information on diet, child's birth and medical history was also obtained from mothers. ***Results:*** The geometric means of hair mercury were 1.65 (range, 0.35-6.32) μg/g for children and 1.62 (0.11-6.86) μg/g for mothers aged of 24-49 years, respectively. Child's hair mercury was significantly correlated with maternal hair mercury. The maternal hair mercury level was significantly correlated with the methylmercury level in umbilical cord. There were no significant dose-effect relationships between the hair mercury levels and outcome variables. Since our study was comparable with the Madeiran cross-sectional study (Murata *et al*, 1999a) regarding maternal hair mercury, BAEP latencies and age, these two data were merged. The significant dose-effect relations of BAEP latencies were found in the combined data. When the benchmark dose and its lower 95% confidence limit (BMDL) were calculated, the BMDLs of mercury exposure for BAEP latencies in the combined data were between 7.8 and 10.4 μg/g, and smaller than those in the Madeiran children alone. ***Conclusions:*** These findings suggest that the current mercury level in maternal hair, if the dietary habit on fish consumption remains unchanged after the pregnancy with the child, may reflect the mercury exposure level at parturition. A significant dose-effect association was not observed in Japanese children alone, but only when higher-level exposures from Madeiran children were included. The BMDL for the combined data was lower than for Madeiran children alone.

Key words: methylmercury, neurodevelopment, prenatal exposure, cross-sectional study

Introduction

Methylmercury is a worldwide contaminant of seafood and freshwater fish. Methylmercury toxicity can produce widespread adverse effects within the nervous system, especially when exposures occur during brain development (IPCS 1990; Clarkson 1992; Igata 1993; National Research Council 2000). Early adverse effects have been characterized by administering neurobehavioral tests to children exposed *in utero* from maternal seafood diets. Thus, the National Research Council (2000) concluded that prenatal exposure was the most critical and emphasized the findings from a prospective birth cohort study carried out in the Faroe Islands (Grandjean *et al.* 1997); nevertheless, such neurodevelopmental risks related to methylmercury exposure from contaminated seafood remain controversial.

Apart from the prospective studies in the Seychelles (Shamlaye *et al.* 1995; Davidson *et al.* 1998; Myers *et al.* 2003), New Zealand (Kjellström *et al.* 1989), and Faroe Islands (Grandjean *et al.* 1997), a cross-sectional study was conducted in 1995, in order to clarify the effects of methylmercury on child neurodevelopment (Murata *et al.* 1999a). One hundred forty-three children, first grade of two elementary schools near the fishing harbor of Câmara de Lobos, Madeira, Portugal, were invited for the study; the child's own hair-mercury concentration was used as an indicator of the current exposure level. For the mother who had not changed her dietary habit, her current hair concentration was used as a proxy for mercury exposure at parturition. However, the Madeiran study could not fully confirm that current mercury concentration in maternal hair reflected the mercury exposure during pregnancy.

As the neurodevelopmental parameters, various neurobehavioral tests such as Wechsler Intelligence Scale for Children, Child Behavior Checklist, McCarthy General Cognitive Test, Preschool Language Scale, Language Development Test, California Verbal Learning Test, Bender Copying Test, Boston Naming Test, McCarthy Motor Test, reaction time and finger tapping, have been used by many researchers addressing risk assessment of methylmercuy (Kjellström *et al.* 1989; Shamlaye *et al.* 1995; Grandjean *et al.* 1997; Davidson *et al.* 1998). Some of the tests have been reported to be associated with exposure biomarkers at parturition, but common tests to the above prospective studies did hardly exist (National Research Council 2000). Accordingly, a mutually comparable study with common tests, as well as specific tests to the exposure, would be required. Also, neurophysiological tests such as the brainstem auditory evoked potential (BAEP) and electrocardiographic (ECG) R-R intervals, may be useful

for the assessment because such measurements have been reported to be sensitive to occupational hazardous substances (Murata & Araki 1996; Araki *et al.* 1997; Counter 2003) and independent of the subjects themselves (*e.g.*, mood, language or education) and socioeconomic factors (Chiappa 1997).

