

Tesi Doctoral

Exposició a compostos organoclorats i efectes sobre la salut infantil durant el primer any de vida



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2003



Departament de Ciències Experimentals i de la Salut
Programa de Doctorat en Ciències de la Salut i de la Vida
Universitat Pompeu Fabra (UPF)

Exposició a compostos organoclorats i efectes sobre la salut infantil durant el primer any de vida

Memòria presentada per la Núria Ribas i Fitó per optar al títol de Doctora per la Universitat Pompeu Fabra. Treball realitzat sota la direcció del Dr. Jordi Sunyer i Deu a la Unitat de Recerca Respiratòria i Ambiental (URRA) de l'Institut Municipal d'Investigació Mèdica (IMIM).
Programa de Doctorat de la Universitat Pompeu Fabra, bienni 1999-2001

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Dipòsit legal: B.7787-2004

ISBN: 84-688-5716-5

PRESENTACIÓ DE LA TESI

“Tot i que la difusió en àmbits científics (reunions de nivells diversos i publicacions) es considera l’etapa final d’una recerca, la publicació dels resultats constitueix el començament d’un procés en què la comunitat científica substancia i corregeix els resultats obtinguts o en desenvolupa de nous en relació amb els primers.” *Punt 5.4. Codi de Bones Pràctiques Científiques. D. CEXS-UPF i IMAS*

Aquesta tesi es presenta com a compendi de publicacions, d’acord amb la Normativa aprovada per la Comissió de Direcció de Doctorat del Departament de Ciències Experimentals i de la Salut de la Universitat Pompeu Fabra durant el mes de juny de 2001. Seguint aquesta normativa, la tesi consta d’un capítol d’introducció, els articles de recerca publicats o enviats per publicació, un capítol de discussió general, les conclusions finals i un resum. Dels 7 articles que es presenten, 6 pertanyen a un mateix projecte destinat a estudiar la transferència dels compostos organoclorats de la mare al nen i a mesurar els efectes d’aquests compostos sobre la salut del nen. El setè article correspon a una revisió sistemàtica sobre l’impacte dels PCBs sobre el desenvolupament neuroconductual del nen. L’estudi sobre “La incorporació de l’hexaclorobenzè (HCB) a través de la lactància i dels seus efectes sobre el desenvolupament neuroconductual del lactant” es va iniciar l’any 1997 gràcies al finançament per part de la Fundació ‘la Caixa’ (‘la Caixa’ 97/009.00) i del Ministeri de Sanitat (FIS-97/1102). Aquest estudi va néixer fruit de la necessitat de conèixer el grau d’exposició a l’HCB en nounats i lactants a través de la mare i dels seus efectes sobre el desenvolupament neurològic del nen en una població de la Ribera de l’Ebre altament exposada a aquest compost.

La meva col·laboració en aquest projecte es va iniciar el novembre de 1998 com a estudiant de 6è curs de Medicina. A partir del setembre de 1999 la meva participació en aquest projecte va ser com a becària predoctoral.

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ACRONIMS

CAP: Centre d'Assistència Primària

COPs: Compostos Orgànics Persistents

CPI: Coproporfirina I

CPIII: Coproporfirina III

CTPs: Compostos Tòxics Persistents

p,p'DDE: Dichlorodipenyldichloroethylene/Diclorodifenildicloroetilè

DDT: Dichlorodipenyiltrichloroethane/Diclorodifeniltricloroetà

EEA: Environmental European Agency/Agència Europea del Medi Ambient

e.s.: error estàndard

GC: Gas Chromatography/Cromatografia de Gasos

GGT: Gamma-GlutamilTransferasa

HCB: Hexaclorobenzè

HCH: Hexaclorociclohexà

HPLC: High Performance Liquid Chromatography/Cromatografia Líquida d'Alta Resolució

