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III. 別刷

内分泌撹乱物質の健康影響に関する疫学研究から

—周産期曝露の影響を中心として—

仲井邦彦*　　佐藤　洋**

要　　旨

内分泌撹乱物質，特に PCB による周産期曝露の健康影響について，心理行動および認知への影響を解析した疫学研究を解説した．母親の PCB 曝露が児の出生後の心理行動，認知の発達に影響することが示されており，児への移行は量的には母乳を介するものが多いが，健康影響では胎児期曝露の比重が大きいことが明らかにされている．PCB による曝露の最大の標的は乳児ではなく胎児と考えられる．またメチル水銀，鉛，カドミウムの摂取も重要な交絡因子であり，このような化学物質の摂取には食習慣，食文化が深くかかわり，我が国独自の疫学の必要性が示唆された．

はじめに

周産期曝露の影響に焦点を当て，特に児の心理行動や認知の発達を指標とした疫学研究を中心に取り上げた．胎児期から新生児期にかけては脳の発生，発達の時期であり，化学物質に対する感受性が高い．内分泌撹乱物質の多くは脂溶性で難分解性であり，永年にわたって母体に蓄積し，胎盤および母乳を介して児に短期間に移行する．そのため，この時期の児の摂取量は成人の１日当たりの摂取量に比較しても極めて高く，胎児や新生児の脳への影響が危惧されるためである．なお，PCB 汚染事故である日本と台湾の油症の疫学からは，今でも甲状腺ホルモンや性成熟について重要なデータが報告されているが，誌面の都合で割愛した．

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キーワード：内分泌撹乱，PCB，胎児期曝露，心理行動，コホート

The Michigan Cohort (The Jacobson Cohort)

母親がミシガン湖の魚を多食する児の発達を追跡した前向きコホート調査が，Jacobson らによって行われた．1980～1981 年にかけて出生した児を対象とし，魚を食べない母親を持つ児を対照に，313 名が登録された（77% が多食群，23% が対照群）．魚種ごとに PCB 汚染の程度を決定し，汚染度が高い魚（マス，サーモン，コイ，ナマズ）の比係数を１とし，魚の摂取状況から PCB 摂取量を推定している．出生後から 11 年間追跡が行われた．まず PCB 曝露群では出生時体重が低く（対照との差は 160～190g），妊娠期間が短く（4.9～8.8 日），それを補正しても

頭囲も小さかった[1]．出生後 60 時間において Neonatal Behavioral Assessment Scale (NBAS) が実施され，多食群において運動の未熟，状態易変化性の低下，驚愕反応の増加，原始反射の低下が確認されている．ただし，臍帯血 PCB と NBAS に相関は見られない．

生後5および7ヵ月に Bayley Scales of Infant Development (BSID) および Fagan's Test of Infant Intelligence (FTII) が実施されている．後者の FTII は映像を見せて判別させる視覚認知試験であり，この時期に実施可能な心理行動試験のうち，成長後の IQ と相関する唯一の試験とも考えられている．BSID は魚摂取量や臍帯血 PCB のいずれとも相関しなかった．一方，FTII も母乳中 PCB と相関しなかったものの，魚摂取量および臍帯血 PCB とよく相関した[2]．したがって，母親から移行する PCB は量的には経母乳が圧倒的であるものの，脳の感受性などを考慮すると胎児期曝露がより重要であるという考察が導かれることとなる．

4歳児の追跡調査は McCarthy Scales of Children's Abilities (MS) を中心とした試験が行われた．最も強く関連したのは臍帯血 PCB であり，言語および数字記憶と相関した[3]．彼らは 11 歳児にも知能試験を実施し，注意と記憶機能の遅れが臍帯血 PCB と相関することを示したが，母乳 PCB との間には関連性は見いだせなかった[4]．

この研究の弱点の1つは，対照群の人数が曝露群の 1/3 程度しかなく，その選択も無作為に選ばれたため，交絡因子が必ずしも制御されていないことにある．特に社会・経済的な要因や母親の IQ などの要因が十分には考慮されていない[5]．また，曝露群ではアルコール，カフェインの摂取が高く，妊娠中の風邪薬の使用も多い．さらに，魚摂取量を PCB 曝露指標の代替指標として用いる場合，胎児期曝露で同じく行動奇形を引き起こしうるメチル水銀も生物濃縮で魚に蓄積するため，メチル水銀が交絡因子となりうる．残念ながらメチル水銀は測定されていない．

The North Carolina Cohort (The North Carolina Breast Milk and Formula Project)

Rogan らによってノースカロライナで行われた疫学では，ハイリスク集団ではなく一般の妊婦から対象者が選ばれた．1978～1982年に 880 組以上の母親－児のペアが登録され，内部比較により対照が設定され，規模も大きい．出生後から5歳まで追跡が行われた．臍帯血 PCB 濃度が低く十分な精度での議論ができなかったため，出生直後の母乳中 PCB を胎児期曝露の指標として用いている．その母乳中 PCB は出生時体重，頭囲と相関しなかったが，3.5mg/ml を超す高濃度群の母親から生まれた児は，生後 72 時間後に実施された NBAS で筋緊張や活動性の低下，原始反射群の低下が観察され[6]，この結果は The Michigan Cohort と同様の傾向であった．また農薬の DDE (DDT の代謝物) が高い場合にも，原始反射の異常が観察された．

