Technol., **42** : 3096-3101, 2008.
13) Trudel, D. et al. : Estimating consumer exposure to PFOS and PFOA. *Risk Analysis*, **28** : 251-269, 2008.
14) Apelberg, B. J. et al. : Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ. Health Perspect.*, **115** : 1670-1676, 2007.
15) Fei, C. et al. : Fetal growth indicators and perfluorinated chemicals : a study in the Danish National Birth Cohort. *Am. J. Epidemiol.*, **168** : 66-72, 2008.
16) Washino, N. et al. : Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. *Environ. Health Perspect.*, 4 Nov. 2008. (Online ; http:/ /www.ehponline.org/members/2008/11681/11681.pdf)
17) Monroy, R. et al. : Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. *Environ. Res.*, **108** : 56-62, 2008.
18) Nolan, L. A. et al. : The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. *Reprod. Toxicol.*, **13** Nov. 2008. [Epub ahead of print]
19) Lau, C. et al. : Perfluoroalkyl acids : a review of monitoring and toxicological findings. *Toxicol. Sci.*, **99** : 366-394, 2007.
20) Emmett, E. A. et al. : Community exposure to perfluorooctanoate : relationships between serum concentrations and exposure sources. *J. Occup. Environ. Med.*, **48** : 759-770, 2006.
21) Grice, M. M. et al. : Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. *J. Occup. Environ. Med.*, **49** : 722-729, 2007.
22) Sagiv, S. K. et al. : Prenatal organochlorine exposure and measures of behavior in infancy using the Neonatal Behavioral Assessment Scale (NBAS). *Environ. Health Perspect.*, **116** : 666-673, 2008.
23) Cao, Y. et al. : Environmental exposure to dioxins and polychlorinated biphenyls reduce levels of gonadal hormones in newborns : results from the Duisburg cohort study. *Int. J. Hyg. Environ. Health*, **211** : 30-39, 2007.
24) Chevrier, J. et al. : Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. *Am. J. Epidemiol.*, **168** : 298-310, 2008.

*　　*　　*

小児環境保健に関する疫学調査の取り組みと課題

秋田大学 村田 勝敬 ・ 東北大学 佐藤 洋

1．はじめに

子どもの健康やライフスタイルは、それを取り巻く家族、地域、学校、職場、国などのあり方に強く影響される。これらの社会環境の改善と良好な状態の維持は子どもの健全な発育・発達にとって不可欠である。しかしながら、昨今の児童犯罪の報道等から察すると、全ての子どもが健全な精神発達を遂げているとは想像しがたい。また、産業構造が変化する中で、ヒトに対する安全性確認が十分に行われていない新たな化学物質や機器が巷に出回り、子どもを含めたヒトの健康を脅かしている。さらに、地球温暖化は温熱曝露に脆弱な子どもや高齢者の生命危機の原因となっている。この自然環境と社会環境からなる“環境”はヒトの生活空間を形成しているが、産業革命以後に激変し、利便性や快適性の向上をもたらした反面、様々な健康リスクを誘発しているように思われる。この時流にあって、次世代を担う子どもの発育・発達に関わる負の要因を取り除くための試みが国際社会の中で進行している。その方策の一つが有害化学物質の同定とその健康影響の解明である。

1997年に米国で開催された先進8カ国の環境大臣会合で、世界中の子どもが環境中の有害因子の脅威に晒されていることが認識され、小児の環境保健を巡る問題に対して優先的に取り組む必要があることが宣言された。これを機に1998年11月に米国ホワイトハウス主催で「メチル水銀曝露による健康影響の評価に関わる科学的問題」ワークショップが開催された[1]。わが国では、平成17年(2005年)12月に「小児の環境保健に関する懇談会」が設置され、小児の環境保健に関する現状と課題について検討を重ね、今後推進すべき施策の方向性についての提言が平成18年8月にまとめられた。環境省は、これに基づき、小児環境保健重点プロジェクトと位置づけて研究の推進を図っている。さらに、これは環境大臣の2大政策の一つとして掲げられた。このプロジェクトの基本計画は「小児環境保健疫学調査に関するワーキンググループ」によって現在作成中であるが、大筋として全国規模で約6万人の出生集団調査を平成22年度以降に立ち上げ、胎児期から学童期(12歳頃)までの健康状態を観察することになっている。

原因物質の定量化とともに、出生から臨床神経学的検査が行える年齢まで追跡した最初の研究報告は、我々が知る限り、イラクのメチル水銀中毒(1971年)に関するものである。このメチル水銀禍では、メチル水銀殺菌剤で処理した種子小麦をパンの原料として使用した農家を中心に多くの犠牲者(入院患者約6,000人、死亡者約500人)を出した[2]。この時、母体内でメチル水銀曝露を受けた18ヵ月児の歩行遅延の有無と母親から採取された毛髪の出産時水銀濃度を統計的に解析し、推定最小影響水銀濃度が算出された[3]。このように、母親の妊娠兆候が医学的に確認される時点の胎児期から一定年齢に達するまでの子ども集団(コーホート)を追跡し、主に子宮内で曝露した推定原因因子によって生じる変化を発育・発達段階毎に影響指標で検証する方法を出生コーホート研究と呼び、環境保健領域で近年盛んに用いられている。本稿では、この手法を用いて実施される「小児環境保健疫学調査」の周辺事情とその概要を紹介する。

2．わが国の環境問題

昭和31年5月1日に公式記録された水俣病は、チッソ工場から水俣湾に排出されたメチル水銀によって汚染された魚介類を摂食した漁民あるいはその家族に発生したメチル水銀中毒症である[2]。これは、日本の高度経済成長期に突入する頃であり、重化学工業化が国の最優先課題であった時代に派生した悲劇の一つと言える。同様の事例として、昭和30年4月以降中国地方を中心とした一帯で粉ミルクを飲んだ乳幼児に被害が出た森永ヒ素ミルク事件[4]、昭和43年に西日本でポリ塩化ビフェニール(PCBs)やポリ塩化ジベンゾフラン(PCDFs)が混入した米ぬか油を摂取した人々に発生したカネミ油症[5]などがこの時期に集中した。前者の事件では、その後の追跡調査で知的発達障害の他、肢体障害、精神障害、てんかんなどの重複神経系障害を抱える被害者が多数見いだされた[6]。後者では、当初は座瘡様皮疹、皮膚・粘膜の色素沈着などが被害者に現れ、その後に台湾で発生した同

様の米ぬか油事件で、胎児期に曝露した子どもに認知機能影響が現れることが明らかにされた[7]。これらの毒性物質、発生場所、原因企業は異なるが、共通項はいずれも犠牲者の多くが胎児ないし乳幼児であり、しかも重篤な神経精神障害を抱えたまま現在も生きているという厳粛な事実である。

胎児性水俣病は熊本大学水俣病研究班がフィールド調査を実施していた昭和33年に見つけられた。成人の水俣病では知覚(感覚)障害、運動失調、視野狭窄などの中枢神経症状を主徴としたが、胎児性水俣病では新生児期から発育・運動機能の発達遅延の他に脳性麻痺に酷似した症状を示し、小児期以後は知能、神経機能両面の発達の遅れが著明であった[8]。この患者の脳の病理組織所見が成人型水俣病と近似していることを病理医が確認し、この病気は胎児期に母親の子宮内で胎盤を介してメチル水銀を取り込んで発症したと判断された。

水俣病は世界的に有名な公害事例であるにもかかわらず、水俣病研究の多くは世界のメチル水銀研究者に殆ど引用されていない。これは原因因子であるメチル水銀濃度が被害者において測定されなかったためである。同様に、体内のヒ素、PCBsおよびPCDFs濃度も当時調べられなかった。発信された“水俣の教訓”のうち、世界が唯一注目したのは「妊婦がメチル水銀に曝露されると、メチル水銀の影響は胎児や生まれてくる子ども(次世代)に現れる」という事実であった[9]。このため、環境基準値等の設定時には、有害化学物質に曝露された労働者や実験動物で観察されたデータから算出されていたものが、胎児の曝露影響所見も考慮されるようになった。そして、ヒト胎児の曝露影響所見を得る方法が出生コーホート研究であり、この端緒を開いたのが胎児性水俣病なのである。

3．何故、小児の研究が必要か

胎児や乳幼児の臓器は脆弱であり、かつ様々な外的因子に感受性が高い。このため、環境からの曝露により種々の健康悪影響が現れる可能性がある。胎児・乳児における神経毒性物質曝露の影響は一生涯残ることが示されている[10]。また、内分泌かく乱作用が疑われる化学物質の妊娠中曝露による発育、アレルギー、停留精巣、矮小陰茎などの影響が示唆されている[11,12]。しかし、多くの環境有害物質の曝露レベルは概して低濃度であり、また子どもの生活環境は大きく異なることから、頻度の低い疾患の場合は臨床医による個々の診断で健康悪影響が見落とされるか、あっても原因不明の異常と片付けられてしまう可能性が高い。逆に、先天異常が見つけられた児について、臨床医が内分泌かく乱化学物質を測定した報告も殆どない。従って、子どもへの環境有害因子の同定とその健康影響の評価のために、大規模な出生コーホート研究が必須であり、さらに臨床医とともに環境保健・公衆衛生専門家の結束がなければ、この究極の目的を完遂することは難しい。

前述したように、メチル水銀の神経影響に関するコーホート研究(追跡調査)としてイラクのメチル水銀禍の報告がある。これは、水俣病と同様、“事故”による曝露であり、摂取量に照らして通常の食生活と大きくかけ離れた状況下で生じたものである。一方、メチル水銀を巡る世界の関心事は次世代に影響が現れ始める濃度(臨界濃度)である。この目的に照らし、日常生活の中で魚を多食する集団のいるニュージーランド、フェロー諸島、セイシェルで出生コーホート研究が1980年代前半にスタートしたのである[13]。また、バックグラウンドレベルにおけるダイオキシン類などの内分泌かく乱化学物質曝露による小児神経発達に関する調査は、1980年代後半から2005年までに、アメリカ、オランダ、ドイツで乳児期から学童期までの縦断研究として行われた[14]。しかし、曝露レベルが比較的低くかつ多様な交絡因子(原因因子および影響指標の両者に関連する居住地、収入、家族構成人員数、保護者の学歴、生活時間など)が介在するためか、一貫性のある結果は得られていない。特に、小規模集団では交絡因子を調整することが困難であり、信頼性の高い結果が得られにくくなる。その上、臨界濃度の推定は食・生活習慣、遺伝子多型、対象集団の平均年齢などで大きく異なり得る。したがって、自国の小児調査の結果に基づいて臨界濃度を推定し、環境基準値などを設定することが望まれる。

わが国で既に「小児環境保健疫学調査」の先行研究となる2つの出生コーホート調査が実施されている。そのうち、東北コーホート調査は難分解性環境汚染物質(POPs)およびメチル水銀による胎児から新生児期の曝露と児の成長・発達との関連性を明らかにするために計画された。登録は2001年1月から2003年9月の間に仙台市の2つの医療機関の協力を得て妊娠22週以降の妊婦約600名を対象に実施された[15]。この調査で調べられた母乳中

のPOPsのうち、有機塩素系殺虫剤のDDTは、1971年5月に農薬登録が失効され1981年に製造・輸入も禁止されたにもかかわらず、高濃度で検出され、環境残留性および生物濃縮の特徴が改めて確認された。現在84ヶ月児調査を行っており、メチル水銀、PCBs、ダイオキシン類等を含むPOPsの小児神経発達に及ぼす影響が報告される予定である。