IC: Interval de Confiança

IgE: Immunoglobulina E

OCs: Organochlorine Compounds/Compostos Organoclorats

OMS: Organització Mundial de la Salut

PCBs: Polychlorinated Biphenyls/Bifenils Policlorats

PCP: Pentaclorofenol

RIE: Risc d'Incidència Estandarditzada

SNC: Sistema Nerviós Central

TSH: Thyroid Stimulating Hormone/Hormona Estimulant de la Tiroide

T4: Tiroxina

RESUM

ANTECEDENTS I OBJECTIUS

L'hexaclorobenzè (HCB) és un compost organoclorat àmpliament distribuït per tot el planeta, altament lipofílic que s'acumula als sistemes biològics. Els nounats s'exposen a aquests compostos organoclorats (OCs) a través de la placenta i de la lactància materna. Tot i que l'HCB és un dels OCs més comuns, la seva transferència a través de la placenta de la mare al fetus durant la gestació i a través de l'alletament matern està poc documentada. El coneixement sobre els seus possibles efectes sobre la salut infantil és també bastant limitat. A Flix, un poble de la Ribera d'Ebre, es van detectar nivells molt elevats d'HCB a l'atmosfera degut a la seva proximitat a una empresa electroquímica. Els objectius del present treball són els d'avaluar l'exposició a OCs a través de la placenta i de la lactància materna i els seus efectes sobre la salut infantil durant el primer any de vida en el conjunt de nounats d'aquesta població.

MÈTODES

Entre març de 1997 i desembre de 1999 es van reclutar els nounats de famílies residents a la població de Flix i a les cinc poblacions veïnes pertanyents a la mateixa Àrea Bàsica de Salut de Flix (Vinebre, La Torre de l'Espanyol, Ascó, Ribarroja d'Ebre i la Palma d'Ebre). Durant aquests mesos van néixer 118 nens dels quals 102 van ser finalment inclosos a la cohort. 98 van participar a l'estudi sobre els efectes d'aquests compostos en les hormones tiroïdees i en les mesures antropomètriques al moment de néixer i 92 van participar en el seguiment fins a l'any d'edat.

Es va dissenyar un estudi longitudinal per tal de mesurar els nivells de compostos organoclorats en el sèrum matern al moment del part, el sèrum de cordó, el sèrum del nen a les 8 setmanes de vida i en el calostre i la llet madura de la mare. Al moment de néixer es va mesurar l'antropometria del nen, als tres dies de vida es van mesurar els nivells de TSH i el patró de les uroporfirines i a l'any de vida es va mesurar el desenvolupament neuroconductual dels nens amb els tests de Bayley i de Griffiths.

RESULTATS

Els resultats van mostrar que tots els nounats presentaven nivells detectables d'HCB, PCBs i p,p'DDE i, en menor percentatge, de β -HCH en el sèrum de cordó. Les concentracions d'HCB van ser les més elevades. La mitjana geomètrica de l'HCB va ser de 1.1 ng/ml, amb un rang de 0.3 a 5.7 ng/ml. Les concentracions d'HCB en sang de cordó es van associar positivament amb les concentracions en la sang materna (coeficient=0.45 $p<0.01$). Els nivells d'OCs es van detectar i quantificar a totes les mostres de calostre i de llet madura (medianes en calostre d'HCB, p,p'DDE i PCBs van ser de 0.90, 1.03 i 0.61 $\mu\text{g/g}$, respectivament). Les concentracions d'aquests compostos en calostre es van correlacionar significativament amb les concentracions del nen a les 8 setmanes de vida.

La concentració de porfirines urinàries va ser de 37.87 $\mu\text{mol/mol}$ creatinina. La coproporfirina I i la coproporfirina III van ser les porfirines més excretades. No es va trobar cap associació positiva entre l'excreció de porfirines urinàries i els nivells d'HCB.