A large scale of study on the neurodevelopmental effects of methylmercury exposure has never been conducted in Japan. We carried out a cross-sectional study with similar tests to the Faroese cohort study (Grandjean *et al.* 1997), to clarify whether Japanese child has any neurodevelopmental impairment due to prenatal methylmercury exposure, and whether the current mercury concentration in maternal hair represents the mercury exposure level at parturition.

Materials and Methods

Subjects

The study protocol was approved by the ethical review committee at the Akita University School of Medicine. The nature of the procedures used in the present study was explained to the parents in Akita and Tottori Prefectures, and mothers and the 7-year-old children were invited for this study during the period of July-September in 2002 and 2003. The children, who were born in the period of April 2nd, 1995 to April 1st, 1997, were chosen in accordance with the preceding study on the risk assessment of methylmercury exposure (Murata *et al.* 1999a). The children were in the first grade of 28 elementary schools, and 14 of them were located in near the fishing harbor. In Japan, there were many mines and smelters thirty years ago, and it was probable that soil or water has been contaminated by lead, copper, cadmium, *etc*; for this reason, the study population did not include those who came from such areas.

Exposure biomarkers

Hair samples were collected, by cutting strands of hair close to the scalp, from the occipital area in all mothers and children. The hair length was generally about 10 cm, ranged from 1 to 30 cm. Total mercury in aliquots of dried hair samples (15 to 20 mg), rinsed with acetone, was determined by the cold vapor atomic absorption spectrophotometry method at the National Institute for Minamata Disease (Akagi and Nishimura 1991). Also, naturally dried umbilical cord samples of children were obtained from parents who consented to our proposal voluntarily; according to Japanese

old traditions, most of families used to preserve a small piece of the cord tissue of the child as a birth memorial. Methylmercury in the cord tissue, after removing blood cells from it, was determined at the same institute by ECD-gas chromatography after the extraction by dithizone (Akagi and Nishimura 1991). Total mercury concentrations in child's and maternal hairs were used as current mercury exposure and a proxy for mercury exposure at parturition, respectively. Methylmercury concentrations in the cord tissue were to check the validity of the proxy for mercury exposure.

Detailed survey on medical records during pregnancy and delivery including smoking and drinking habits, gestation period and birth weight, past and present history of illness in child, dietary habits in mother, and frequency and amount of fish intake was conducted by a trained interviewer at the schools or civic centers where examinations on child neurodevelopment were also done. Also, a questionnaire on artificial hair waving was collected from the mothers to clarify the effect on exposure biomarkers.

Outcome variables

Concerning outcome variables for methylmercury exposure, we considered the following points: (1) sensitive to toxic exposure, (2) reflecting functional domains, (3) reasonably specific, with limited potentials for confounding, (4) appropriate for age and culture, (5) skilled examiners, and (6) computer-assisted objective methods (Grandjean *et al.* 1997). We examined tremor, postural sway, ear-hand coordination, and reaction time (at the Station A); corrected QT (QTc) interval on ECG, heart rate variability, and eye-hand coordination (at the Station B); and, brainstem auditory evoked potential latencies (at the Station C) for totally one hour per child, by using the Neurobehavioral Test System (CATSYS 2000, Danish Product Development Ltd, Denmark), ECG-Amplifier 1271SP (NEC-Sanei, Japan), electrocardiography (ECG-9202, Nihon Kohden Co, Japan), and four-channel electromyography (Neuropack μ, Nihon Kohden Co, Japan). These tests were conducted by three trained examiners.

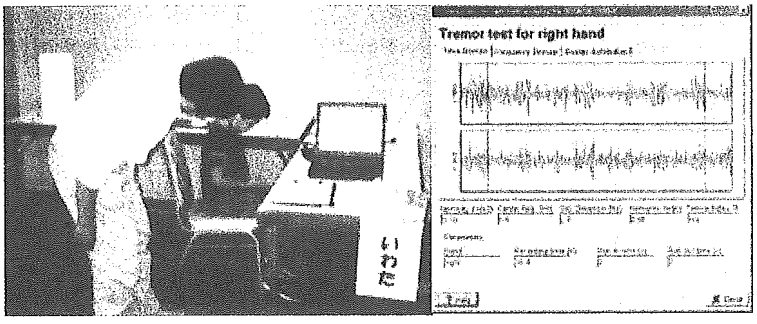


Figure 1 Measurement of tremor test

Hand tremor was measured successively for each hand during 16.4 sec, by asking the