もう一つは「環境と子どもの健康に関する北海道スタディ」である。これは札幌市内の1産院コーホート(対象者514名)と、北海道全域での大規模コーホート(妊婦の参加目標2万人、現在1万7千人が登録)の2つからなる。前者は母体血、臍帯血、毛髪、母乳のダイオキシン類、PFOSと呼ばれる有機フッ素系化合物、水銀等の化学物質による小児の出生時体格、神経発達、アレルギー疾患など健康影響を詳細に検討する目的で2002年に開始され、後者は先天異常発生と化学物質曝露の因果関係、化学物質代謝酵素等の遺伝子多型に基づく個人感受性素因の相違と先天異常との関連を解明する目的で2003年に立ち上げられた。これらの成果として、北海道における先天異常を有する児の出産頻度が2%であることの他、妊娠中母の喫煙と関連する遺伝子多型によって出生体重への影響が大きく異なること[16]、ダイオキシン類曝露による神経発達や有機フッ素系化合物の出生体重への影響などが報告されている[17,18]。

4．疫学調査の研究課題

小児環境保健疫学調査の主目的は化学物質の曝露や生活環境が、胎児期から小児期の子どもの健康にどのように関わり、どのような影響を与えるのか検討する。推定原因因子は環境中に存在する化学物質であり、主に母子の生体試料から測定される。結果因子として小児保健で問題となっている幾つかの健康事象が想定されており、出産時や乳児健診・アンケート調査時に調べられる。併せて、化学物質の曝露以外の交絡因子も検討予定であり、遺伝要因、人間生態要因、社会要因、生活習慣要因等がこれらに含まれる。実施体制としては、コアセンターを国立環境研究所に設置して各種データの集約を図り、またユニットセンターは全国複数箇所に公募・設置して、調査対象者の登録と生体試料の採取、小児の追跡を行うことになる。

小児環境保健疫学調査の仮説の設定に当たっては、広く国民の意見を伺うとともに、専門家からの具体的な調査仮説が公募された。これらに国の環境保健行政に関わる仮説を加えて基本計画が公表される。同時期に進行している米国のNational Children's Study(米国の10万人の小児を対象として出生前から21歳まで追跡し、小児の健康と発達に及ぼす環境影響を明らかにする調査 http://www.nationalchildrensstudy.gov/)を参考にすると、以下のような仮説が考えられよう。①化学物質曝露と性の決定(性比、性成熟の異常、脳における性分化など)、②化学物質曝露と妊娠異常(早産、妊娠高血圧症候群、切迫流産、流産、死産)、③化学物質曝露と発育障害(低出生体重・出生週数の早期化、身体発達)、④化学物質曝露と先天異常、⑤化学物質曝露と精神発達障害、⑥化学物質曝露と免疫系異常(小児アレルギー、感染症)、⑦化学物質曝露と代謝・内分泌系異常(甲状腺の異常、耐糖能異常、若年性糖尿病、若年性肥満)、⑧化学物質曝露と不妊、⑨化学物質曝露と脳の形態・機能異常、⑩騒音曝露と精神神経発達異常。これらの仮説の採否は、研究の実行可能性とともに、6万人の全数調査、2〜3万人規模の詳細調査、ユニットセンター内調査、コーホート内症例対照研究、付随研究等を念頭に置きながら取捨選択される。なお、わが国での12歳以降の継続最終判断は、期間内の研究成果、社会的要請、コーホートの追跡率等を勘案して下されることになろう。

5．調査への期待と不安

殺虫剤を多量に使用するエクアドルのバラ栽培地域に住む子どもにおいて、母親の妊娠中に殺虫剤曝露を受けた子どもは収縮期血圧が対照群と比べて高く、また殺虫剤曝露した子どもで神経行動学的検査や幾つかの神経心理検査の成績が低下していたことが報告されている[19]。殺虫剤、除草剤、殺菌剤、防カビ剤等の総称である農薬には、有機リン系農薬、カーバメイト系農薬、有機塩素系農薬、パラコートなどがある。このため、単に農作物に噴霧・撒布する農薬だけでなく、家庭菜園や観葉植物における病害虫駆除剤、シロアリ・ゴキブリ駆除剤、虫除けスプレー等を含めると我々の生活空間に残留・使用されている農薬は枚挙に暇がない。これらの毒性検査は動物実験結果のみで評価されているので、ヒトへの毒性は有害影響が実際に現れるまで不明なことが多い。今回の調査の推定原因因子に、どれほどの農薬・ダイオキシン類あるいはその代謝産物の測定が含まれるのかは定かでないが、ヒトへの催奇形性を含む健康影

響の解明が進むことを期待してやまない。

近年注目されている小児の精神発達障害や問題行動として、自閉症、アスペルガー症候群、注意欠陥・多動性障害、学習障害、感情障害、適応障害等がある。これらの多くは原因不明のままであるが、一部において環境中の化学物質曝露の関与が示唆されている。したがって、妊娠中あるいは出生時の生体試料を用いた化学物質の測定により、因果関係を推定することは意義深い。しかしながら、これらの精神発達上の障害の解明を6万人規模で実施しようとする場合、診断基準の設定の難しさとともに診断者の違いによる測定誤差も無視できず、単に曝露評価を正確に行えば万全と考えるのは軽率の誹りを免れない。

米国では小児における鉛影響(たとえば神経行動障害)に関心が集まっている。最近の鉛の疫学・的研究によると、血中鉛濃度は10mg/dl以下であっても、3〜10歳児の知能指数(IQ)得点と負に関連することが報告されている[20,21]。小児は指を舐めたり、食物以外の物を口に入れる習性があるので、鉛の吸収や貯留は成人に比べて多い。平成18年6月にイタリア・ブレシアで開催された国際労働衛生委員会(ICOH)の2つの合同科学委員会の鉛、水銀およびマンガンの神経毒性に関する国際ワークショップは、小児の血中鉛レベルを5mg/dlまで下げるべきとする宣言を採択した[22]。わが国で2003〜2004年に調べられた3ヶ月児から15歳児の平均血中鉛濃度は2mg/dl以下であり[23]、鉛の健康影響があまり問題視されていない。しかし、鉛精錬工場やバッテリー再生工場付近では鉛がヒュームの形で大気中に排出され、また橋梁や自動車の錆止め顔料として使用されている鉛化合物は腐蝕すると大気中に埃として飛散することもありうる。今回の調査で父親からの血液採取が行われるなら、不妊に関わる鉛の生殖毒性についてもある程度類推することが可能となるかもしれない。

この種の疫学調査で最も考慮されるべきことは、環境中の有害化学物質の曝露レンジが広くなるよう対象集団を集める必要があるということである。当該有害化学物質の曝露濃度に関して、既知の安全基準値を超える対象者が集団内に含まれ、かつ全集団に有意な量−反応(影響)関係が認められるならば、因果関係の推定は比較的容易である。しかし、安全基準値以下の集団で統計的に有意な量−反応(影響)関係が見られる場合、我々は(重要な交絡因子の影響を完全に否定するまで)その因果関係を保証することが難しくなるのである。例えば、鉛の臨界濃度は成人の血中鉛で30〜40mg/dlと考えられてきたが[24]、米国の一般成人13,946人の血中鉛濃度は0.05〜10mg/dl(幾何平均値、2.58mg/dl)であった[25]。この集団を12年間追跡した研究によると、血中鉛濃度1.94mg/dl未満群(平均年齢36.7歳)に比べ、3.62mg/dl以上群(平均年齢50.7歳)の全死因死亡リスクは1.25倍(95%信頼区間、1.04〜1.51)であり、心血管系死亡リスクは1.55倍(同、1.08〜2.24)であった。一方、鉛と無関係の論文の中で、睡眠が5時間以下の成人群の冠動脈性心疾患死亡リスクは、7時間睡眠の対照群と比べ、1.57倍(同、1.32〜1.88)高くなり、また9時間以上の睡眠群で1.79倍(同、1.48〜2.17)と発表されている[26]。すなわち、上の鉛論文では、平均年齢が14歳も違う集団の睡眠時間が交絡因子として調整されておらず、鉛と睡眠時間のいずれが死亡リスクに影響したのか、また血中鉛10〜40mg/dl間で曝露量の増加に伴い死亡リスクがさらに高くなるのか不明のままなのである。

6. おわりに

小児環境保健疫学調査は、化学物質の有害性に科学的根拠を提供し、日本の将来を担う次世代を環境中の脅威から守り、健やかに成長できるようにする小児環境保健政策として行われる国家事業である。このため、全国で一般公募される各ユニットセンターが集める出生コーホートを用いて本事業と直接関連しない研究を行うことを否定するものではないが、本事業の中断はあってはならない。参加を希望する大学・研究機関(研究者)はこの趣旨を理解された上、疫学調査を支援して頂きたいと思う。また、追跡期間は当面12年間であり、これを維持できる人的体制を構築することも求められる。かかる意味で、長期に及ぶこの調査の成功への鍵は、ユニットセンターの小児科、産婦人科、環境保健・公衆衛生の専門家チームの連携の持続性とともに、対象地域の市町村自治体とその住民の息の長い参加・協力が握っている。

参考文献

1) 村田勝敬・嶽石美和子、日衛誌、**60**, 4-14 (2005)
2) 佐藤洋(編)、Toxicology Today−中毒学から生体防御の科学へ、金芳堂、pp.93-108 (1994)
3) Cox C, et al., Environ Res, **49**, 318-332 (1989)
4) 浜本英次(編)、昭和30年8月岡山県における粉乳砒素中毒症発生記録、岡山県衛生部 (1957)

5) 小栗一太他(編)、油症研究－30年の歩み、九州大学出版会 (2000)
6) Dakeishi M, et al., Environ Health, **5**, 31 (2006)
7) Lai TJ, et al., Br J Psychiatry, **40** (Suppl), 49-52 (2001)
8) 有馬澄雄(編)、水俣病—20年の研究と今日の課題、青林舎 (1979)
9) Social Scientific Study Group on Minamata Disease. In the hope of avoiding repetition of a tragedy of Minamata disease. National Institute for Minamata Disease (2001)
10) Murata K, et al., Am J Ind Med, **50**, 764-771 (2007)
11) Hosie S, et al., Eur J Pediatr Surg, **10**, 304-309 (2000)
12) Klip H, et al., Lancet, **359**, 1102-1107 (2002)
13) National Research Council. Toxicological effects of mehtylmercury. National Academy Press (2000)
14) 岸玲子他、日衛誌、**63**, 285-292 (2008)
15) Nakai K, et al., Tohoku J Exp Med, **202**, 227-237 (2004)
16) Sasaki S, et al., Am J Epidemiol, **167**, 719-726 (2008)
17) Nakajima S, et al., Environ Health Perspect, **114**, 773-778 (2006)
18) Washino N, et al., Environ Health Perspect, **117**, 660-667 (2009)
19) Grandjean P, et al., Pediatrics, **117**, 546-556 (2006)
20) Canfield RL, et al., N Engl J Med, **348**, 1517-1526 (2003)
21) Lanphear BP, et al., Environ Health Perspect, **113**, 894-899 (2005)
22) Landrigan PJ, et al., Am J Ind Med, **50**, 709-711 (2007)
23) Kaji M., Biomed Res Trace Elements, **18**, 199-203 (2007)
24) International Programme on Chemical Safety, Inorganic lead, WHO (1995)
25) Menke A, et al., Circulation, **114**, 1388-1394 (2006)
26) Shankar A, et al., Am J Epidemiol, **158**, 1367-1373 (2008)

村田 勝敬 (むらた かつゆき)
秋田大学大学院医学系研究科社会環境医学系環境保健学講座 教授
(〒010-8543 秋田市本道1-1-1)
主な研究：ヒトの化学物質の曝露・影響評価

佐藤 洋 (さとう ひろし)
東北大学大学院医学系研究科環境保健医学 教授
(〒980-8575 宮城県仙台市青葉区星陵町2-1)