2歳までの追跡調査では BSID と Mental Development Scales (MDS) が採用され，PCB は BSID の心理行動面のスコアーのみと相関し，著者らは胎児期曝露によって運動系の機能の成長がわずかではあるが有意に遅延したと考察している．3歳から5歳にかけての追跡調査では MS が採用されたが，PCB 曝露との間に相関は見いだされておらず[7]，したがって The Michigan Cohort で報告された短期記憶への影響も確認されていない．また，本研究では2歳までの時点で運動面の成熟の遅延が示されていたが，検査法が異なるとはいえ3歳以上では運動面の遅れはなく，2歳での遅延は解消されたものと理解される．

本研究は対照の取り方，標本数，交絡因子の定義など疫学的にも信頼度が高いものであるが，母親の IQ や家庭内環境など児の認知行動の発達に影響しうると考えられる要因についての考慮が十分ではない[9]．また臍帯血 PCB 測定値の多くが検出限界以下となり，胎児期曝露の指標として出生直後の母乳中 PCB の値で代替したこと，その出生直後の母乳を採取できなかった場合は，欠落データを回避するために追跡調査の過程で得られた母乳の値から推定した「予想値」を用いたこと[8]を指摘しておきたい．最後に，The Michigan Cohort に比べ人口構成から見ると本研究では高学歴の女性の構成比が高く，そのため本研究の対象者の母乳保育の割合も高いと予想される．実際に，母乳保育により MS の成績が良くなることが確認されており[9]，母乳保育のプラス面が環境汚染というマイナス面を代償したことも想定される．

The Oswego Study（The Oswego Newborn and Infant Development Project）

ニューヨーク州 Oswego 郡において，The Michigan Cohort の再現性確認を意識した疫学が 1991 年より進行している．この調査の特徴は，PCB，DDE，HCB に加え，Mirex，鉛，および毛髪総水銀なども解析されたことである．鉛やメチル水銀も行動奇形を引き起こしうる化学物質であり，特にメチル水銀は PCB と同様に魚に生物濃縮するため，PCB などの影響を評価するうえで重要な交絡因子となる．

対象者はオンタリオ湖の魚を習慣的に食べる母親 141 人と食べない母親 152 人であり，母親の魚摂取状況の聞き取りが行われている．心理行動検査は生後 1 ～ 2 日目に NBAS が実施され，慣れ反応（睡眠中に与えられる光や音刺激に対する反応の漸減を見るもの），自律系の安定性，原始反射において，スコアーの減少が母親の魚摂取の習慣とよく相関した．臍帯血 PCB は総 PCB では NBAS スコアーと相関しないが，PCB の結合塩素数で細分し，高塩素化（塩素数 7 ～ 9 個）PCB が NBAS の成績とよく相関することが示されている[10]．この報告は PCB の中に生物活性を有する PCB 化学種が存在する可能性を示し，その特定のための基礎研究と特異的な分析手法の確立の必要性を強調するものである．

次に，児が 67 週（230 人）または 92 週（216 人）にて FTII が行われた．PCB と FTII の成績はよく相関するものの，Mirex，DDE，鉛および毛髪総水銀は相関しない[11]．一方，母乳中 PCB の分析は容易で，高濃度の PCB が検出されたものの，母乳中 PCB と FTII に関連性は見いだされなかった．授乳期の曝露よりも胎児期曝露の重要性を示唆するものであり，The Michigan Cohort の結果とよく一致する．ただし，PCB 曝露で変動する FTII の成績の絶対値は小さく，心理行動検査の鋭敏性の良さを示唆するものではあるものの，健康影響の程度を考慮するうえで慎重な取り扱いが必要であろう．

The Dutch Study

オランダの工業都市 Rotterdam，およびその比較で都市化がそれほど進んでいない Groningen の 2 地域を対象に，1990～1992 年にかけて合計 400 人程度の児が登録され，前向きコホートが進行中である．母乳保育群と人工栄養群から構成されており，母体血および臍帯血から 4 つの代表的な非コプラナー PCB を分析し，生後 2 週目の母乳からダイオキシン類，26 種類の PCB の分析が行われた．

生後 10 ～ 21 日目に行われた心理行動検査では，NBAS に類似の The Prechtle Neurological Examination（PNE）が採用されてい

る．その成績は，母体血と臍帯血 PCB との間に相関はないものの，母乳中ダイオキシン類，PCB とよく相関し，特に PCDD＋PCDF の TEQ，または PCB を加えた総 TEQ との間に高い相関が観察された[12]．

追跡調査では，生後3，7および 18 ヵ月の時点で BSID が実施されている．臍帯血 PCB およびダイオキシン は生後3ヵ月までの心理行動指標と関連し，また母乳 PCB およびダイオキシンは生後7ヵ月の知能関連指標と相関したが，18 ヵ月では相関は認められなかった[13]．なお，母乳保育と人工栄養の結果を比較しており，行動の成熟度は母乳栄養群で得点が高く，母乳によって化学物質の移行も進むが，母乳にはプラス面があることも強調されている．次に，生後 18 ヵ月[14]および 42 ヵ月[15]における追跡調査では，独自に開発された発達試験も採用され，握，着座，腹ばい，起立，および歩行について評価が行われた．18 ヵ月目では母乳 PCB 類は発達試験の成績と相関せず，臍帯血 PCB がわずかに相関した．一方，生後 42 ヵ月では PCB やダイオキシン曝露との関連性は消失していたという．生後 42 ヵ月では言語能力も試験されているが，この場合は母体血 PCB 濃度と言語能力がわずかに相関していることが示されている[16]．オランダの疫学研究の結果は，全体として母乳栄養の優位性を示し，曝露時期としては胎児期がより重要であることを示すものであろう．