subjects to hold a light stylus as they would hold an ordinary pen, with the elbow joint bent at a right angle and free of body contact or any obstacles as shown in Fig. 1 (Despres *et al.* 2000). The stylus was held horizontally, parallel to the abdomen at approximately 10 cm in front of the navel and the index finger was positioned about 1 cm from the tip of the stylus. Ear-hand coordination was examined with the CATSYS System, which was composed of a drum that records hand pronation-supination movements (Despres *et al.* 2000). The test was performed with right and left hands under the following standard condition: hand pronation-supination at a constant slow (1 Hz) and a constant fast (2.5 Hz) metronome beat. Eye-hand coordination was examined by operating the mouse in front of the portable computer (Fig. 2). Reaction time to a sound stimulus was measured with right and left hands (Despres *et al.* 2000).

The postural sway was quantitatively measured with flat floor using the computerized posturography (Despres *et al.* 2000). Subjects were asked to stand quietly on the platform without foam under eyes-open and eyes-close conditions; again, they were asked to stand on the platform with foam in the same manner.

Figure 2 Measurement of eye-hand coordination test

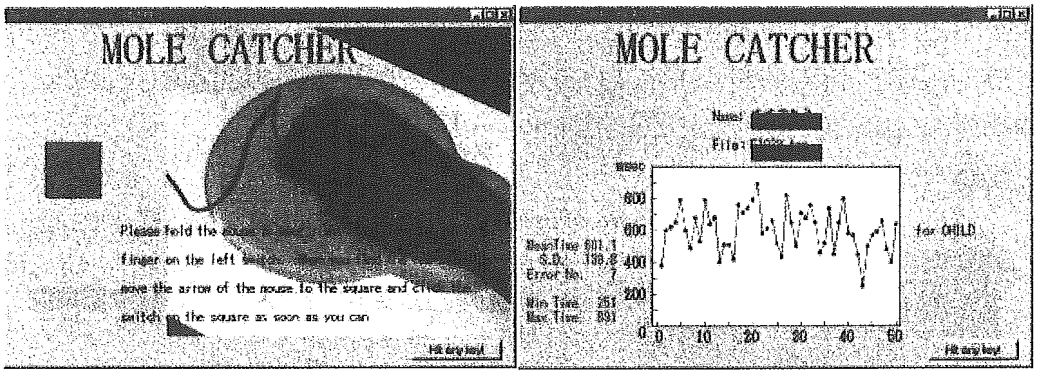
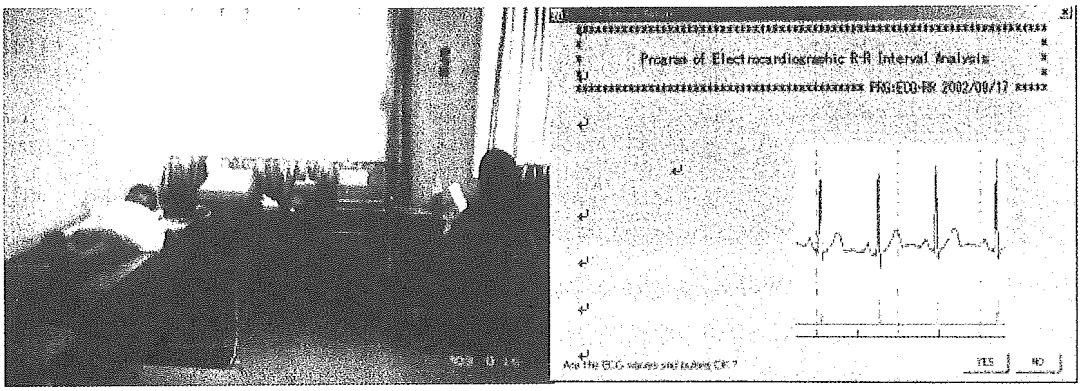