日衛誌 (Jpn. J. Hyg.), 64, 747-748 (2009)

特集 第78回日本衛生学会
公募シンポジウム10『環境と子どもの健康に関するコーホート研究の現状と課題』

特集「環境と子どもの健康に関するコーホート研究の現状と課題」

村田 勝敬, 那須 民江, 岸 玲子*

生殖次世代影響研究会世話人 (*代表)

平成20年3月に開催された第78回日本衛生学会総会で「環境と子どもの健康に関するコーホート研究の現状と課題」と題する生殖次世代影響研究会のシンポジウムが組まれた。そこで, わが国において出生コーホート研究の先鞭となった2つの調査, すなわち「東北コーホート調査」と「環境と子どもの健康に関する北海道スタディ」が紹介された。その後, これら調査の方法論, 測定された曝露指標と影響指標, 得られた証拠, そして今後の課題が議論された。多くのシンポジウム参加者の関心は環境省主導で行われる「小児環境保健疫学調査」がどのような形で一般公募されるのかに向けられていたが, わが国で実施されている2つの先行研究の現状と今後の課題を整理していく中で小児環境保健に求められる科学的本質も明示された。

子どもの健康や生活習慣は, それを取り巻く家族, 地域, 学校, 職場, 国などのあり方に強く規定される。これら社会環境の改善と良好な状態の維持は子どもの健全な発育・発達にとって不可欠である。しかしながら, 昨今の児童犯罪の報道等から察すると, 全ての子どもが健全な精神発達を遂げているとは想像しがたい。また, 産業構造が変化する中で, ヒトに対する安全性確認が十分に行われていない新たな化学物質や機器が巷間に出回り, 子どもを含めたヒトの健康を脅かしている。さらに, 地球温暖化は温熱曝露に脆弱な子どもや高齢者の生命危機の源となっている。この自然環境と社会環境からなる“環境”はヒトの生活空間を形成しているが, 産業革命以後激変し, 利便性や快適性の向上をもたらした反面, 様々な健康リスクも誘発しているように思われる。この時流にあって, 次世代を担う子どもの発育・発達に関わる環境中のマイナス要因を取り除くための試みが国際社会の中で進行している。その方策の一つが有害化学物質の同定とその健康影響の解明である。

胎児や乳幼児の臓器は脆弱であり, かつ外的因子に感受性が高く, このため環境からの曝露により種々の健康影響を受け易い。その上, その頃の神経毒性物質 (例えば, 鉛, メチル水銀) の曝露影響は一生涯残り得ることが報告されているし, PCBsやダイオキシン類など内分泌かく乱作用が疑われる化学物質の妊娠中曝露による発育・発達, アレルギー, 停留精巣, 矮小陰茎などの影響が示唆されている。一方, 多くの環境中の有害化学物質の曝露レベルは概して低濃度であり, 頻度の低い疾患の場合は臨床医による診断でその影響が見落とされるか, あっても原因不明の異常と片付けられてしまう可能性もある。逆に, 先天異常の見つけられた児において内分泌かく乱化学物質の測定を臨床医が行った報告も殆どない。子どもの健康に及ぼす有害化学物質の同定とその健康影響の解明のために, 我々が大規模な出生コーホート研究を必要としている理由はここにある。

例えば, 殺虫剤, 殺菌剤, 防カビ剤等の総称である農薬は単に農作物に噴霧・撒布されるだけでなく, 家庭菜園や観葉植物の病害虫駆除剤, シロアリ・ゴキブリ駆除剤, 虫除けスプレー等として我々の生活空間で多用されている。これらの毒性検査は動物実験結果のみで評価されているので, ヒトへの毒性は有害影響が実際に現れるまで不明である。また, 自閉症, アスペルガー症候群, 注意欠陥・多動性障害, 学習障害, 適応障害等の小児の精神発達障害や問題行動が近年注目されているが, 多くは原因不明であり, 一部に化学物質曝露の関与が示唆されているのみである。したがって, 妊娠中あるいは出生時の生体試料から化学物質を測定し, 現れる健康障害との因果関係を推定することは非常

に意義深い。ただ，これらの障害の診断基準の設定は難しくかつ診断者の違いによる測定誤差も無視できないので，産科，小児科および環境保健・公衆衛生の専門家チームの構築とその連携の持続性が重要な鍵となろう。

このたび，講演で語られた内容を総説として日本衛生学雑誌に特集されることとなりました。コーホート研究にさらなる関心を寄せて頂くとともに，わが国の環境リスク評価に寄せる環境省の熱い取り組みに注目して頂ければ幸いです。まもなくスタートする小児環境保健疫学調査は，化学物質の有害性に科学的根拠を提供することを目指しているものの，日本の将来を担う次世代を環境中の脅威から守り，健やかに成長できるようにする小児環境保健政策として行われる国家事業です。妊婦登録から始まり，その出生コーホートを12歳頃まで追跡するので，研究者にかかる負担も多いと予想されます。しかし，出生コーホート研究を継続することで，多くの仮説が証明されるとともに，新たな人間生物学的証拠も得られるかもしれません。世界に向けてこれらの情報を日本から発信して頂きたく思います。

Environmental Research 110 (2010) 699–704

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Neurobehavioral effects of prenatal exposure to methylmercury and PCBs, and seafood intake: Neonatal behavioral assessment scale results of Tohoku study of child development

Keita Suzuki [a,b,1], Kunihiko Nakai [a], Tomoko Sugawara [a], Tomoyuki Nakamura [a], Takashi Ohba [a], Miyuki Shimada [a,b], Toru Hosokawa [c], Kunihiro Okamura [d], Takeo Sakai [e], Naoyuki Kurokawa [a], Katsuyuki Murata [f], Chieko Satoh [a], Hiroshi Satoh [a,*]

[a] Environmental Health Sciences, Tohoku University, Graduate School of Medicine, 2-1 Seiryo-machi, Sendai, Miyagi 980-8576, Japan
[b] Japan Society for the Promotion of Science, Tokyo, Japan
[c] Human Development and Disabilities, Tohoku University, Graduate School of Education, Sendai, Japan
[d] Obstetrics and Reproductive Medicine, Tohoku University, Graduate School of Medicine, Sendai, Japan
[e] Miyagi Children's Hospital, Sendai, Japan
[f] Department of Environmental Health Sciences, Akita University, School of Medicine, Akita, Japan

ARTICLE INFO

Article history:
Received 5 October 2009
Received in revised form
22 June 2010
Accepted 2 July 2010
Available online 1 August 2010

Keywords:
Methylmercury
Polychlorinated biphenyls (PCBs)
Maternal seafood intake
Neurobehavioral function
Neonatal Behavioral Assessment Scale (NBAS)
Neonates

ABSTRACT

As factors affecting neonatal neurodevelopment, methylmercury, polychlorinated biphenyls (PCBs), and maternal seafood intake reflecting *n*-3 polyunsaturated fatty acids (PUFAs) are believed to have adverse or beneficial effects, but there are a few reports addressing such factors simultaneously. We carried out a birth cohort study to clarify the effects of these three factors on the Neonatal Behavioral Assessment Scale (NBAS), administered 3 days after birth. In a total of 498 mother–neonate pairs, the total mercury level (median, 1.96 μg/g) in maternal hair at parturition and the ∑PCB level (45.5 ng/g-lipid) in cord blood were analyzed, and maternal seafood intake was estimated using a semi-quantitative food frequency questionnaire. A negative relationship between the hair mercury level and the motor cluster of NBAS was observed, even after adjusting for PCBs, maternal seafood intake, and possible confounders such as maternal age, birth weight, and parity. The ∑PCB level was negatively correlated with the motor cluster, but this association was attenuated after adjusting for mercury and the confounders. There was seen to be a positive association between maternal seafood intake and the motor cluster when considering the effects of mercury and PCBs. In conclusion, our data suggest that prenatal exposure to methylmercury adversely affects neonatal neurobehavioral function; in contrast, maternal seafood intake appears to be beneficial. The neurobehavioral effect of prenatal exposure to PCBs remains unclear in our study. Further research is necessary to elucidate interactive effects of methylmercury, PCBs, and *n*-3 PUFAs, originating from fish, on child neurodevelopment.

1. Introduction

Methylmercury (MeHg), synthesized in the natural environment, is known to be a widespread environmental neurotoxicant (**National Research Council, 2000**). In aquatic ecosystems, the MeHg is bioaccumulated and bioconcentrated as it passes up the aquatic food chain. All fish contain some MeHg and vertebrates (fish and sea mammals) at the top of the food chain contain larger quantities. For this reason, concern had long been expressed for populations consuming large amounts of fish and sea mammals, and special attention was directed to the developmental effect of prenatal exposure. Then, several birth cohort studies were conducted to elucidate the effect. In a study carried out in the Faroe Islands, over 900 children were followed to 14 years of age, and the associations of increased cord blood mercury with lower scores of several neurodevelopmental parameters were demonstrated (**Grandjean et al., 1997; Debes et al., 2006**). Another study in the Seychelles, by contrast, did not support such associations between maternal hair mercury at parturition and developmental performances in more than 700 children followed from 6 to 107 months of age (**Davidson et al., 1995, 1998; Myers et al., 2003**), although the average mercury levels in maternal hair at parturition were somewhat higher in the Seychelles (6.8 μg/g) than in the Faroe Islands (4.3 μg/g).

Polychlorinated biphenyls (PCBs) have been reported to have adverse effects on neurobehavioral development (**Rogan et al.,**

* Corresponding author. Fax: +81 22 717 8106.
E-mail address: h.satoh@ehs.med.tohoku.ac.jp (H. Satoh).
[1] Currently at Faculty of Education, Kochi University, Kochi, Japan.

0013-9351/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2010.07.001

1986; Jacobson and Jacobson, 1996; Patandin et al., 1999; Darvill et al., 2000; Walkowiak et al., 2001; Sagiv et al., 2008). Since PCBs accumulate in fish and sea mammals, there can be concomitant exposures to PCBs and MeHg in fish-eating populations. Indeed, Faroese children had been exposed to PCBs *in utero*, along with MeHg, via maternal dietary intake of seafood (Grandjean et al., 2001). Also, these neurotoxicants may have an additive or synergistic effect mutually (Weihe et al., 1996; Fischer et al., 2008). A recent animal study reported that coexposure to both PCB (PCB153) and MeHg could induce motor behavioral defects in rats (Roegge et al., 2004), but another study did not find such interactive effects on neurobehavioral development in mice (Sugawara et al., 2008). Thus, the effect of coexposure to MeHg and PCBs on child neurodevelopment has not always been conclusive.

Fish supply *n*-3 polyunsaturated fatty acids (PUFAs). Since *n*-3 PUFAs cannot be synthesized in humans, we must obtain them from our diet (Cohen et al., 2005). Several studies demonstrated the beneficial effect of fish intake or *n*-3 PUFA supplementation during pregnancy on child development (Helland et al., 2003; Colombo et al., 2004; Bouwstra et al., 2006; Hibbeln et al., 2007). Two cohort studies in U.S. and England also reported that maternal fish intake was associated with higher scores in neurodevelopmental outcomes (Daniels et al., 2004; Oken et al., 2005, 2008), though there was disagreement concerning the adverse effect of prenatal exposure to MeHg on child neurodevelopment. Such results may have been due to the assumption that the nutritive factors of fish, presumably *n*-3 PUFAs, can compensate for the detrimental effect of neurotoxins such as MeHg or PCBs.