TWI Study（Toxic Waste Incinerator）

ドイツにおけるこの調査は胎児期曝露の疫学ではないが，交絡因子として血中カドミウムが重要であることを示す貴重な報告である[17]．焼却場周辺での小児を対象とした調査にて，636 人の小学生（7～10 歳）が登録され，320 人から末梢血が採取された．甲状腺機能との関連性が調べられ，総 PCB または幾つかの PCB と TSH，遊離 T_3 が正に相関した（T_4 は相関せず）．興味あることに，血中カドミウムが TSH と正に，遊離 T_4 と負に相関することが示されている（鉛，総水銀は相関しない）．我が国でも，カドミウム汚染地区の女性で腎尿細管障害を有する者では遊離 T_4 が低下していることが報告されており[18]，甲状腺はカドミウム曝露の標的臓器であるかもしれない．脳の発達には T_4 が必須であり，甲状腺機能の先天的低下は知能低下を招くクレチン症として知られる．PCB による脳作用の機序は，この甲状腺機能撹乱が1つの仮説である．実際に国内で 36 人の母乳保育児を対象とした調査では，母乳中ダイオキシン類 TEQ と T_4，T_3 が負に相関するという[19]．ただし，The North Carolina Cohort で臍帯血 TSH，T_4，T_3 と PCB の関連性が遡及的に解析されたが，有意な相関は見られていない[20]．

メチル水銀の疫学研究から

PCB やダイオキシンの主な摂取経路は魚であるが，すでに述べたとおり，メチル水銀もまた魚に生物濃縮され摂取される．低濃度のメチル水銀による胎児期曝露の影響について，クジラを多食するハイリスク集団を対象に Faroe 諸島で調査が行われている．初期の調査では 1986～1987 年に出生した 1,000 人以上の児が登録され，出生時の母親の毛髪総水銀と臍帯血総水銀の分析が行われた．すでに多くの報告があるが，特徴的な点は，7歳の時点でも，The Boston Naming Test, The California Verbal Learning Test, The Continuous Performance Test で胎児期曝露との間に関連性が認められ，出生時の母親の毛髪総水銀が 10ppm を超える場合，児の言語能力，注意や短期記憶，空間認知とそれに関連する運動系の発達に影響を残すことが示されたことである．毛髪総水銀が 10ppm を

表1 PCBによる周産期曝露と児の心理行動，認知の発達に及ぼす影響

心理行動検査	主な結果	曝露との相関		
		魚摂取	胎児期 PCB*1	出生後 PCB*1
The Michigan Cohort				
NBAS（出生後 60 時間）	運動↓，状態易変化性↓，驚愕反応↑，原始反射↓	○	×	
BSID（5，7ヵ月）	関連性認めず	×	×	
FTII（5，7ヵ月）	視覚認知機能↓	○	○	×
MS（4歳）	言語・数字記憶↓		○	
知能試験（11歳）	注意と記憶機能↓		○	×
The North Carolina Cohort				
NBAS（出生後 72 時間）	運動の筋緊張↓，活動性↓，原始反射↓		○*2	
BSID（2歳まで）	運動系↓		○*2	
MDS（2歳まで）	関連性認めず		×*2	
MS（3～5歳）	関連性認めず		×	
The Oswego Study				
NBAS（出生後 48 時間）	慣れ反応↓，自律系安定性↓，原始反射↓	○	○	×
FTII（67, 92 週）	視覚認知機能↓		○	×
The Dutch Study				
PNE（生後 10～21 日）	筋緊張↓，総合スコアー↓		×	○
BSID（3ヵ月）	心理行動指標↓		○	×
〃（7ヵ月）	知能関連指標↓		×	○
機能発達試験（18ヵ月）	発達↓		○	×
機能発達試験（42ヵ月）	関連性認めず		×	×
言語試験（42ヵ月）	言語能力の発達↓		○*3	

*1 胎児期曝露の指標は特に記載がなければ臍帯血 PCB を指し，出生後曝露は母乳中 PCB を指す．
*2 出生直後の母乳中 PCB であり，胎児期曝露を反映すると考えられる．
*3 母体血中 PCB であり，胎児期曝露を反映すると考えられる．
心理検査法は便宜上以下のように略した．
NBAS：Neonatal Behavioral Assessment Scale，BSID：Bayley Scales of Infant Development，
FTII：Fagan's Test of Infant Intelligence，MS：McCarthy Scales，MDS：Mental Development Scales，
PNE：The Prechtl Neurological Examination

超える妊婦の割合は，国内でも数% 程度は存在すると危惧されている．

ただし，同様の疫学研究がインド洋の Seychelles 島でも行われたが，メチル水銀の胎児期曝露と認知行動面の指標との間に相関は確認されていない（検査は BSID，FTII，MS などが用いられている）[21]．この2つの研究の差異については，Seychelles 島ではより多様な魚が消費され，セレンや不飽和脂肪酸などの有益な成分に加え，PCB など他の有害化学物質による曝露も Faroe 諸島とは異なったものと想定されている．採用された検査法の差異や，原語から現地語への翻訳なども大きな要因であろう．両研究では，その後 PCB やダイオキシンをも考慮した新たな疫学が開始されており，Faroe 諸島からはすでに 435 人の7歳児での報告が出されている[22]．児の出生時における臍帯組織 PCB は臍帯血総水銀と高い相関を有し（r=0.42），臍帯組織 PCB は児の認知行動の成長の遅れとよく相関した．著者らはメチル水銀の影響がより大きいとするものの，PCB との間に