Els nounats prematurs van presentar majors concentracions de p,p'DDE [2.40 ng/ml versus 0.80 ng/ml ($p<0.05$)]. Aquells infants nascuts amb una talla baixa per l'edat gestacional van presentar nivells més alts d'HCB en sèrum de cordó que aquells nounats amb una talla adequada per l'edat gestacional [1.64 ng/ml versus 1.00 ng/ml ($p<0.05$)]. A més, els nivells d'HCB en sèrum de cordó es van associar negativament, amb una relació de dosi-resposta, amb la talla del nounat.

Tots els nounats van presentar concentracions de TSH dins del rang de normalitat dels valors de referència (<25 mU/l). Els nivells d'HCB no es van associar amb els nivells de TSH.

Els nivells de p,p'DDE en sèrum de cordó es van associar negativament amb el desenvolupament mental i psicomotor del nen a l'any de vida. Per cada doblada de la dosi de p,p'DDE es va trobar un decrement resultant de 3.50 (e.s.=1.39) punts en l'escala mental i de 4.01 (e.s.=1.37) punts en l'escala psicomotora. L'exposició prenatal a l'HCB no va tenir cap efecte sobre el desenvolupament neurològic del nen. La lactància materna de llarga durada es va associar amb una millor resposta en les escales mental i psicomotora.

CONCLUSIONS

Els nivells d'HCB i de p,p'DDE en el sèrum de cordó i en el calostre de les mares d'aquesta població són dels més alts mai descrits en poblacions occidentals. L'HCB, de la mateixa manera que altres compostos organoclorats, es transfereix a través de la placenta implicant que les mares

més exposades tinguin fills amb majors concentracions. La lactància materna implica un canvi d'aquests contaminants al cos i incrementa el grau de contaminació dels lactants durant les primeres setmanes de vida.

Les concentracions d'HCB al moment de néixer s'han associat amb una disminució de la talla del nounat al naixement. Tot i que el grau d'exposició a HCB en aquesta població és molt elevat no s'han trobat associacions amb el patró d'excreció de porfirines, les concentracions de TSH al néixer i el desenvolupament neuroconductual a l'any de vida.

Per contra, l'exposició prenatal a p,p'DDE s'ha associat amb prematuritat dels nens i amb un retard en el desenvolupament mental i psicomotor al primer any de vida.

La lactància materna de llarga durada s'ha associat amb una millor resposta en les escales mental i psicomotora.

INTRODUCCIÓ

1. INFÀNCIA I MEDI AMBIENT

“Jo sóc jo i les meves circumstàncies”
Ortega y Gasset

a. INFÀNCIA I MEDI AMBIENT: Un Recorregut Per La Història

Malauradament la història ha posat en evidència en nombroses ocasions l'impacte directe de la contaminació ambiental sobre la salut dels infants. L'any 1955 a Minamata (Japó) una emissió accidental de metilmercuri a les aigües marines va produir que nombroses persones ingerissin aquest compost a partir del consum d'animals marins contaminats (Harada, 1976). En adults es van diagnosticar molts casos de patologia neurològica i, a partir del 1958, es va observar que molts dels nens nascuts després de l'accident presentaven retards cognitius molt severos (figura 1). Aquest fet va posar en evidència la vulnerabilitat de l'ésser humà durant la vida intrauterina, ja que pels nens nascuts després de l'accident l'única via d'incorporació al metilmercuri hauria estat la de la mare durant la gestació. Va ser en aquell moment quan es va començar a especular per primera vegada sobre la possibilitat de que el metilmercuri hagués passat la barrera transplacentària o la llet materna provocant els efectes neurològics tan severos observats en els nens (Myers et al., 1998).

A finals dels anys 50, a Turquia, es va estar utilitzant l'hexaclorobenzè (HCB) com a fungicida en el tractament de les llavors de blat. Al ser una època d'escassetat, molts dels nadius van utilitzar aquestes llavors tractades com a aliment directe. Les conseqüències del consum no intencionat d'HCB en els nens van ser deplorables. Almenys un 95% dels nens afectats van morir en el període d'un any i, en moltes poblacions, entre 1955 i 1960, no va quedar cap nen viu d'entre 2 i 5 anys (Peters, 1976). La majoria d'aquests nens va morir amb debilitat, convulsions i un eritema cutani anomenat “Pembe Yara” (Peters et al., 1966).