Japanese diet relies heavily on steamed rice, seafood, and vegetables. Since the Japanese eat a great amount of fish, they are exposed to both MeHg and PCBs from fish products (Iwasaki et al., 2003; Mato et al., 2007). Also, when a workshop on scientific issues relevant to assessment of health effects from exposure to MeHg was held in 1998, the committee developed a series of questions (Committee on Environment and Natural Resources, Office of Science and Technology Policy and the White House, 1999). One of them was "What are the confounders that affect health endpoints positively (e.g., [selenium], omega-3 fatty acids) and negatively (e.g., PCBs; alcohol use; health conditions with neurological effects, such as diabetes)?" Therefore, we have performed a birth cohort study (Tohoku study of child development, TSCD) focusing on both the potential risks and benefits of fish eating during pregnancy to clarify the effects of neurotoxicants such as MeHg and PCBs on child development in Japan (Nakai et al., 2004). In this study, we conducted the Neonatal Behavioral Assessment Scale (NBAS) test in neonates and examined the concomitant effects of prenatal exposure to MeHg and PCBs, as well as of the maternal seafood intake, on neurobehavioral function.

2. Materials and methods

2.1. Cohort establishment

The study protocol of TSCD has been described elsewhere (Nakai et al., 2004). The study has been carried out in an urban area and a coastal area in the Tohoku district of Japan. In the present study, we employed the study population in the urban area. We initially recruited 1500 pregnant women making antenatal visits to obstetric wards of hospitals. Eligibility criteria included a singleton pregnancy and Japanese as the mother tongue. To establish an optimal study population, only neonates born at term (36–42 weeks of gestation) without congenital anomalies or diseases were included. In addition, only neonates with a birth weight of 2400 g or more were included.

We enrolled 687 women (45.8%) with their written informed consent to participate in the study between January 2001 and September 2003. All women delivered by March 2004, and 599 mother–neonate pairs were registered in the cohort study according to the eligibility criteria. Finally, we obtained complete information on mercury analysis, PCBs analysis, maternal seafood intake, the NBAS, and maternal/neonate characteristics from 498 mother–neonate pairs.

2.2. Measurement of exposure

Maternal hair samples were obtained from the back of the head near the occipital protuberance 2 days after delivery, and 3-cm hair close to the scalp was used for analysis of the total mercury (THg) level to avoid the effect of permanent waving (Ohba et al., 2008). THg levels in maternal hair were measured by cold vapor atomic absorption spectrophotometry (CVAAS, HG-201, Sanso Seisakusho Co. Ltd., Tokyo, Japan) according to the method described by Akagi and Nishimura (1991). The method involves sample digestion with HNO_3, $HClO_4$, and H_2SO_4 (1:1:5) at 200–230 °C for 30 min, followed by reduction to elemental Hg vapor by adding 10% $SnCl_2$. The limit of detection (LOD) was less than 0.1 ngHg. The precision and accuracy of THg measurement in maternal hair was assured by interlaboratory calibration exercises with the National Institute for Minamata Disease (NIMD: Minamata, Japan). Hair samples and the standard reference material for hair, NIES CRM No. 13 human hairs (National Institute for Environmental Studies, Ministry of the Environment: Tsukuba, Japan) were repeatedly analyzed and verified at the NIMD.

Cord blood samples were collected immediately after birth, and stored at −80 °C until the analysis. They were analyzed for all 209 PCB congeners by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using the isotope dilution method. Laboratory analytical methods and quality control procedures were described elsewhere (Nakamura et al., 2008): All sample analyses were performed by IDEA Consultants, Inc. (Tokyo, Japan) and Shimadzu Techno-Research, Inc. (Kyoto, Japan). The quality of PCB analyses in the two laboratories was validated using an external quality assurance program, the German external assurance scheme at IDEA Consultants, Inc., and the Fukuoka Institute of Health and Environmental Sciences at Shimadzu Techno-Research, Inc. We also assured the analytical quality of the two laboratories by comparing the analytical results for the same samples. The results of two laboratories did not show significant differences (paired t test, $t=2.5$, $p>0.05$), and showed high correlations (Pearson product–moment correlation coefficient $r=0.87$). Therefore, it was thought that the two sets of the data were comparable. The calculated LOD was 0.03 pg/g-wet, which was identified by the signal-to-noise ratio. The amount of congeners below the LOD was set at zero. We used the sum of all the measured congeners (209 congeners, ∑PCB), expressed as ng/g-lipid.

Thyroid functions, including thyroxin (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH), were measured in cord plasma samples by SRL Inc. (Tokyo, Japan) using the ECLusys electrochemiluminescence immunoassay on a Modular Analytics analyzer (Roche Diagnostics K.K., Tokyo).

2.3. Maternal seafood intake

Maternal fish/seafood intake during pregnancy was assessed using a food frequency questionnaire (FFQ) that was administered by trained interviewers 4 days after delivery. Detailed information on eating frequency and the amounts of 13 specific seafood types was collected. The specific 13 seafood types were tuna, bonito, whale, salmon, eel, yellowtail, silvery blue fish (which included sardines, saury, and mackerel), white meat fish (which included cod, bream, and flat fish), and other seafood types (which included sweetfish, conger, and sierra, squid and octopus, shellfish, salmon roe, and canned tuna). These seafood types were selected from fish and shellfish that were often found at the fish market in the study area, and it is thought that they are representative of almost all the varieties of seafood/shellfish consumed in this area (Yaginuma-Sakurai et al., 2009). We used the total seafood intake (g/year), which was calculated as the sum of the intake of the 13 seafood types.

2.4. Maternal and neonatal characteristics

Information was obtained about demographics, cigarette smoking and alcohol consumption during pregnancy through interviews and a questionnaire 4 days after delivery. Information on the delivery condition and neonatal characteristics such as gestational age, delivery type, sex, birth weight, and Apgar scores 1 and 5 minutes after delivery was obtained from medical records before discharge from the hospital.

2.5. Neonatal neurobehavioral assessment

Neurobehavioral function was examined with the NBAS. The scale combines neurological items with an extended behavioral repertoire of the neonates in an interactional process (Brazelton and Nugent, 1995). The basic score is composed of 28 behavioral items and 18 reflex items. To compare the evaluations of the neonate's behavior, items that interact in similar ways have been classified into

seven clusters describing global functions (**Lester et al., 1982**). (1) habituation, which includes items assessing the infant's reactivity to stimulation from a rattle, bell, light, and mild pin pick, followed by response decrement while in a light sleep state; (2) orientation, which includes attention to visual and auditory stimuli during alert states; (3) motor, which measures the quality of muscle tone and movement; (4) range of state, which includes items related to the level of arousal; (5) regulation of state, which reflects the quality of the infant's responses when aroused and ability to control arousal in response to environmental stimulation; (6) autonomic stability, which includes items assessing physiologic responses to stress; and (7) reflexes, which reflects the number of abnormally elicited reflexes. Higher scores in the 6 clusters other than the reflex cluster indicate a better behavioral response, whereas higher scores for the reflex cluster indicate a more abnormal response.

The NBAS was administrated when neonates were 3 days of age. All examinations took place in a quiet room under predetermined lighting, and temperature conditions. Examinations were also performed under predetermined timing in the day. Examiners were blinded to the exposure status of the participants. Neonatal assessments were performed by six examiners trained in administration and scoring according to the inter-rater reliability criteria established by the NBAS manual (**Brazelton and Nugent, 1995**). The mean weighted κ statistics of inter-rater reliability for examiners was 0.88. Moreover, these examiners for the NBAS were certified to administer the NBAS by the Training Center for the NBAS in the Nagasaki University School of Health Sciences in Japan.

2.6. Statistical analysis

The Pearson product–moment correlation coefficient (r) was obtained to examine the relationship between the NBAS clusters and exposure markers of the hair THg level, cord blood $\sum$PCB level, and maternal seafood intake. These exposure markers were normalized by logarithmic transformation. When the above coefficient was statistically significant, multiple regression analysis was used to adjust for possible confounders. The NBAS clusters were set as dependent variables, and hair THg and cord blood $\sum$PCB were set as independent variables (model 1). Then, total seafood intake was added to the independent variables (model 2), and finally confounders were also added into the independent variables (model 3). Possible confounders were selected from maternal and neonatal characteristics (**Table 1**). There were strong correlations between birth weight and length at birth ($r=0.71$, $p<0.05$), and between the T3 and T4 level in cord blood ($r=0.46$, $p<0.05$). Considering multicollinearity, length at birth and the T4 level in cord blood were excluded from the confounders. Six examiners of the NBAS and two institutes performing PCB analyses were adjusted in the above three models. All analyses with two-sided p values (significance level, $p=0.05$) were performed using the SPSS 17.0 (SPSS Inc., Tokyo, Japan).

3. Results

Information about maternal and neonatal characteristics in the 498 mother–neonate pairs is shown in **Table 1**. Forty (8.0%) mothers smoked during pregnancy, and 156 (31.3%) drank alcoholic beverages at least once a month during pregnancy.

Table 1
Characteristics of the study subjects.

	No. (%)	Mean	SD
Maternal characteristics			
Maternal age (years)	498	31.4	4.3
Body weight (before pregnancy) (kg)	498	52.9	7.9
Body height (cm)	498	158.7	4.8
Body Mass Index (kg/m^2)	498	21.0	2.8
Education ($\leq$12 years)	122 (24.5)		
Alcohol drinking during pregnancy (yes)	156 (31.3)		
Cigarette smoking during pregnancy (yes)	40 (8.0)		
Delivery type (Spontaneous)	364 (73.1)		
Parity (first)	255 (51.2)		
Total energy intake (kcal/day)	498	2344	707
Neonatal characteristics			
Sex (boy)	257 (51.6)		
Gestational age (weeks)	498	39.5	1.2
Birth weight (kg)	498	3081.9	331.4
Length at birth (cm)	498	49.0	1.8
Apgar score 1 min after birth	498	8.2	0.8

Table 2
Exposure level, maternal seafood intake, and thyroid function in 498 mother–neonate pairs.

	Median	Mean	SD	Min–Max
Hair THg (μg/g)	1.96	2.22	1.16	0.29–9.35
Cord blood $\sum$PCB (ng/g-lipid)	45.5	52.4	32.6	7.0–273.8
Total seafood intake (g/year)	15,890	18,576	12,693	15–113,632
T3 level in cord blood (ng/dL)	0.50	0.53	0.15	0.28–1.82
T4 level in cord blood (μg/dL)	8.53	8.66	1.38	5.20–17.7
TSH level in cord blood (mU/L)	8.96	11.30	7.91	1.71–55.9

Hair THg: total mercury in maternal hair
$\sum$PCB: sum of all 209 congeners in cord whole blood.
Total seafood intake: sum of 13 seafood type intake assessed by the FFQ.

The mean gestational age at birth and mean birth weight were 39.5 weeks and 3.08 kg, respectively.

The distributions for the mercury level, $\sum$PCB level, maternal seafood intake, as well as thyroid function are presented in **Table 2**. The median THg level was 1.96 μg/g in maternal hair, similar to data of Japanese pregnant and non-pregnant women (**Sakamoto et al., 2007; Yasutake et al., 2003**). The medians were 45.5 ng/g-lipid for $\sum$PCB level in cord blood, and 15,890 g/year for total seafood intake. According to the FFQ, 16 mothers of the 498 mother–neonate pairs ate whale meat during pregnancy and the median of cord blood $\sum$PCB levels in their neonates was 43.5 (range, 7.0–79.0) ng/g-lipid. The mean levels of T3, T4 and TSH in cord blood were 0.53 ng/dL, 8.66 μg/dL, and 11.3 mU/L, respectively. The T3 level had a positive correlation with $\sum$PCB ($r=0.139$, $p=0.02$) and the TSH level seemed to decrease with increasing $\sum$PCB ($r=-0.078$, $p=0.09$).