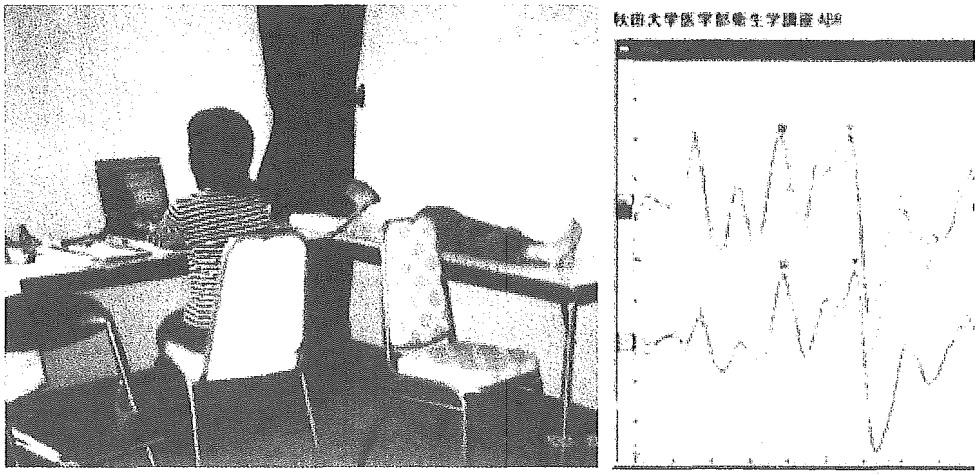
Figure 3 Measurement of the R-R intervals on ECG



After the subject had lain quietly supine, ECG 300 R-R intervals were measured in

real time and stored on the hard disk (Fig. 3). Consecutive 100 R-R intervals with the minimal standard deviation (SD) were automatically extracted from the obtained data to avoid non-stationarities. The CV_{RR} was defined as the ratio of the standard deviation of the R-R intervals to the average value (RR_{mean}, ms). The power spectrum of R-R intervals was computed by autoregressive spectral analysis (Hayano 1988; Murata *et al.* 1992). The spectrum of each of two components, *i.e.*, the high frequency (HF) component at the center frequency of 0.15-0.4 Hz and low frequency (LF) component at 0.01-0.15 Hz, was separated by component analysis. As the square root of the total spectral power (*i.e.*, the sum of each component power) is equal to the standard deviation of the R-R intervals, each component coefficient of variation (*i.e.*, CCV_{HF} and CCV_{LF}) was defined as the ratio of the square root of each component power spectral density (PSD_k, ms^2) to the RR_{mean}: $CCV_k=100\cdot(PSD_k)^{1/2}/RR_{mean}$, where k=HF or LF. The daily variation in the CV_{RR} was 7.5 (Murata *et al.* 1992). As parasympathetic blockade with atropine abolishes the HF component but beta-sympathetic blockade has no effect on it, the CCV_{HF} reflects the parasympathetic activity; and, the LF component is considered to be derived from the fluctuation in the vasomotor activity through the baroreflex mechanism and show a beta-adrenergically mediated increase in the standing posture (Pagani *et al.* 1986; Hayano 1988; Ewing 1992). The CV_{RR} reveals higher functions of the autonomic nervous system as well as both parasympathetic and sympathetic activities (Ewing 1992; Murata *et al.* 1992).

Figure 4 Measurement of brainstem auditory evoked potential



The BAEP was recorded in subjects lying comfortably (Fig.4). Click signals with an intensity of 65 dB HL were presented to the right ear through electromagnetically shielded earphones at 20 Hz and 40 Hz independently (Murata *et al.* 1999a); the other ear was masked with white noise of intensity of 30 dB HL. Evoked potentials were

recorded using three standard EEG electrodes placed on the vertex, the right mastoid ipsilateral to stimulation and the left mastoid (ground). The responses were averaged 2,000 times after amplification and filtration (bandpass, 200-2,000 Hz), with one replication for each rate. Peaks I, III and V are thought to reflect the volume-conducted electric activity from the acoustic nerve, pons (superior olivary nucleus) and midbrain (inferior colliculi), respectively (Stockard *et al.* 1986). The coefficients of variation (daily variation) in the BAEP latencies at 20 and 40 Hz in a 20-year-old student for 14 days were 3.0% and 3.4% for peak I latencies; 1.4% and 1.6% for peak III latencies; 0.9% and 1.6% for peak V latencies, respectively.