L'any 1968, més de 1000 persones a Kyushu (Japó) van consumir olis contaminats per bifenils policlorats (PCBs) (Kuratsune et al., 1971), donant lloc a una malaltia anomenada *Yusho* (malaltia de l'oli), que va consistir en cloracnè i diferents anomalies hepàtiques i del sistema nerviós. A més, els nens més exposats a PCBs durant la vida intrauterina van presentar un retard important en el creixement intrauterí (figura 2) i un lleuger retard mental posterior (Harada, 1976).

L'any 1979 es va produir un accident similar a Taiwan amb olis de consum contaminats per PCBs i dibenzofurans. La malaltia derivada del consum d'aquests olis es va anomenar *Yucheng* i va consistir en cloracnè, hiperpigmentació de la pell i canvis en el desenvolupament neuroconductual dels nens (Hsu et al., 1985; Rogan et al., 1988). Els nens es van exposar a aquests contaminants únicament a través de la placenta, ja que, com a mesura preventiva es va recomanar a les mares afectades que substituïssin la lactància materna per l'artificial. El grau d'intoxicació a Taiwan no va ser tan important com el del Japó i, per tant, els efectes sobre la salut dels nens no van ser tan severos i clínicament rellevants.



Figura 1. Nen de 14 anys. Retard mental, microcefàlia incoordinació, nistagmus, hiperquinèsia, deformitats als dits i a la columna, disàrtria i hipersalivació. Exposició intrauterina al mercuri (Japó, 1955) (Harada, 1976).

Figura 2. Nounat amb retard del creixement intrauterí i hiperpigmentació de la pell en comparació amb un nounat normal. Exposició intrauterina als PCBs (Japó, 1968) (Harada, 1976).



b. Infància i MEDI AMBIENT: Compostos Tòxics Persistents

Els éssers humans vivim en contacte permanent amb els contaminants ambientals ja des del moment de la concepció. Quasi la totalitat dels nounats de qualsevol racó del món presenten nivells detectables de compostos tòxics persistents (CTPs). Els CTPs són compostos químicament molt estables, de volatilitat mitja, molt hidròfobs i tòxics (Solomon et al., 2002a). Tenen una vida mitjana de 10 anys o més, una tensió de vapor suficientment gran com per poder ser transportats a través de l'atmosfera i una elevada hidrofobicitat que fa que s'acumulin a la matèria orgànica de sòls i sediments i en els organismes vius (Ockende et al., 2003). Les mares passen aquests compostos als seus fills a través de la placenta i de la lactància materna (Rhainds et al., 1999). Aquests compostos van crear alarma als anys 60 després de la publicació del llibre "Silent Spring" de Raquel Carson que advertia de la desaparició de diverses famílies d'ocells degut a l'acció del DDT i de la possible transcendència que aquest fet podria tenir en els humans (Carson, 1962). Als anys 90 Theo Colborn al seu llibre "Our Stolen Future" advertia dels efectes d'aquests compostos com a disruptors hormonals (Colborn, 1996). D'aquestes dues reflexions se'n podia derivar que l'ésser humà podria arribar a estar afectat per agents químics així com ho estaven els animals, que la disfunció en el funcionament d'alguns sistemes podria implicar un impacte de magnituds similars al de malalties com el càncer i, que els contaminants podrien tenir un efecte transgeneracional i afectar a les generacions següents a la contaminada (Goldman et al., 2000).