Table 3 presents the Pearson product–moment correlation coefficients between seven clusters of the NBAS and exposure markers of hair THg, $\sum$PCB, and total seafood intake. The motor cluster was negatively correlated with hair THg ($p=0.01$) and $\sum$PCB ($p=0.002$). In contrast, a marginally positive correlation between the motor cluster and total seafood intake was found ($p=0.1$). Positive correlations between $\sum$PCB and the orientation cluster ($p=0.03$), and $\sum$PCB and the cluster of regulation of state were found ($p=0.02$).

We focused on the motor cluster that had close relations to hair THg and $\sum$PCB in the simple correlation analysis (**Table 3**), and performed multiple regression analysis (**Table 4**). In every model, maternal hair THg was negatively related to the motor cluster ($p=0.047$ in model 1; $p=0.01$ in model 2; and, $p=0.01$ in model 3). $\sum$PCB tended to relate negatively to the motor cluster in models 1 and 2 ($p=0.07$ and 0.07, respectively), whereas this relationship was not observed in model 3 ($p>0.1$). Total seafood intake was positively related to the motor cluster in model 2 ($p=0.03$), whereas no significant relationship was observed in model 3 ($p>0.1$). Of the confounders, maternal age, birth weight, and parity showed significant relations to the motor cluster of the NBAS (standardized $\beta=-0.165$, 0.201 and 0.136, respectively). As for the orientation cluster and the cluster of regulation of state, no significant relationship with $\sum$PCB, hair THg or total seafood intake was observed in any model.

4. Discussion

This may be the first report addressing the neurobehavioral effects of prenatal exposure to MeHg, PCBs, and seafood intake in Japanese neonates. Results of the present study indicated that prenatal exposure to MeHg at maternal hair levels of less than 10 μg/g had a negative relation to one cluster of the NBAS (i.e., motor cluster), whereas the impact of PCBs was not confirmative.

Table 3
Correlations between seven clusters of the NBAS and exposure markers (maternal hair total mercury (THg), cord blood ∑PCB, and total seafood intake)

	Scores Mean ± SD	Pearson product–moment correlations with seven clusters of the NBAS		
		Hair THg (μg/g)	Cord blood ∑PCB (ng/g-lipid)	Total seafood intake (g/year)
Habituation	6.67 ± 1.49	0.010	0.039	-0.034
Orientation	4.42 ± 1.13	0.058	0.100*	-0.042
Motor	4.69 ± 0.64	-0.126*	-0.140*	0.073[a]
Range of state	3.91 ± 0.77	0.076	-0.043	0.012
Regulation of state	4.15 ± 1.15	-0.008	0.102*	0.011
Autonomic stability	5.86 ± 0.96	0.003	-0.066	0.030
Reflex	2.66 ± 2.00	0.068	0.030	-0.026

THg, ∑PCB, and total seafood intake were logarithmically transformed.

* $p < 0.05$.

[a] $p = 0.10$

Table 4
Relations of motor cluster of NBAS to hair THg, cord blood ∑PCB, total seafood intake, and confounders: results of multiple regression analysis.

	Model 1[b]	Model 2[c]	Model 3[d]
R^2 of the model	0.070*	0.079*	0.151*
Standardized β coefficient			
Hair THg (μg/g)[a]	−0.090*	−0.119*	−0.116*
Cord blood ∑PCB (ng/g-lipid)[a]	−0.083+	−0.084+	0.034
Total seafood intake (g/year)[a]		0.102*	0.078

All models were adjusted for six examiners of the NBAS and two institute of the PCB analysis.

* $p < 0.05$, $+0.05 < p < 0.10$.

[a] Logarithmic transformation, $\log_{10}X$, was used on ∑PCB in cord blood, THg in hair, and total seafood intake.

[b] Model included hair THg and cord blood ∑PCB.

[c] Model included hair THg, cord blood ∑PCB, and total seafood intake.

[d] Model included hair THg, cord blood ∑PCB, and total seafood intake, and confouders. Confounders were maternal age, maternal BMI, education (≤ 12 years, > 13 years) , alcohol drinking during pregnancy (no, yes), cigarette smoking during pregnancy (no, yes), delivery type (spontaneous, others), parity (first, second and more), total energy intake, T3 and TSH level in cord blood, child's sex (girl, boy), gestational age, birth weight, and Apgar score 1 min after birth.

Also, maternal seafood intake, that is the primary route of exposure to MeHg and PCBs, seemed to have a positive relation to the same cluster. These findings suggest that maternal seafood intake during pregnancy may bring not only benefits but also risks to neurobehavioral function in neonates.

In this study, a negative association between maternal hair THg and the motor cluster of the NBAS was observed while the THg level in maternal hair (median 1.96 μg/g) was lower than those in the Faroe Islands and Seychelles (**Grandjean et al., 1992**; **Davidson et al., 1995**). This significant association remained preserved even after adjusting for PCBs, maternal seafood intake, and other confounders. The motor function was more discernible to trained examiners than other functions, and the dysfunction such as deficits in finger tapping speed and reaction time on a continued performance task was associated with prenatal MeHg exposure indicators (**Debes et al., 2006**), implying that the motor cluster of the NBAS may be more sensitive and reliable endpoint in neonates than other clusters. Also, retarded walking seemed to be more susceptible to MeHg exposure than central nervous signs (**Cox et al., 1989**). Additionally, this finding is similar to the following three reports, though the measured endpoint and age were different among the study populations: an increase of the cord-blood THg concentration was associated with a decreased neurologic optimality score of the Prechtl neurologic examination at 2 weeks of age (**Steuerwald et al., 2000**); and, cognitive effects of prenatal exposure to MeHg were found at 6 months of age in the U.S. (**Oken et al., 2005**) and at 12 months of age in Poland (**Jedrychowski et al., 2006**). In Seychellois children aged 6 months (**Davidson et al., 1995**) or in British children aged 18 months (**Daniels et al., 2004**), however, there was no consistent relationship between prenatal exposure to MeHg and cognitive function. Still, the Seychelles child development study did not use either PCBs or maternal seafood intake as a crucial confounder (**Davidson et al., 1995, 1998**; **Myers et al., 2003, 2006**). Taken together, prenatal exposure to MeHg, even at low doses, appears to impair neurobehavioral function in neonates.

The cord blood ∑PCB levels of the current study are similar to those in Japanese pregnant women (**Todaka et al., 2008**; **Kawashiro et al., 2008**) and seem to be considerably lower than in the Faroese Islands study (**Grandjean et al., 2001**; **Longnecker et al., 2003**). Sixteen mothers of our cohort would have eaten whale meat, but not blubber, during pregnancy because the cord blood ∑PCB levels in their neonates were close to the population mean. As shown in **Table 3**, a negative association between the motor cluster score and cord blood PCBs was apparently seen, but it disappeared when adjusting for all possible confounders including maternal hair THg (**Table 4**). A similar finding was observed in Faroese children at age 7 years (**Grandjean et al., 2001**); i.e., the cord PCBs were associated with deficits on the Boston naming test and the continuous performance test reaction time, but the association of PCBs was not significant after the adjustment for mercury concentration. Although many studies have demonstrated the adverse effects of prenatal exposure to PCBs on child neurodevelopment (**Rogan et al., 1986**; **Jacobson and Jacobson, 1996**; **Patandin et al., 1999**; **Boersma and Lanting, 2000**; **Darvill et al., 2000**; **Sagiv et al., 2008**), most of them have not taken MeHg exposure into account as a confounder or effect modifier. Accordingly, the adjustment for MeHg, other than the selection of cognitive assessment methods (**Boucher et al., 2009**), should be crucial in assessing the neurodevelopmental impact of prenatal exposure to chemical substances such as PCBs and dioxins, because such effects are generally subtle (**Longnecker et al., 2003**).

In this study, a marginally significant association between the motor cluster and maternal seafood intake was observed in correlation analysis ($p = 0.1$), but the association became significant in multiple regression analysis including hair THg and ∑PCB ($p < 0.05$ in model 2). This result is almost concordant with one report (**Oken et al., 2005**), which detected a positive effect of fish intake on offspring cognitive function in a statistical model taking mercury exposure into account. In this way, nutritive factors, including *n*-3 PUFAs, can contribute to neonatal neurobehavioral function.

In the present study, interactive effects of THg in maternal hair, ∑PCB in cord blood, and maternal seafood intake (a surrogate of *n*-3 PUFA), on neonatal neurodevelopment were confirmed using multivariate analysis, as mentioned above. The Faroes birth

cohort study used maternal hair THg and cord blood THg as exposure variables and whale and/or fish intake as confounders in structural equation model analysis, and demonstrated the adverse effect of prenatal exposure to MeHg on neurobehavioral endpoints (**Budtz-Jørgensen et al., 2002, 2007**). Similarly, in the Seychelles child development nutrition study with 229 mother–infant pairs, **Strain et al. (2008)** observed not only a significant correlation coefficient (r=0.31) between maternal hair mercury and n-3 PUFA, but also a significant adverse association between prenatal MeHg and 30-month psychomotor developmental index of the Bayley Scales of Infant Development when the n-3 PUFA measures were included in the regression analysis. **Davidson et al. (2008)** also suggested a possible confounding role of maternal nutrition in studies examining associations between prenatal MeHg exposures and developmental outcomes in children. Thus, in assessing subtle health effects of MeHg exposure at low levels in children, different from at high levels (e.g., Minamata disease and Iraqi methylmercury poisoning), special attention should be paid to all crucial factors affecting the endpoints positively and negatively.

There may have been potential limitations in the current study. We could not directly analyze the level of n-3 PUFAs because of the limited specimens. Nor did we employ any markers of exposure to dioxins, pesticides, or heavy metals such as arsenic. A low level of lead in cord blood was detected, but no significant associations were found between lead and any cluster of the NBAS (data not shown). Concerning the covariates or confounders affecting the endpoints, we included possible confounders in the data analysis, such as maternal age, birth weight, and thyroid related hormones (**Table 3**). Mean scores of seven NBAS clusters, employed in our study, were similar to other reports in Japan (**Akiyama et al., 2001; Loo et al., 2005**) and our inter-rater reliability was relatively high (weighted κ statistics=0.88). Therefore, it is suggested that our data was not heavily influenced by such confounders or measurement bias.

In conclusion, our findings suggest that prenatal exposure to MeHg adversely affects neonatal neurobehavioral function; in contrast, maternal seafood intake during gestation appears to carry benefits. The neurobehavioral effect of prenatal exposure to PCBs remains unconfirmed in this study. Further research is required to elucidate the interactive effects of MeHg, PCBs, and n-3 PUFAs on child neurodevelopment directly, because a significant association was observed only between prenatal exposure to MeHg and one cluster of NBAS in this study and fish contain such beneficial, but also toxic, substances (**Sakamoto et al., 2004**).

Funding source

This research was funded by the Japan Ministry of Health, Labor and Welfare and the Ministry of the Environment. The funding sources had no role in the study protocol, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication. For this reason, the findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the above government agencies. All procedures of this study were approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine.

Acknowledgments

We thank all the families for their participation in the cohort study. We would like to acknowledge all the staff members of Environmental Health Sciences, Tohoku University Graduate School of Medicine, for their help with the organization of data collection for the cohort study.

References

Akagi, H., Nishimura, H., 1991. Specification of mercury in the environment. In: Suzuki, T., Imura, N., Clarkson, T.W. (Eds.), Advances in Mercury Toxicology. Plenum Press, New York, pp. 53–76.

Akiyama, T., Ogi, S., Takahashi, T., Fukuda, M.., 2001. The neonatal behavioral assessment scale (NBAS) as a predictor of cerebral palsy among low birth weight infants. Jpn. J. Rehabil. Med. 38, 211–218 in Japanese.