複合的な影響があることを認めている．なお，この調査では臍帯血 PCB と臍帯組織 PCB がよく相関することをあらかじめ確かめた後に，曝露指標として臍帯組織 PCB が用いられた．臍帯や胎盤は採取も容易で試料としても大きく，かつ廃棄される臓器であり，疫学研究では魅力的な試料である．

我が国独自の疫学調査の必要性

内分泌撹乱物質と同様に，メチル水銀も魚を介して摂取される．特に日本人はメチル水銀の汚染度が高いマグロなどを好み，メチル水銀の摂取量は欧米に比べて高い．さらに，TWI Study からはカドミウムが甲状腺機能撹乱の重要な要因であることが示唆されたが，日本人は米を主食とし，日本の米のカドミウム含有量は世界的にも高レベルである．欧米などと比較して，日本人は固有の食習慣を有し，栄養学的にも有害化学物質の摂取においても特徴的と理解され，したがって内分泌撹乱物質による健康影響を論ずるうえで独自の疫学データに基づく検証が必要であろう．

欧米の調査からは，母乳を介した曝露ではなく胎児期曝露がより重要であることが示された．国内では母乳汚染をモニタリングする疫学が進められており，母乳中の化学物質と甲状腺ホルモンとの関連性を解析した優れた疫学研究が長山らによって行われている[19]．しかしながら，臍帯血を利用した疫学調査は少なく，指標として児の心理行動，認知を解析したものもない．我が国でも，児の心理行動面の発達を追跡する前向きコホート調査に基づく基礎資料が希求されよう．

最後に

出生児や流産児の性比異常，精子数減少，性成熟の早熟化，乳がんの増加，子宮内膜症の増加，アトピーの増加などの異常現象が観察されており，内分泌撹乱物質による曝露との因果関係が取りざたされているが，実証的な疫学データは必ずしも明確ではない．しかし，本稿のテーマである胎児期曝露に起因した児の心理行動や認知への影響は，かなり確からしいと思われる．その際に胎児期曝露が重要であり，母乳には PCB などが含まれるものの，母乳保育の優位性がその汚染の影響を凌駕するものと考えられ，授乳行為を否定するものではない．しかし，母親の体内に蓄積した PCB 量は自身が新生児期に哺乳で受け取った部分もあると考えられ，その意味では次世代影響が考慮されるべきである．いずれにしても，胎児期曝露を軽減するには女性の化学物質による曝露を抑える必要があり，社会的な対応が望まれる．そのような議論を進めるには科学的で具体的な基礎データが必須であり，疫学のみならず基礎科学を含めた研究の進展が望まれる．

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A Review of Epidemiological Studies on the Neurodevelopmental Effects of Perinatal Exposure to Endocrine Disruptors

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Tohoku J. Exp. Med., 2002, **196**, 89-98

Review

Developmental Neurotoxicity Following Prenatal Exposures to Methylmercury and PCBs in Humans from Epidemiological Studies

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NAKAI, K. and SATOH, H. *Developmental Neurotoxicity Following Prenatal Exposures to Methylmercury and PCBs in Humans from Epidemiological Studies* Tohoku J. Exp. Med., 2002, **196** (2), 89-98 —— Adverse health effects following prenatal exposures to methylmercury (MeHg) have been apparent from several prospective cohort studies conducted in a fish-eating population. A prospective study in a Faroese birth cohort documented subtle deficits of several functional domains at prenatal MeHg exposure levels previously thought to be safe. Recent additional studies also showed neurobehavioral deficits associated with exposures to polychlorinated biphenyls (PCBs) with concomitant MeHg poisoning. In contrast, a prospective study in the Seychelles did not detect a similar association between MeHg exposure and neurodevelopmental deficits; children of the highest MeHg exposure group showed better scores in some developmental tests than those of the lower exposure groups for both prenatal and postnatal MeHg exposures. This paradoxical difference between both studies is summarized herein. The primary source of human exposure to MeHg is fish. Since a considerable number of pollutants, including polychlorinated biphenyls (PCBs) and pesticides, are also present in fish, and since some organochemical substances including PCBs are also well documented to be neurotoxic to the developing brain from epidemiological studies, the combined effects of these pollutants should be considered in discussing the neurotoxicity of MeHg. In this article, therefore, major prospective cohort studies focusing on the exposures to PCBs were reviewed. ———— brain development; epidemiology; methylmercury; neurotoxicology; polychlorinated biphenyls; prenatal exposure

Received January 31, 2002; revision accepted for publication February 28, 2002.

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This paper was presented at the Sendai Symposium: Paradigm Shift in Heavy Metal Toxicology held on November 21-22, 2001.

The neurotoxicity of high levels of methylmercury (MeHg) was well documented from the severe epidemic in the small Japanese fishing community, Minamata. Since the developing fetal brain is highly susceptible to MeHg, severe neurotoxicity had been observed in the children of mothers exposed to MeHg during pregnancy, although the mothers themselves did not show clinically evident symptoms of MeHg poisoning. Until recently, public health workers have considered that such high-level exposures to MeHg which cause clinically apparent disease are only the range of adverse health effects of MeHg poisoning. However, in the 1990s, a new understanding has emerged regrding the adverse neurodevelopmental effects of MeHg that may be detectable at the prenatal exposure levels currently considered to be safe (Grandjean et al. 1998). Such adverse effects can be recognized only when a group of individuals are examined in a large cohort study. In this context, several prospective studies have been conductd in fish-eating populations, but the data obtained so far are not consistent. The differences between these studies are summarized in this article. In addition, polychlorinated biphenyls (PCBs) have also been recognized to be potent neurotoxicants when children were prenatally exposed, and to cause a delay in neurodevelopment. The primary source of human exposure to MeHg is fish. Considering that significant amounts of PCBs and related halogenated aromatic hydrocarbons are also ingested through fish consumption, the effects of concomitant exposures to several pollutants should be considered. Several major prospective studies regarding the hazardous effects of PCBs on human health are also reviewed.