La majoria d'aquests compostos han estat sintetitzats per les seves propietats com a pesticides o per usos industrials (Carpenter et al., 1998). Degut a les seves propietats químiques tenen una gran capacitat per arribar a zones remotes. El fenomen de la distribució planetària dels CTPs és potser un dels exemples que justifica de manera més clara la màxima de "Pensa globalment, actua localment", ja que l'ús local d'aquests compostos té efectes globals que poden arribar a ser més intensos en zones aïllades que en els seus punt d'ús. Als anys 80 va semblar que el problema dels CTPs quedava resolt amb la prohibició del seu ús per part de la majoria de països desenvolupats. De totes maneres, actualment se sap que, tot i que els nivells han disminuït, aquests compostos segueixen àmpliament distribuïts per tot el planeta (Solomon et al., 2002b).

c. INFÀNCIA i Medi Ambient: Els Nens No Són Petits Adults

Cada vegada existeixen més evidències que exposicions ambientals (per exemple al plom (Bellinger et al., 1987) o al mercuri (Davidson et al., 1998; Grandjean et al., 1998) poden afectar el creixement i el desenvolupament neuroconductual de l'infant en períodes crítics, implicant efectes sobre la funció sensorial, cognitiva o motora (Tilson et al., 1998), fins i tot a nivells d'exposició baixos.

Els infants tenen un risc major de patir els efectes dels contaminants ambientals ja que es comporten de manera diferent als adults i perquè físicament són més vulnerables. Els seus sistemes neurològic, immunològic i digestiu, junt amb altres sistemes del cos humà, es troben encara en vies de desenvolupament (Olsen, 2000). A més a més, els nens consumeixen més aliments, beuen més líquids i respiren més aire que els adults en proporció a la massa del seu cos, i els seus patrons de conducta, com gatejar o col·locar-se objectes a la boca, poden implicar una major exposició a alguns contaminants ambientals (www.epa.gov).

En aquests moments hi ha molt motius per pensar que els factors etiològics de moltes malalties cròniques s'han acumulat a l'organisme des de les primeres etapes de la vida i, que els factors relacionats en les etapes embrionària i fetal són de vital importància (Weiss, 2000a). Es coneix per models experimentals que les exposicions a contaminants durant la vida intrauterina s'associen a retards en el desenvolupament i el creixement de les cries (Eriksson, 1996). No és descabellat

pensar, doncs, que l'estructura cel·lular dels òrgans depengui parcialment de l'ambient intrauterí i no només de la càrrega genètica.

El sistema nerviós central (SNC) en desenvolupament és el sistema orgànic on s'hi ha observat una major prevalença d'anomalies congènites. La majoria de tòxics són més eficaços mentre el cervell es troba en fases de desenvolupament que quan ja és madur, ja que el SNC no ha desenvolupat encara les barreres suficients. Donat que el sistema nerviós requereix un temps de desenvolupament major que la resta d'òrgans (figura 3), és presumible pensar que el període on es pot ocasionar dany sigui major que per la resta d'òrgans i que fins i tot arribi al període perinatal (Rodier, 1994).

L'exposició a teratògens, a part de ser responsable d'algunes malformacions, també pot conduir a simples dèficits en el funcionament d'algun òrgan. Aquests canvis funcionals poden ser suficients per causar una patologia concreta a llarg terme (Weiss, 2000a). La recerca epidemiològica s'ha basat clàssicament en l'estudi de la causalitat de diferents processos patològics clarament definits com a malalties o síndromes. En els darrers anys hi ha hagut un creixent interès en l'estudi de trastorns funcionals que, més que estudiar patologies, estudia petites alteracions que tot i estar dins la normalitat poden implicar alteracions a nivell poblacional si la variable d'exposició es troba present en tots els éssers humans. L'estudi d'aquests trastorns funcionals a èpoques molt primerenques de la vida obra les portes a un camp molt important per la salut pública: la possibilitat de poder aplicar mesures de prevenció per reduir o eradicar el problema.