Boersma, E.R., Lanting, C.I., 2000. Environmental exposure to polychlorinated biphenyls (PCBs) and dioxins. Consequences for longterm neurological and cognitive development of the child lactation. Adv. Exp. Med. Biol. 478, 271–287.

Boucher, O., Muckle, G., Bastien, C.H., 2009. Prenatal exposure to polychlorinated biphenyls: a neuropsychologic analysis. Environ. Health Perspect. 117, 7–16.

Bouwstra, H., Dijck-Brouwer, J., Decsi, T., Boehm, G., Boersma, E.R., Muskiet, F.A., Hadders-Algra, M., 2006. Neurologic condition of healthy term infants at 18 months: positive association with venous umbilical DHA status and negative association with umbilical trans-fatty acids. Pediatr. Res. 60, 334–339.

Brazelton, T.B., Nugent, J.K., 1995. Neonatal Behavioral Assessment Scale 3rd ed. Mac Keith Press, London.

Budtz-Jørgensen, E., Keiding, N., Grandjean, P., Weihe, P., 2002. Estimation of health effects of prenatal mercury exposure using structural equation models. Environ. Health 1, 2.

Budtz-Jørgensen, E., Grandjean, P., Weihe, P., 2007. Separation of risks and benefits of seafood intake. Environ. Health Perspect. 115, 323–327.

Cohen, J.T., Bellinger, D.C., Connor, W.E., Kris-Etherton, P.M., Lawrence, R.S., Savitz, D.A., Shaywitz, B.A., Teutsch, S.M., Gray, G.M., 2005. A quantitative risk-benefit analysis of changes in population fish consumption. Am. J. Prev. Med. 29, 325–334.

Colombo, J., Kannass, K.N., Shaddy, D.J., Kundurthi, S., Maikranz, J.M., Anderson, C.J., Blaga, O.M., Carlson, S.E., 2004. Maternal DHA and the development of attention in infancy and toddlerhood. Child Dev. 75, 1254–1267.

Committee on Environment and Natural Resources, Office of Science and Technology Policy and the White House, 1999. Report of the Workshop on Scientific Issues Relevant to Assessment of Health Effects from Exposure to Methylmercury. National Institute of Environmental Health Science, Research Triangle Park.

Cox, C., Clarkson, T.W., Marsh, D.O., Amin-Zaki, L., Tikriti, S., Myers, G.J., 1989. Dose-response analysis of infants prenatally exposed to methylmercury. An application of a single compartment model to single-strand hair analysis. Environ. Res. 31, 640–649.

Daniels, J.L., Longnecker, M.P., Rowland, A.S., Golding, J., 2004. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology 15, 394–402.

Darvill, T., Lonky, E., Reihman, J., Stewart, P., Pagano, J., 2000. Prenatal exposure to PCBs and infant performance on the Fagan test of infant intelligence. Neurotoxicology 21, 1029–1038.

Davidson, P.W., Myers, G.J., Cox, C., Shamlaye, C., Choisy, O., Sloane-Reeves, J., Cernichiari, E., Marsh, D.O., Berlin, M., Tanner, M., Clarkson, T.W., 1995. Neurodevelopmental test selection, administration, and performance in the main Seychelles child development study. Neurotoxicology 16, 665–676.

Davidson, P.W., Myers, G.J., Cox, C., Axtell, C., Shamlaye, C., Sloane-Reeves, J., Cernichiari, E., Needham, L., Choi, A., Wang, Y., Berlin, M., Clarkson, T.W., 1998. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles child development study. JAMA 280, 701–707.

Davidson, P.W., Strain, J.J., Myers, G.J., Thurston, S.W., Bonham, M.P., Shamlaye, C.F., Stokes-Riner, A., Wallace, J.M., Robson, P.J., Duffy, E.M., Georger, L.A., Sloane-Reeves, J., Cernichiari, E., Canfield, R.L., Cox, C., Huang, L.S., Janciuras, J., Clarkson, T.W., 2008. Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy. Neurotoxicology 29, 767–775.

Debes, F., Budtz-Jørgensen, E., Weihe, P., White, R.F., Grandjean, P., 2006. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. Neurotoxicol. Teratol. 28, 536–547.

Fischer, C., Fredriksson, A., Eriksson, P., 2008. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244, 157–165.

Grandjean, P., Weihe, P., Jørgensen, P.J., Clarkson, T., Cernichiari, E., Viderø, T., 1992. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch. Environ. Health 47, 185–195.

Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sorensen, N., Dahl, R., Jorgensen, P.J., 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 19, 417–428.

Grandjean, P., Weihe, P., Burse, V.W., Needham, L.L., Storr-Hansen, E., Heinzow, B., Debes, F., Murata, K., Simonsen, H., Ellefsen, P., Budtz-Jørgensen, E., Keiding, N., White, R.F., 2001. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol. Teratol. 23, 305–317.

Helland, I.B., Smith, L., Saarem, K., Saugstad, O.D., Drevon, C.A., 2003. Maternal supplementation with very-long-chain *n*-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 111, e39–44.

Hibbeln, J.R., Davis, J.M., Steer, C., Emmett, P., Rogers, I., Williams, C., Golding, J., 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 369, 578–585.

Iwasaki, Y., Sakamoto, M., Nakai, K., Oka, T., Dakeishi, M., Iwata, T., Satoh, H., Murata, K., 2003. Estimation of daily mercury intake from seafood in Japanese women: Akita cross-sectional study. Tohoku J. Exp. Med. 200, 67–73.

Jacobson, J.L., Jacobson, S.W., 1996. Intellectual impairment in children exposed to polychlorinated biphenyls *in utero*. N. Engl. J. Med. 335, 783–789.

Jedrychowski, W., Jankowski, J., Flak, E., Skarupa, A., Mroz, E., Sochacka-Tatara, E., Lisowska-Miszczyk, I., Szpanowska-Wohn, A., Rauh, V., Skolicki, Z., Kaim, I., Perera, F., 2006. Effects of prenatal exposure to mercury on cognitive and psychomotor function in one-year-old infants: epidemiologic cohort study in Poland. Ann. Epidemiol. 16, 439–447.

Kawashiro, Y., Fukata, H., Omori-Inoue, M., Kubonoya, K., Jotaki, T., Takigami, H., Sakai, S., Mori, C., 2008. Perinatal exposure to brominated flame retardants and polychlorinated biphenyls in Japan. Endocrine Journal (Japan) 55, 1071–1084.

Lester, B.M., Als, H., Brazelton, T.B., 1982. Regional obstetric anesthesia and newborn behavior: a reanalysis toward synergistic effects. Child Develop. 53, 687–692.

Longnecker, M.P., Wolff, M.S., Gladen, B.C., Brock, J.W., Grandjean, P., Jacobson, J.L., Korrick, S.A., Rogan, W.J., Weisglas-Kuperus, N., Hertz-Picciotto, I., Ayotte, P., Stewart, P., Winneke, G., Charles, M.J., Jacobson, S.W., Dewailly, E., Boersma, E.R., Altshul, L.M., Heinzow, B., Pagano, J.J., Jensen, A.A., 2003. Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. Environ. Health Perspect. 111, 65–70.

Loo, K., Ohgi, S., Zhu, H., Howard, J., Chen, L., 2005. Cross-cultural comparison of the neurobehavioral characteristics of Chinese and Japanese neonates. Pediatr. Int. 47, 446–451.

Mato, Y., Suzuki, N., Katatani, N., Kadokami, K., Nakano, T., Nakayama, S., Sekii, H., Komoto, S., Miyake, S., Morita, M., 2007. Human intake of PCDDs, PCDFs, and dioxin like PCBs in Japan, 2001 and 2002. Chemosphere 67, S247–S255.

Myers, G.J., Davidson, P.W., Cox, C., Shamlaye, C.F., Palumbo, D., Cernichiari, E., Sloane-Reeves, J., Wilding, G.E., Kost, J., Huang, L.S., Clarkson, T.W., 2003. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 361, 1686–1692.

Myers, G.J., Davidson, P.W., Shamlaye, C.F., 2006. Developmental disabilities following prenatal exposure to methyl mercury from maternal fish consumption: a review of the evidence. In: Davidson, P.W., Myers, G.J., Weiss, B. (Eds.), Neurotoxicity and Developmental Disabilities. Elsevier Academic Press, San Diego, pp. 141–169.

Nakai, K., Suzuki, K., Oka, T., Murata, K., Sakamoto, M., Okamura, K., Hosokawa, T., Sakai, T., Nakamura, T., Saito, Y., Kurokawa, N., Kameo, S., Satoh, H., 2004. The Tohoku study of child development: a cohort study of effects of perinatal exposures to methylmercury and environmentally persistent organic pollutants on neurobehavioral development in Japanese children. Tohoku J. Exp. Med. 202, 227–237.

Nakamura, T., Nakai, K., Matsumura, T., Suzuki, S., Saito, Y., Satoh, H., 2008. Determination of dioxins and polychlorinated biphenyls in breast milk, maternal blood and cord blood from residents of Tohoku, Japan. Sci. Total Environ. 394, 39–51.

National Research Council, 2000. Toxicological Effects of Methylmercury. National Academic Press, Washington, DC.

Ohba, T., Kurokawa, N., Nakai, K., Shimada, M., Suzuki, K., Sugawara, N., Kameo, S., Satoh, C., Satoh, H., 2008. Permanent waving does not change mercury concentration in the proximal segment of hair close to scalp. Tohoku J. Exp. Med. 214, 69–78.

Oken, E., Radesky, J.S., Wright, R.O., Bellinger, D.C., Amarasiriwardena, C.J., Kleinman, K.P., Hu, H., Gilman, M.W., 2008. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. Am. J. Epidemiol. 167, 1171–1181.

Oken, E., Wright, R.O., Kleinman, K.P., Bellinger, D., Amarasiriwardena, C.J., Hu, H., Rich-Edwards, J.W., Gillman, M.W., 2005. Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. Environ. Health Perspect. 113, 1376–1380.

Patandin, S., Lanting, C.I., Mulder, P.G., Boersma, E.R., Sauer, P.J., Weisglas-Kuperus, N., 1999. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J. Pediatr. 134, 33–41.

Roegge, C.S., Wang, V.C., Powers, B.E., Klintsova, A.Y., Villareal, S., Greenough, W.T., Schantz, S.L., 2004. Motor impairment in rats exposed to PCBs and methylmercury during early development. Toxicol. Sci. 77, 315–324.

Rogan, W.J., Gladen, B.G., McKinney, J.D., Carreras, N., Hardy, P., Thullen, J., Tinglestad, J., Tully, M., 1986. Neonatal effects of transplacental exposure to PCBs and DDE. J. Pediatr. 109, 335–341.

Sagiv, S.K., Nugent, J.K., Brazelton, T.B., Choi, A.L., Tolbert, P.E., Altshul, L.M., Korrick, S.A., 2008. Prenatal organochlorine exposure and measures of behavior in infancy using the Neonatal Behavioral Assessment Scale (NBAS). Environ. Health Perspect. 116, 666–673.

Sakamoto, M., Kubota, M., Liu, X.J., Murata, K., Nakai, K., Satoh, H., 2004. Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. Environ. Sci. Technol. 38, 3860–3863.

Sakamoto, M., Kaneoka, T., Murata, K., Nakai, K., Satoh, H., Akagi, H., 2007. Correlations between mercury concentrations in umbilical cord tissue and other biomarkers of fetal exposure to methylmercury in the Japanese population. Environ. Res. 103, 106–111.

Steuerwald, U., Weihe, P., Jørgensen, P.J., Bjerve, K., Brock, J., Heinzow, B., Budtz-Jørgensen, E., Grandjean, P., 2000. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. J. Pediatr. 136, 599–605.