Neurotoxicity of MeHg

The Faroese birth cohort

Two large prospective cohort studies have been conducted in fish-eating communities; the Faroese birth cohort and the Seychelles Child Development Study.

The Faroese birth cohort was initially generated in 1986-1987 at three hospitals in the Faeroes Islands (Grandjean et al. 1992). This consisted of 1022 consecutive singleton births, and MeHg exposures mainly originated from pilot whale consumption. At approximately seven years of age, 917 of the children underwent detailed neurobehavioral examination. Clinical examination did not reveal any clear-cut MeHg-related abnormalities. However, when a subgroup of 112 children whose mothers had hair mercury concentrations of 10-20 ppm was compared with a subgroup of children whose mothers had lower (<3 ppm) hair mercury concentrations, mild decrements were observed, especially in the domains of motor function, language, and memory (Grandjean et al. 1997; Grandjean et al. 1998). Among the motor function tests, one finger tapping condition and the overall hand-eye coordination average showed a deficit in the exposed group. A small visuospatial deficit was apparent in block designs in the revised version of Wechsler Intelligence Scale for Children. With regard to language, results of the Boston Naming Test were lower in the exposed children than in the nonexposed ones. On the long-term delayed reproduction of the California Verbal Learning Test, increased MeHg exposure was associated with a deficit. Neurophysiological tests also showed significant MeHg-associated delays of the peak III latency and the I-III interpeak latency of the auditory brainstem evoked potentials (Murata et al. 1999)

In this cohort, prenatal exposure to PCBs was re-examined by analysing cord tissues from 435 children (Grandjean et al. 2001b). Maternal exposure to MeHg was through consumption of fish and intermittent higher level exposure, through consumption of pilot whale meat, while consumption of pilot whale blubber resulted in maternal exposure to PCBs. Among 17 neuropsychological outcomes determined at seven years of age, the cord PCB concentration was associated with deficits in the three outcomes,

namely, the Boston Naming Test, the Continuous Performance test reaction time, and possibly, the long-term reproduction of the California Verbal Learning Test. However, the cord blood mercury concentration was associated with seven outcomes in the 17 measures. This suggests that in the Faroese population, MeHg neurotoxicity may be a greater hazard than that associated with PCBs.

In the next cohort, generated from 1994–1995, 182 singleton births were newly evaluated at two weeks of age (Steuerwald et al. 2000). PCBs were determined in maternal serum and breast milk samples, and mercury was determined in cord blood, cord serum, and maternal hair. Infant's neurological optimality score in the Prechtle Neurological Examination was determined with the age adjusted for gestational age. Only cord blood mercury concentration was significantly associated with the neurological optimality score, while PCB, w3 fatty acid, and selenium had no effect on this outcome. Thyroid function was normal. These findings also support the idea that MeHg is the major hazardous material responsible for transplacental neurotoxicity in this fishing community.

The Seychelles Child Development Study

The most direct comparison with the Faroese cohort is the longitudinal assessment of children from a fish-consuming population that has been conducted in the Republic of Seychelles in the Indian Ocean, where 85% of the population daily consumes ocean fish daily (Myers et al. 1997; Davidson et al. 1998). This cohort consisted of 711 mother-child pairs. The prenatal exposure to MeHg was estimated by determining the total mercury level in the mother's hair; the mean maternal hair total mercury level was 6.8 ppm. Postnatal exposure to MeHg was estimated by determining the mercury level in the children's hair; the mean mercury level was 6.5 ppm. Children were assessed at 66 months of age by administering six neurodevelopmental tests: McCarthy Scales of Children's Abilities (MS), the Preschool Language Scale, the Woodcock-Johnson Applied Problems, Letter and Word Recognition Tests of Achievement, the Bender Gestalt test, and the Child Behavior Checklist. No adverse outcomes were associated with prenatal MeHg exposure. Furthermore, a subgroup of children with higher prenatal and postnatal exposures to MeHg had statistically significant increases in test scores on several developmental outcomes compared with other subgroups of lower MeHg exposures. When children were re-examined at 108 months of age, similar results were obtained; enhanced performance in males in the Boston Naming Test and two tests of visual motor coordination was associated with increased prenatal MeHg exposure (Davidson et al. 2000). The authors discussed these paradoxical findings in terms of the beneficial nutritve factors closely associated with fish consumption other than the intake of MeHg.

Differences between the Faroese birth cohort and the Seychelles Child Development Study

One possible difference is that the Seychellois population is in some way buffered from the adverse effects of prenatal exposure to low levels of MeHg and benefits from a high level of fish consumption (Davidson et al. 1998). One possible candidate for the beneficial nutrition is the essential fatty acids present in seafood since docosahexaenoic acid is known to be essential for early neurodevelopment. However, this point is contradicted by a report showing that an increased intake of marine fat appeared to decrease the birthweight adjusted for gestational age (Grandjean et al. 2001a). Potential differences in the kinds of seafood between the two communities may also be related to the difference in the exposures to other neurotoxicants; this may be the case for the mothers in the Faroese Islands because they may ingest more PCBs by consuming whale

blubber. However, in the recent cohorts in the Faroese Islands, MeHg neurotoxicity has been shown to be more potent than PCB neurotoxicity (Steuerwald et al. 2000; Grandjean et al. 2001b). Other possible differences include the age of the children at the time of testing, differences in the test batteries, genetic/ethnic differences in the populations studied, and potential differences in timing, magnitude, and duration of MeHg exposure (Mahaffey 1998).