Si bé és cert que l'enverinament sever amb conseqüències clíniques importants queda, afortunadament, restringit a un nombre limitat d'individus, el nombre d'individus amb manifestacions subclíniques (p.e. en el cas d'afectacions neurològiques només detectables amb tests neuropsicològics) afectaria a una població molt més àmplia. I encara molt més gran seria la població exposada de forma latent que només presentaria un quadre clínic si hi hagués un factor de risc afegit (p.e. exposició a un altre compost, o altres problemes de salut). Aquest fenomen queda molt ben il·lustrat a la piràmide que Weiss presenta al seu article (Weiss, 2000a) (figura 4). Una disminució petita en el nombre de connexions nervioses al principi de la vida podria ampliar-se al final i avançar de forma notable l'aparició de malalties com les demències (figura 5).

Figura 3. Desenvolupament del sistema nerviós durant el període prenatal i postnatal (Herschkowitz et al., 1997).

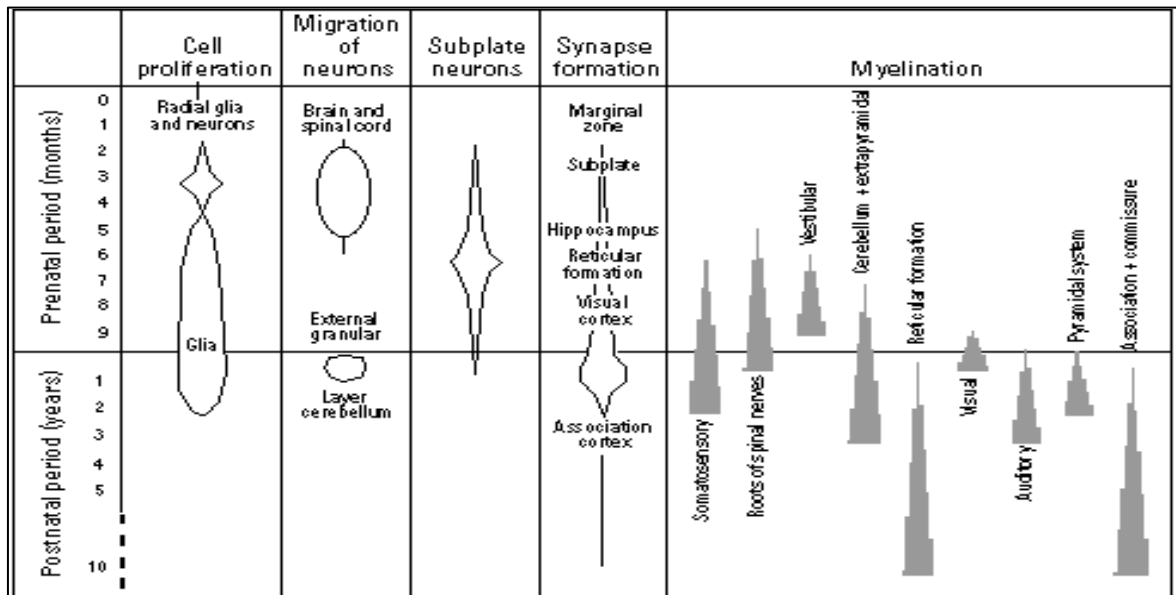


Figura 4. Piràmide de toxicitat pels pesticides (Weiss, 2000a).