Strain, J.J., Davidson, P.W., Bonham, M.P, Duffy, E.M., Stokes-Riner, A., Thurston, S.W., Wallace, J.M., Robson, P.J., Shamlaye, C.F., Georger, L.A., Sloane-Reeves, J., Cernichiari, E., Canfield, R.L., Cox, C., Huang, L.S., Janciuras, J., Myers, G.J., Clarkson, T.W., 2008. Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles child development nutrition study. Neurotoxicology 29, 776–782.

Sugawara, N., Ohba, T., Nakai, K., Kakita, A., Nakamura, T., Suzuki, K., Kameo, S., Shimada, M., Kurokawa, N., Satoh, C., Satoh, H., 2008. Effects of prenatal coexposure to methylmercury and polychlorinated biphenyls on neurobehavioral development in mice. Arch. Toxicol. 82, 387–397.

Todaka, T., Hori, T., Hirakawa, H., Kajiwara, J., Yasutake, D., Onozuka, D., Kato, S., Sasaki, S., Nakajima, S., Saijo, Y., Sata, F., Kishi, R., Iida, T., Furue, M., 2008. Congener-specific analysis of non-dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. Chemosphere 73, 923–931.

Walkowiak, J., Wiener, J.A., Fastabend, A., Heinzow, B., Kramer, U., Schmidt, E., Steingruber, H.J., Wundram, S., Winneke, G., 2001. Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 358, 1602–1607.

Weihe, P., Grandjean, P., Debes, F., White, R., 1996. Health implications for Faroe islanders of heavy metals and PCBs from pilot whales. Sci. Total Environ. 186, 141–148.

Yaginuma-Sakurai, K., Shimada, M., Ohba, T., Nakai, K., Suzuki, K., Kurokawa, N., Kameo, S., Satoh, H., 2009. Assessment of exposure to methylmercury in pregnant Japanese women by food frequency questionnaire. Public Health Nutr. 12, 2352–2358.

Yasutake, A., Matsumoto, M., Yamaguchi, M., Hachiya, N., 2003. Current hair mercury levels in Japanese: survey in five districts. Tohoku J. Exp. Med. 199, 161–169.

THE ASSOCIATION OF PERINATAL EXPOSURE TO PERSISTENT ENVIRONMENTAL POLLUTANTS WITH CHILD DEVELOPMENT: TOHOKU STUDY OF CHILD DEVELOPMENT

Tatsuta N[1,2], Nakai K[1], Shimada M[1,3], Yaginuma K[1,3], Suzuki K[1,2,4], Kurokawa N[1], Murata K[5], Hosokawa T[2], Satoh H[1]

[1] Environmental Health Sciences, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, 980-8575 Japan; [2] Human Development and Disabilities, Tohoku University Graduate School of Education, 27-1 Kawauchi, Aoba-ku, Sendai, 980-8576 Japan; [3] Research Fellow of the Japan Society for the Promotion of Science, Tokyo, Japan; [4] Faculty of Education, Kochi University, 2-5-1 Akebono-cho, Kochi, 780-8520 Japan; [5] Environmental Health Sciences, Akita University Graduate School of Medicine, 1-1 Hondo, Akita, 010-8543, Japan

Introduction

The neurobehavioral effects of perinatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), dioxins, and pesticides, are of great concern worldwide. Several studies reported the association between perinatal exposure to MeHg or PCBs and poorer cognitive functions. These chemicals accumulate in humans mostly through the consumption of food, especially fish and shellfish. From the nutritional point of view, fish is beneficial for pregnant women and unborn babies because it is rich in nutrients such as polyunsaturated fatty acids (PUFA), essential for the developing brain. Therefore, these health hazard issues are particularly important in fish-eating populations. A prospective cohort study, the Tohoku Study of Child Development (TSCD)[1], has been established to examine the effects of perinatal exposure to MeHg, PCBs and dioxins on child development.

We registered 599 mother-infant pairs from January 2001 to September 2003 at obstetrical wards of two hospitals in Sendai, Japan. Maternal peripheral blood, placenta, cord blood, maternal hair, and breast milk were collected for chemical analyses. Maternal diet was estimated with a semi-quantitative food frequency questionnaire (FFQ). Previously, we reported associations of neonatal neurobehavioral status with maternal hair mercury (hair Hg), total PCBs concentration in cord blood and maternal fish intake[2].

In this study, we examined the associations of the Kyoto Scale of Psychological Development (KSPD)[3] and Bayley Scales of Infant Development second edition (BSID-II)[4] in infants 7 months old with the total PCBs concentration in cord blood, maternal hair mercury and maternal fish intake.

Materials and Methods

The participants were 360 mother-infant pairs whose variables, including the total PCBs concentration in cord blood, KSPD and BSID-II, and other confounders, were available. Mean maternal age at delivery was 31.4. The infants consisted of 188 boys and 172 girls, and they were all singletons and had full-term (36-42 weeks) gestation (body weight of more than 2400g, and when the term was 36 weeks of gestation, body weight of more

than 2500g was included) without congenital anomalies or diseases. Information was obtained about pregnancy, delivery conditions and infant characteristics from medical records.

The PCBs concentration in whole cord blood collected immediately after delivery was measured. All congeners were analyzed using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) (IDEA Consultants, Inc. Tokyo, Japan and Shimazu Techno-Research Inc. Kyoto, Japan). The total PCBs concentration represented the sum of the all measured congeners, expressed as pg/g-wet or ng/g-lipid. The mercury concentration was analyzed from maternal hair samples taken at two days after delivery.

Maternal fish intake was estimated using the FFQ for 122 individual foods and recipes and 13 additional items regarding fish and shellfish. The FFQ was administered at four days after delivery. Trained examiners showed mothers a real-size photograph of each food, and the mothers answered about the frequency and the amount of intake per meal.

The KSPD is a standardized developmental assessment tool for Japanese infants and it is widely used at child counseling centers and public health centers. This scale consists of 324 items covering the Cognitive-Adaptive (C-A), Language-Social (L-S) and Postural-Motor (P-M) areas. From scores in these three areas, the total score is calculated and converted to the Developmental Age (DA). By dividing the DA with the chronological age and then multiplying by 100 the Developmental Quotient (DQ) is calculated.

The BSID-II was used in over 20 countries as a tool assessing development in children. It is categorized into two main domains; the Mental Scale and the Psychomotor Scale. The Mental scale yields a normalized standard score, the Mental Development Index (MDI), and is intended to assess cognitive functions such as memory, habituation, problem solving, primary numeric concepts, generalization, classification, vocalization, language, and social strategy. The Psychomotor scale also yields a standard score, the Psychomotor Development Index (PDI), and is intended to evaluate body control as well as fine and gross motor functions. Since there is no Japanese version of the BSID-II, we translated the original assessment items and the manual into Japanese.

Other major potential confounders include age at examination (months), period of gestation (weeks), birth length, birth weight, gender, maternal age at delivery, delivery type, birth order, alcohol consumption/smoking habits during pregnancy, paternal/maternal education level, and the period of breast feeding. The quality of the home environment was assessed using a questionnaire, the Evaluation of Environmental Stimulation (EES)[5], which has been established in Japan, modified after the Home Observation for Measurement of the Environment (HOME) score. The mother was asked to fill in the EES when the children were 18 months old. The maternal intelligence quotient was measured using the Raven standard progressive matrices and the results were analyzed using the raw score because it has not been standardized in Japan.

In the statistical analysis, Pearson's product-moment correlation coefficients (r) were calculated to determine the association between the total PCBs concentration in cord blood, hair Hg and the scores of KSPD, BSID-II. Multiple regression analyses were performed for adjustment of confounders. All statistical analyses were performed in SPSS version 17.0 for Windows (SPSS Japan, Tokyo) and statistical significance was set at $p < 0.05$.

Results and Discussion

The mean total PCBs concentration in cord blood was 132.0 pg/g-wet (median 118.6), 51.1 ng/g-lipid (median 45.8), the mean total hair Hg level was 2.2 μg/g (median 2.0), and the mean maternal total fish intake was 22.3 kg/year (median 19.7). These were transformed into logarithmic ($\log_{10}$) values.

The total PCBs concentration (pg/g-wet) was significantly correlated negatively with the C-A area of the KSPD ($r=-0.11$, $p<0.05$), DQ of the KSPD ($r=-0.13$, $p<0.05$) and PDI of the BSID-II ($r=-0.11$, $p<0.05$). On the other hand, the total PCBs concentration (pg/g-wet) was not significantly correlated with the L-S or P-M areas of the KSPD or the MDI of the BSID-II. The same results were found with pg/g-wet and ng/g-lipid. Total hair Hg and total fish intake was not correlated significantly with the scores of the KSPD and BSID-II.

Multiple regression analysis was used to examine the association between the scores for the C-A area and DQ of the KSPD and the PDI of the BSID-II (Table 1). The first step was to examine the exposure index for independent values, which revealed a negative association between these developmental scores (the C-A area and DQ of the KSPD and the PDI of the BSID-II) and the total PCBs concentration. The second step was to examine the confounders and exposure index for independent values, which revealed a negative association between the PDI of the BSID-II and birth order. The negative relationship between the PDI of the BSID-II and the total PCBs concentration was not found after adjustment. No statistically significant correlations were found between other two developmental scores (the C-A area and DQ of the KSPD) and the total PCBs concentration.

Previous studies reported adverse effects of prenatal PCB exposure on neurodevelopment[6,7]. Our findings confirmed these results. Compared to previous studies, the PCB level in this study was lower, though the total PCBs concentration was negatively associated with developmental scores, especially the score for the C-A area and DQ of the KSPD, and the PDI of the BSID-II. However, after other confounders were included as independent variables, birth order was associated with the PDI of the BSID-II, but the total PCBs concentration was not. These results showed that the PDI of the BSID-II was low in the first-born child.

The PCBs concentration was associated with the mental part of the KSPD and the motor part, but not the mental part of the BSID-II. Several possibilities may account for this discrepancy. First, although these two assessment tools were constructed based on Gesell's developmental diagnosis, the number of assessment items is different. The KSPD consists of fewer items than the BSID-II. Second, to divide the child development into two or three parts is difficult. The BSID-II manual announces that there are some items that are difficult to place in the Mental or Motor Scale. It was suggested that one item could extend into both the Mental and Motor Scales.

In previous studies, an adverse effect of prenatal MeHg exposure on neurodevelopment was found in the Faroe Islands[8] and Boston[9], but not in the Seychelles[10]. Our findings agree with the latter study. Japanese people often eat fish that contains PUFA, so we need to inquire into the possible interaction between PUFA and MeHg.

Further studies will require consideration of the potential risks of fish intake in the context of potential benefits. Since the TSCD study is a prospective cohort study, we will readdress these health issues when the children become older.

Acknowledgements

We thank all the families who participated in the cohort study. The Medical Ethics Committee of the Tohoku University Graduate School of Medicine approved the study protocol. The Japan Ministry of Health, Labour, and Welfare, Research on Risks of Chemical Substances funded this research.