Exposure reference dose for MeHg

Fetuses are considered to be the most sensitive subpopulation because of the vulnerability of the developing brain. However, still unknown is the lowest dose that impairs neurodevelopment. A reference dose (RfD) of 0.1 mg MeHg/kg/day had been established by the U.S. Environmental Protection Agency based on a study on Iraqi children exposed to MeHg in utero (Marsh et al. 1987). The RfD is an estimate of the daily exposure to the human population that is likely to be without appreciable risk of deleterious effects during a lifetime. However, the exposure in Iraqi occurred at high levels for a limited period of time, and consequently were not typical of chronic lower exposure levels associated with fish consumption. Major obstacles for understanding such a low-level chronic MeHg exposure include the delayed appearance of the neurodevelopmental effects following prenatal exposure and limited knowledge of cellular and molecular processes underlying these neurological changes. In this context, the National Research Council had started evaluating new epidemiological data that were not available to USEPA at the time it derived the RfD, and finally concluded that the Faroese study was the most appropriate study for discussing the MeHg exposure among sensitive subpopulations (National Research Counsel 2000). Using a technique called benchmark dose (BMD) analysis (Mahaffey 2000), based on cord blood mercury concentrations, the lowest BMD for a neurobehavioral endpoint that the committee considered to be sufficiently reliable was 58 ppb of mercury in cord blood, which corresponds to a maternal hair mercury concentration of apptoximately 12 ppm. A daily intake of 1 mg MeHg/kg/day would result in a maternal hair mercury concentration of 10 ppm. Assuming that an uncertainty factor of 10 was applied, 0.1 mg MeHg/kg body weight per day would be a scientifically justified exposure RfD. Consequently, the NRC Mercury Committee found that USEPA's current RfD for MeHg was a justifiable level for protection of public health.

Neurotoxicity of PCBs and related halogenated aromatic hydrocarbons

The polychlorinated biphenyls (PCBs) and related halogenated aromatic hydrocarbons such as the chlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) are a family of widely dispersed, environmentally persistent organic compounds. The potential neurotoxicity of PCBs was first recognized in 1968 when a number of Japanese people became ill after ingesting rice oil that was contaminated with PCBs (Yusho). Later, a similar exposure occurred in Taiwan (Yu-Cheng). Children born to Taiwanese mothers who consumed PCB-contaminated rice oil were examined and a number of neurodevelopmental abnormalities, including behavioral problems and lower intelligence quotient (IQ) scores, were observed (Chen et al. 1992).

The Michigan Cohort

Since the Yusho and Yu-Cheng incidents, several cohort studies have been initiated to assess the potential neurobehavioral effects of in utero and lactational exposure to low levels of PCBs in the environment. One of the biggest cohort studies was a longitudinal prospective study of maternal PCB exposure from the food chain (Lake Michigan fish) and its effects on the developmental outcomes in the children. This study was generated by screening more than 800

women who delivered babies in 1980-1981. The final samples consisted of 313 women. The children were evaluated at birth, five months, seven months, and four years of age. Neonatal behavioral function was assessed using the Brazelton Neonatal Behavioral Assessment Scale (NBAS) (Jacobson et al. 1984). PCB exposure, measured by maternal fish consumption, was associated with several adverse outcomes on the NBAS. However, a more direct measure of exposure, umbilical cord serum PCB level, was not related to any adverse behavioral scores. Infant cognitive function was assessed at five and seven months of age (Jacobson et al. 1985, 1986). The Bayley Scales of Infant Development (BSID) was administered at five months of age, and Fagan Test of Infant Intelligence (FTII) was administered at seven months of age. Neither maternal fish consumption nor umbilical cord serum PCB level was related to scores on BSID. In contrast, both exposure indices were associated with less preference for the novel stimulus on the FTII. Postnatal PCB exposure, determined by measuring PCB level in breast milk, was not related to the scores in FTII. Children were also assessed at four years of age (Jacobson et al. 1990). Higher levels of prenatal PCB exposure, determined by measuring umbilical cord serum PCB levels, were associated with poorer scores on two subtests of the MS that measure verbal and numerical memory. In contrast, neither the quantity of breast milk consumed nor the child's current serum PCB concentrations were related to any of the outcomes. Children were finally reassessed at 11 years of age (Jacobson and Jacobson 1996). Prenatal exposure to PCBs was still associated with lower full-scale and verbal IQ scores, while postnatal exposure to PCB had no impact on the IQ scores.

Development after exposure to PCBs and dichlorodiphenyl dichloroethane transplacentally and through human milk

The North Carolina Cohort

Another important cohort study was carried out in North Carolina. This cohort consisted of 880 pregnant women, and these subjects differed from those in the Michigan cohort in that they were selected from the general population and had not been exposed to any known dietary source of PCBs other than the background levels that contaminate the general food supply. The PCB levels in umbilical cord blood were nearly all below the detection limit, and therefore, the investigators used the PCB level in maternal milk at birth as an indicator of the child's prenatal exposure. In addition, the investigators examined the decline in maternal milk PCB levels at birth and with time, and obtained a rationale to estimate and adjust the maternal milk PCB levels of mothers who did not provide milk samples at birth.