Data analyses

To make an international comparison, the data obtained from Madeiran children (Murata *et al.* 1999a, 2002) were merged into this study, because the Madeiran cross-sectional study seemed to be entirely comparable with our study in Japan, in regard to the exposure biomarkers and outcome variables, such as the maternal mercury level in scalp hair, BAEPs and age of the study population.

The relationships among exposure biomarkers were assessed by the Spearman rank correlation coefficient (r_s). The multiple regression analysis was performed to examine the dose-effect relations of neurobehavioral and neurophysiological tests to mercury exposure. The differences in outcome variables both between boys and girls and between Japanese and Madeiran children were analyzed by the analysis of covariance to control for age (and gender).

The benchmark dose (BMD) was defined as the mercury concentration in maternal hair that resulted in an increased probability of abnormal test performance by a benchmark response (BMR), *i.e.*, from P_0 to P_0+BMR at the BMD (National Research Council 2000), when the P_0 and BMR represented an abnormal probability in unexposed population and an excess risk in exposed population, respectively. The BMD and cutoff value (C) were calculated using a statistical dose-effect model based on power functions ($g(d)=d^K$) for the dependence (μ) of the outcome variable on the mercury concentration (d) and confounders such as age, gender and race (Budtz-Jørgensen *et al.* 2001): (1) $\mu(d)=\beta_0+\beta_1 \cdot g(d)+\beta_2 \cdot [age]+\beta_3 \cdot [gender]+\beta_4 \cdot [race]$, (2) $P_0=1-\Phi([C-\beta_0]/\sigma)$, and (3) $BMD=g^{-1}\{[\Phi^{-1}(1-P_0)-\Phi^{-1}(1-P_0-BMR)]\sigma/\beta_1\}$ (the Φ and σ indicated the normal cumulative distribution function and standard deviation of the outcome variable in unexposed population, respectively). The normalized value for each confounder was employed in the above regression model. A lower confidence limit for BMD (BMDL) was then calculated as the statistical 95% lower bound of the

BMD (Budtz-Jørgensen *et al.* 2001). The power parameter K has been restricted to values equal to or above 1, thus allowing the dose effect curve to be nonlinear. Since previous applications of this method have used a P_0 of 5% and a BMR of 5% (Budtz-Jørgensen *et al.* 2001; Murata *et al.* 2002), we applied the linear (K=1) and K-power dose effect curves, set at the same P_0 and BMR. All analyses were performed using the Statistical Package for the Biosciences (SPBS V9.51) (Murata & Yano 2002).

Results

Exposure biomarkers

The participating subjects, from whom informed consent was obtained, were 327 mothers at 35.8±4.5 (range, 24~49) years of age, and the children at 6.9±0.3 (range, 6.3~7.5) years of age. The summary of exposure biomarkers is shown in Table 1. Geometric means of mercury in hair were 1.62 (median 1.63) μg/g for 327 mothers and 1.65 (median 1.65) μg/g for the children, and the maximum was 6.86 μg/g for the mothers and 6.32 μg/g for the children; also, there was no difference between the mother and child (Wilcoxon signed rank test, p>0.5). No significant differences in hair mercury concentrations were found either between subjects residing in cities and towns or between those in non-fishing and fishing areas (two-way analysis of variance, p>0.05). Also, hair mercury concentrations (0.11~6.86, mean 1.62 μg/g) in the 108 mothers with artificial hair waving were significantly lower than those (0.39~5.83, mean 2.04 μg/g) in the 219 mothers without (Mann-Whitney U test, p<0.0001).

Table 1 Summary of 327 participating subjects in Japan

	Participating subjects	Prefecture (number)	Hair mercury in mother (μg/g) (Mean, range)	Hair mercury in child (μg/g) (Mean, range)
Urban areas (Cities)	181	Akita 135*1 Tottori 46*2	1.87, 0.11~6.86 1.66, 0.44~5.62	1.85, 0.35~5.32 2.20, 0.43~5.83
Rural areas (Towns & villages)	146	Akita 108*3 Tottori 38*4	2.06, 053~5.38 1.85, 0.42~4.79	1.79, 0.56~6.32 1.90, 0.67~4.39

*1 boys = 64, girls = 71; *2 boys = 31, girls = 15; *3 boys = 55, girls = 53; *4 boys = 17, girls = 21