References

1. Nakai K, Suzuki K, Oka T, Murata K, Sakamoto M, Okamura K, Hosokawa T, Sakai T, Nakamura T, Saito Y, Kurokawa N, Kameo S and Satoh H. (2004); *Tohoku J Exp Med.* 202: 227-237
2. Suzuki K, Nakai K, Nakamura T, Hosokawa T, Okamura K, Sakai T, Kurokawa N, Kameo S, Murata K, and Satoh H. (2006); *Organohalogen Compounds.* 68: 1201-1204
3. Ikuzawa M, Matushita Y and Nakase A. (1995); *Nakanishiya Publishers.* (in Japanese)
4. Bayley N. (1993); *The Psychological Corporation.*
5. Anme T, Ueda R and Hirayama M. (1986); Shoni Hoken Kenkyu. 45:556-560 (in Japanese)
6. Stewart P, Reihman J, Lonkey E, Darvill T and Pagano J. (2000); *Nurotoxicol and Teratol.* 22: 21-29
7. Vreugdenhil H, Van Zanten G, Mulder P, and Weisglas-Kuperus N. (2004); *Dev Med Child Neurol.* 46: 398-405
8. Grandjean P, Weihe P, White R, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, and Jorgensen P. (1997). *Neurotoxicol Teratol.* 19: 417-428
9. Oken E, Wright R, Kleinman K, Bellinger D, Amarasiriwardena C. Hu H, Rich-Edwards J and Gillman M. (2005). *Environ Health Perspect.* 113: 1376-1380
10. Myers G, Davidson P, Cox C, Shamlaye C, Palumbo D, Cernichiari E, Sloane-Reeves J, Wilding G, Kost J, Huang L and Clarkson T. (2003). *Lancet.* 361: 1686-1692

Table 1 Results of multiple regression analyses (Standard β)

	C-A of KSPD		DQ of KSPD		PDI of BSID-II		
Adjusted r^2	0.02	0.06	0.02	0.06	0.02	0.06	0.04
Total PCBs (pg/g wet)[a]	-0.13 *	-0.06	-0.15 *	-0.06	-0.12 *	-0.01	-0.07
Total hair Hg (μg/g)[a]	0.08	0.08	0.07	0.07	0.00	0.02	0.03
Total fish intake (kg/year)[a]	0.04	-0.03	0.03	-0.07	0.06	0.02	0.03
Birth weight		0.12		0.08		0.04	0.03
Child gender		-0.08		-0.08		-0.06	-0.06
Maternal age at delivery		-0.11		-0.07		-0.12	-0.05
Birth order		-0.09		-0.12		-0.17 *	
Alcohol consumption during pregnancy		0.01		0.06		0.00	0.00
Smoking habits during pregnancy		-0.03		-0.04		0.08	0.09
Period of breast feeding		-0.11		-0.11		0.07	0.06
Score of Raven standard matrices		0.06		-0.01		-0.03	-0.05
Score of EES		0.08		0.08		0.10	0.10

* $p<0.05$

[a] Log translations, Log_{10X}, were used on values of total PCBs, hair Hg, and total fish intake

KSPD: Kyoto Scale of Infant Development, C-A: Cognitive-Adaptive, DQ: Developmental Quotient

BSID-II: Bayley Scales of Infant Development, PDI: Psychomotor Developmental Index

日衛誌 (Jpn. J. Hyg.), 65, 516-523 (2010)

原　著

日本語版不適応行動尺度の作成の試み

龍田　希[*1,2]，仲井　邦彦[*1]，鈴木　恵太[*2,3]，黒川　修行[*1]，島田　美幸[*1,4]，
柳沼　梢[*1,4]，佐藤　洋[*1]，細川　徹[*2]

[*1]東北大学大学院医学系研究科環境保健医学分野
[*2]東北大学大学院教育学研究科人間発達臨床科学講座
[*3]日本学術振興会特別研究員（PD）
[*4]日本学術振興会特別研究員（DC）

Construction of Maladaptive Behavior Scale in Japanese

Nozomi TATSUTA[*1,2], Kunihiko NAKAI[*1], Keita SUZUKI[*2,3], Naoyuki KUROKAWA[*1], Miyuki SHIMADA[*1,4], Kozue YAGINUMA[*1,4], Hiroshi SATOH[*1] and Toru HOSOKAWA[*2]

[*1]*Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine*
[*2]*Department of Human Development and Disability, Tohoku University Graduate School of Education*
[*3]*Research Fellow of the Japan Society for the Promotion of Science (PD)*
[*4]*Research Fellow of the Japan Society for the Promotion of Science (DC)*

Abstract　**Objectives:** To evaluate child development, social competence, like intellectual ability, is an important aspect. The social competence of a child is prescribed by behaviors suitable for the society (adaptive behaviors) and behaviors not suitable for the society (maladaptive behaviors). The Vineland Adaptive Behavior Scales (VABS) have been widely administered to children in a semi-structural interview to evaluate social competence. The Social Competence test (S-M test) widely used in Japan is a translated version of the Vineland Social Maturity Scale for adaptive behaviors. Since only the region of adaptive behaviors was translated into Japanese, we attempted to develop a Maladaptive Behavior Scale in Japanese based on the VABS to evaluate the social competence of children.

Methods: The Maladaptive Behavior Scale of the VABS was translated into Japanese and back-translated to ensure appropriate translation. It was administered to children belonging to the birth cohort of the Tohoku Study of Child Development at the age of 66 months. The subjects for analysis in this study were 451 children (230 boys, 221 girls).

Results: To assess internal consistency, we used the standardized Cronbach alpha coefficient and the result was 0.81. The correlation coefficient between the scores of the S-M test and those of the Maladaptive Behavior Scale was −0.15. The correlation coefficient between the scores of the Child Behavior Checklist at 30 months and those of the Maladaptive Behavior Scale was 0.44.

Conclusion: These results suggest that the Maladaptive Behavior Scale may be a reliable instrument for assessing maladaptive behavior in Japanese children.

Key words: Vineland Adaptive Behavior Scales, Maladaptive Behavior Scale（不適応行動尺度），questionnaire（質問票），preschool children（就学前の子ども）

緒　言

子どもの成長と発達を捉える上で，知的能力のみならず社会生活能力からみた評価が重要と考えられている(1)。子どもの社会生活能力は，社会生活を営む上で，その社会に適した行動（適応行動）と適応できずに表れた行動（不適応行動）により規定されるもので，人と社会環境の関係の中で調和している状態を適応，緊張と葛藤

受付2010年1月15日，受理2010年3月29日
Reprint requests to: Nozomi TATSUTA
Tohoku University, Graduate School of Medicine Environmental Health Sciences, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
TEL: +81(22)717-8102, FAX: +81(22)717-8106
E-mail: tatsuta@ehs.med.tohoku.ac.jp

が生じている状態を不適応という (2)。不適応状態にある子どもでも社会環境が変われば適応できる子どももおり (2)，適応行動と不適応行動の量や質は必ずしも反比例するとは限らず，子どもが属している環境によって適応行動と不適応行動はそれぞれ独立して出現すると考えられる。

社会生活能力は，知能指数（Intelligence Quotient, IQ）で表される知的能力とは異なる (1, 2)。現代において，IQに顕著な遅れはみられないものの，集団行動がとれない・落ち着きがない等の行動を示す子どもの存在が確認されており，実際に社会生活能力指数は，IQや言語能力との関連が低いことが示されている (3)。そのため，社会生活能力の測定が望まれ (1)，そのことによって原因探求や支援が実現できると考えられる。

American Association on Mental Retardation（AAMR）(2007年1月にAmerican Association on Intellectual and Developmental Disabilities（AAIDD）に改称）においても知的能力と社会生活能力を区別して考え，精神遅滞を知的機能と適応行動の著しい制約によって特徴づけられる障害 (4) と定義しており，その診断に日常生活上の適応能力を評価することのできるVineland Adaptive Behavior Scales（VABS）(5) が広く利用されてきた。VABSは，知的発達に遅れのみられない発達障害等のある子どもの社会生活能力を把握するために使用されることが多く，自閉症の診断 (6) やその生活を支援する際に子どもの社会生活能力を把握することを目的として用いられてきた (7)。近年では，ADHD，アスペルガー症候群，脆弱X症候群における行動特性 (8-10)，自閉症の投薬治療の効果 (11) の指標としても応用され，このように医学分野，教育分野および福祉分野で幅広く使用されている。一方，我が国には，適応行動については，新版S-M社会生活能力検査があるが，不適応行動に関しては，半構造化面接法であるVABSの日本語版作成の試みが始まっているものの (12)，質問票は存在しない。

S-M社会生活能力検査は，1935年にDollが開発した半構造化面接法で用いる質問票であるVineland Social Maturity Scale（VSMS）(13) が原型となっており，そのうちの適応行動部分のみを日本語に翻訳し，養育者が回答する質問票に応用したものである。その後，社会環境の変化に合わせて1980年に新版S-M社会生活能力検査が刊行された (14)。新版S-M社会生活能力検査は，子どもが自立した生活を営むために必要な能力がどの程度身に付いているかを評価する質問票として位置づけられており，1）身辺自立，2）移動，3）作業，4）意志交換，5）集団参加，6）自己統制の6尺度130項目から構成されている。

米国では，VSMSがS. Sparrowらによって VABSへと発展した (5)。VABSは，子どもの日常生活上の社会生活能力を評価するために子どもの行動を適応行動（Communication, Daily Living Skills, Socialization），運動能力（Motor Skills），および不適応行動（Maladaptive Behavior）の3つの領域から評価される。採点は，「いいえ」(0点)，「どちらともいえない」(1点)，「はい」(2点) の3つの選択肢からなり，それぞれの領域の合計得点が高い方が適応能力が高い，運動能力が優れている，あるいは不適応状態にあると判断される。現在ではVABSは絶版となっており，VABS-IIの開発が行われている (15)。

半構造化面接法では，検査者（面接者）があらかじめ定められた枠組みを守りながらも，面接の細部に関しては柔軟な対応が可能であるが，多数を対象とした疫学的なアプローチを行う上では質問票を用いることが望ましい。今回，疫学調査において，子どもの適応行動ならびに不適応行動を評価するため，適応行動については新版S-M社会生活能力検査をそのまま活用し，不適応行動についてはVABSのMaladaptive Behavior Domain（不適応行動尺度）の日本語版を作成し，養育者が回答する質問票とすることを試みた。なお，日本語版の作成について，VABSの開発者であるDr. S. Sparrowより承認を得た。

VABSの不適応行動尺度は2部構成であり，PartIでは主要な不適応行動（27項目），Part IIでは特殊な不適応行動（9項目）を評価するものである (5)。Part IIを構成する項目は，障害のない子どもには滅多にみられない行動であり，VABSの標準化が行われた際にもPart Iのみで信頼性や妥当性が検討された経緯がある。そこで，本研究においてもPart Iのみを活用した。

今回の検討では，第一に，日本の社会環境に適した日本語版不適応行動尺度を作成した。第二に，日本語版不適応行動尺度を構成する項目の内的整合性を検討した。第三に，日本語版不適応行動尺度の得点と関連のある発育，発達状況等の要因を検索した。第四に，適応能力との関連性を明らかにするため新版S-M社会生活能力検査を同時に実施し，社会生活能力指数と日本語版不適応行動尺度の得点との関連性を検討した。第五に，行動上の問題との関連性を明らかにするため日本語版Child Behavior Checklist age for 2-3（CBCL）(16, 17) の得点と日本語版不適応行動尺度の得点の関連性を検討した。

方　法

1. VABSの日本語版不適応行動尺度の作成

VABS原版の日本語への翻訳は，海外生活経験10年以上の日本人研究者1名が担当した。その上で，日本語に翻訳した質問項目を日本人翻訳家とカナダ人翻訳家に依頼し，逆翻訳を行った。この逆翻訳の結果を考慮し，原版の質問の本意と言外語句の意味を損なわないように，翻訳しにくい単語や文章はより正確な日本語表現になるよう考慮しながら日本語版不適応行動尺度を決定した。回答方法は原版のまま3件法とし，得点が高い場合に不適応状態にあると判定される。本研究では，就学前の生後66ヶ月（5歳6ヶ月）の子どもの社会生活能力を測定したため，対象児の年齢を考慮し，学校に関する項目