PCB exposure was associated with several adverse outcomes on the NBAS (Rogan et al. 1986). Infants whose mothers had the highest PCB concentrations in their milk had less muscle tone and lower activity levels, and were hyporeflexive. Infant cognitive and motor development was assessed by administering BSID at six, 12, 18, and 24 months of age (Gladen et al. 1988; Rogan and Gladen 1991). Higher transplacental exposure to PCBs was associated with lower psychomotor scores at six, 12, and 24 months of age. There was no relationship between transplacental PCB exposure and scores on the Mental Development Scale, and postnatal exposure through breast feeding was unrelated to performance on either scale. The children were later assessed on the MS at three, four, and five years of age, and neither transplacental nor breast-feeding exposure was related to the scores (Gladen and Rogan 1991).

Other PCB prospective cohorts

A number of additional prospective longitudinal cohort studies are now under way in the United States and Europe. In a cohort study conducted in Oswego in the United States, women who consume sports-caught Great Lakes fish were recruited. Their infants were assessed at birth by administering NBAS and at six and 12 months of age by administering FTII. The investigators found that heavily (Cl 7 to 9) chlorinated PCB levels were highly associated with poorer performance on the habituation and autonomic clusters of the NBAS, while lightly chlorinated PCB levels were unrelated to NBAS performance (Stewart et al. 2000). A similar relationship was observed between the total umbilical cord blood PCB levels and poorer FTII performance, while there was no significant relationship between total PCBs in breast milk and FTII performance (Darvill et al. 2000).

The relationship between in utero and lactational PCB exposure and later neuropsychological function of children is also being studied in a Dutch cohort. This study consisted of approximately 400 children. They were assessed at birth by administratering the Prechtle Neurological Examination (Huisman et al. 1995a). The umbilical cord plasma and maternal plasma PCB levels were not related to neurological function. In contrast, higher levels of planar PCBs, PCDDs, and PCDFs in human breast milk were related to reduced neonatal neurological optimality. The children were assessed at three, seven, and 18 months of age by administering BSID (Koopman-Esseboom et al. 1996). Higher transplacental exposure to PCBs was associated with lower psychomotor scores at three months. Another neurological examination also showed a negative relationship with the level of transplacental exposure to PCBs (Huisman et al. 1995b). A similar phenomenon was observed when the children were assessed at 42 months of age by administering the Kaufman Assessment Battery for Children (Patandin et al. 1999). In utero exposure to PCBs was associated with poorer cognitive function, while lactational exposure to PCB and dioxins was not related to the performance. The investigators, therefore, suggest a beneficial effect of breast-feeding on the fluency of movements.

Implications of cohort studies on the perinatal MeHg and PCBs

These cohort studies presented evidence of a delay in psychomotor development in children who were exposed to MeHg and PCBs during the perinatal periods. MeHg is mainly transferred to the children through the transplacental passage (Sakamoto et al. 2001). In contrast, the absolute quantity of PCBs transferred via breast milk is substantially higher than the quantities transferred via the placenta, and therefore the postnatal exposure would pose a greater threat to the infants. However, the findings from the PCB cohort studies appear to contradict this prediction, suggesting that the exposure during the prenatal period poses the primary threat. These suggest that the prenatal exposures to MeHg and PCBs are most hazardous for the neurodevelopment of the human fetus.

One may debate that although the prenatal exposures to those pollutants are essential in affecting the neurodevelopment in the children, the magnitude of the effects is small. For example, in the North Carolina PCB cohort, the difference in BSID between the lowest and highest PCB exposure groups was only 4-9 points depending on the age of assessment (Rogan and Gladen 1991). Nevertheless, the public health implications of an effect of this magnitude could potentially be very significant. Perinatal lead exposure has been shown to cause lead-associated intellectual deficits. In the lead cohorts, the similar viewpoint has been emphasized (Needleman et al. 1982). At the population level, it was evident that a shift of

TABLE 1. *Perinatal PCB exposure and outcomes of neurological and cognitive tests*

Test	Major finding	Correlation			Reference
		Fish intake	Prenatal PCB[1]	Postnatal PCB[1]	
The Michigan Cohort					
NBAS (60 hours)	Motor immaturity, Poorer lability of states A greater amount of startle, Hypoactive reflexes	Relate	None		Jacobson et al. 1984
BSID (5, 7 mo)	No relation	None	None		Jacobson et al. 1986
FTII (5, 7 mo)	Less performance	Relate	Relate	None	Jacobson et al. 1985
MS (4 years)	Poorer scores in verbal and numerical memory		Relate	Relate	Jacobson et al. 1990
IQ test (11 years)	Intellectual impairment		Relate	None	Jacobson and Jacobson 1996
The North Carolina Cohort					
NBAS (72 hours)	Less muscle tone, Lower activity levels, hyporeflexive		Relate[2]		Rogan et al. 1986
BSID (2 years)	Lower psychomotor scores		Relate[2]		Gladen 1988, Rogan and Gladen 1991
MDS (2 years)	No relation		None[2]		Rogan and Gladen 1991
MN (3-5 years)	No relation		None		Gladen and Rogan 1991
The Oswego Study					
NBAS (48 hours)	Lower scores in habituation, autonomic and reflex	Relate	Relate	None	Stewart et al. 2000
FTII (6 and 12 mo)	Less performance		Relate	None	Darvill et al. 2000
The Dutch Study					
PNE (10-21 days)	Less muscle tone, Reduced neurological optimality		None	Relate	Huisman et al. 1995a
BSID (3 mo)	Lower psychomotor scores		Relate	None	Koopman-Esseboom et al. 1996
BSID (7 mo)	Lower psychomotor scores		None	Relate	Koopman-Esseboom et al. 1996
Neurological examination (18 mo)	Lower optimality		Relate	None	Huisman et al. 1995b
Neurological examination (42 mo)	No relation		None	None	Lanting et al. 1998
K-ABC (42 mo)	Intellectual impairment		Relate[3]	None	Patandin et al. 1999

[1]Cord bllod 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's Test of Infant Intelligence (FTII), McCarthy Scales (MS), Mental Development Scales (MDS), the Prechtle Neurological Examination (PNE), Kaufman Assessment Battery for Children (K-ABC).
mo, months.

4 points in the mean of the normal distribution of IQ had marked effects on the properties of the tails of the distribution; children with elevated lead levels were three times more likely to have a verbal IQ below 80. Although 5% of those with low lead level had IQ in the superior range (>125), no child with an elevated lead level had scored in this range. A shift in mean performance on the BSID of only 4 points would result in a 50% increase in the number of children with subnormal scores (Schantz 1996). The costs to society to solve this problem would be large.

The exposures to MeHg and PCBs occur mainly through the intake of foods, especially through fish-eating. The Japanese population like eating fish. USEPA's public health guidance on MeHg intake, as mentioned above, has issued several proposals to the Japanese society to avoid the possible health hazards in high-risk populations such as young females and children (Mahaffey 2001). Fish consumption is widely recognized as a good source of important nutrients, but simultaneously it should be acknowledged that some species and sizes of fish contain MeHg at levels associated with adverse developmental effects. Since the Japanese food culture has characteristics different from those of other countries, the Japanese society should have its own evidence to discuss the hazardous effects of perinatal and low-dose exposures to MeHg, PCBs, and other pollutants.

Finally, one possible confounding factor should be mentioned. Japanese people like rice. Since cadmium (Cd) concentration in Japanese rice is high, the Japanese are known to be exposed to high levels of Cd. Although the typical target organ of Cd exposure has been considered to be the kidney, a recent epidemiological study on exposure to a toxic waste incineration plant revealed that blood Cd concentration was associated with an increase in TSH and a decrease in free T4 (Osius et al. 1999). Considering that one of the possible mechanisms by which PCB disturbs the normal development of fetal brain is suspected to be the disruption of the pituitary thyroid feedback regulation (Porterfield 2000), the exposure to Cd is likely an important confounding factor. In Japan, we must consider these confounding factors including food intake to elucidate the exact effects of neurotoxicity of MeHg and PCBs.

Acknowledgments

This work was supported in part by a Grant-in-aid for Scientific Research (C, #13897002) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a Health Sciences Research Grant on Research on Environmental Health from the Ministry of Health, Labor and Welfare of Japan, and by a grant from the Japan Public Health Association.

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Int Arch Occup Environ Health (2004) 77: 571–579
DOI 10.1007/s00420-004-0542-1

ORIGINAL ARTICLE

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Effects of methylmercury on neurodevelopment in Japanese children in relation to the Madeiran study

Received: 14 January 2004 / Accepted: 25 May 2004 / Published online: 20 November 2004

Abstract ***Objectives***: A cross-sectional study was carried out to assess the effects of methylmercury exposure on neurodevelopment in Japanese children, in relation to the Madeiran cross-sectional study, and to estimate benchmark dose (BMD) levels using the data of two studies. ***Methods***: Mercury levels in hair samples obtained from 327 Japanese mothers and their 7-year-old children, and methylmercury levels in the umbilical cord, were determined. Neurodevelopmental examinations, including the brainstem auditory evoked potential (BAEP), were performed on the children. ***Results***: The medians of hair mercury were 1.63 (0.11–6.86) μg/g for mothers and 1.65 (0.35–6.32) μg/g for children, and a significant correlation was seen between the hair mercury levels in mothers and children. The maternal hair mercury was significantly correlated with the methylmercury in the umbilical cords obtained from 49 children. In 210 children whose mothers had not changed their dietary habits since pregnancy, most of the neurodevelopmental variables were not significantly related to hair mercury levels. The BAEP latencies were significantly shorter in the Japanese children than in the 113 Madeiran 7-year-old children, whose mothers had hair mercury of 1.12–54.5 (median 10.9) μg/g. Significant relationships between the maternal hair mercury level and BAEP latencies (peaks III and V, and interpeak I–III) were found only in the merged data of Japanese and Madeiran children. When the lower 95% confidence limit of BMD (BMDL) was calculated, the BMDLs of mercury exposure for BAEP latencies in the merged data were between 6.9 and 10.5 μg/g, and lower than those in the Madeiran children. ***Conclusions***: It is suggested that Japanese children may ingest similar doses per body weight of methylmercury to their mothers. If maternal hair mercury was used as a proxy for mercury exposure at birth, no significant dose–effect associations with the BAEP latencies were observed in Japanese children with exposure levels below 6.9 μg/g of hair mercury, but only when higher-level exposures from Madeiran children were included. The BMDL was lower for the merged data than for Madeiran children alone.

Keywords Methylmercury · Child neurodevelopment · Dose–effect relationship · Benchmark dose · Brainstem auditory evoked potential

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Introduction

Methylmercury is a worldwide contaminant of seafood and freshwater fish. Its toxicity can produce widespread adverse effects within the nervous system, especially when exposure occurs during brain development (Igata 1993; International Programme on Chemical Safety 1990; National Research Council 2000). Early adverse effects have been characterized by administering neurobehavioral tests to children exposed in utero from maternal seafood diets (Grandjean et al. 1997; Kjellström et al. 1989). The National Research Council