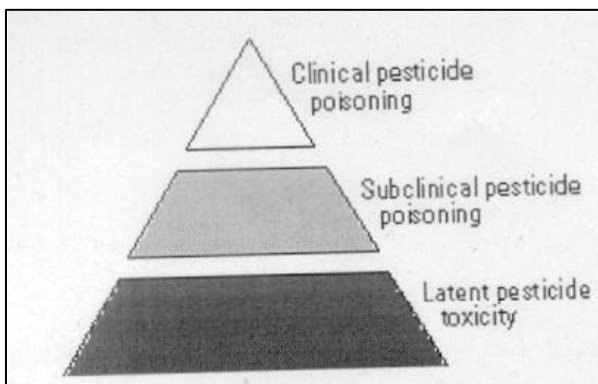
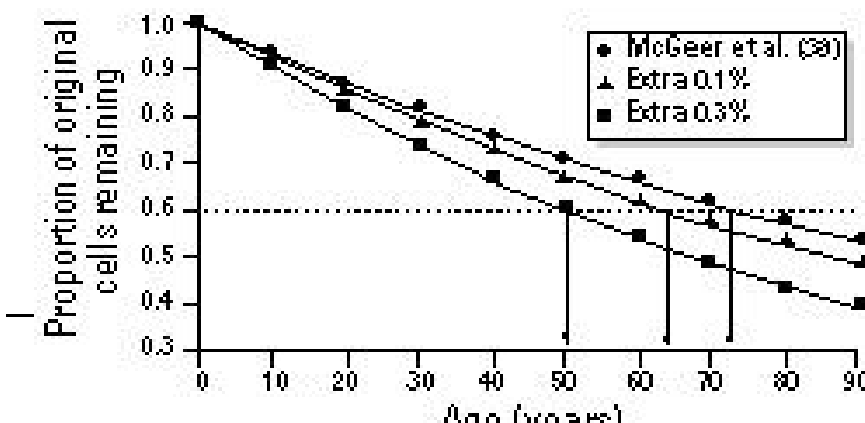


Figura 5. Pèrdua de la proporció de connexions interneuronals en funció de l'edat i del nivell inicial (Weiss, 2000a).



2. LA POBLACIÓ DE FLIX

“- Hi ha coses que no s’esborren mai de la vida.

- Mai.

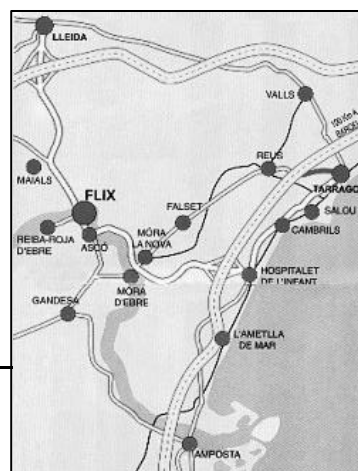
- Són com l’Ebre i com el Segre. - va intervenir la Teresa - L’aigua mai no acaba de passar.”

Jesús Moncada. Fragment de *Camí de Sirga*

a. FLIX: El Poble

Flix és un municipi de la Ribera d'Ebre, a la província de Tarragona, a banda i banda de l'Ebre, amb una extensió de 116,29 km², al límit de les comarques de les Garrigues i el Segrià (figura 6). Té una població relativament estable des dels anys 70, amb uns 5.000 habitants. La seva activitat econòmica bàsica és la indústria, a la qual s’hi dedica la majoria de la població. L'agricultura, actualment secundària en l'economia del poble, és principalment de secà. Els conreus més importants es distribueixen de la següent manera: domina l'olivera, seguida dels ametllers i en menys proporció, la vinya i els cereals. La vinya havia estat el conreu més important fins als anys 70.

Els orígens de Flix es remunten als poblats ibers. Els romans sembla que li donaren el seu nom, *Flexus* o retomb, en referència al tomb del riu envoltant la vila. Ja a les acaballes del segle XIX es construí l'Electroquímica de Flix, S.A. (actualment anomenada Ercros) amb capitals i tècnics alemanys, construint-se una de les primeres electròlisis d'Europa. Aquest fet ha marcat el caràcter industrial de la població de Flix, una població que a partir d'aquest nucli fabril ha sabut organitzar-se a nivell associatiu (www.flix.altanet.org).



b. FLIX: L'Empresa

Els orígens de la fàbrica de Flix (figura 7) es remunten al 1897 quan un grup d'industrials espanyols, alemanys i suïssos es van associar per constituir la Societat Electroquímica de Flix, a la ribera del riu Ebre a la província de Tarragona. Aquest complex químic va ser el pioner en el sector de la indústria química bàsica a Espanya i antecedent dels polígons de Tarragona.

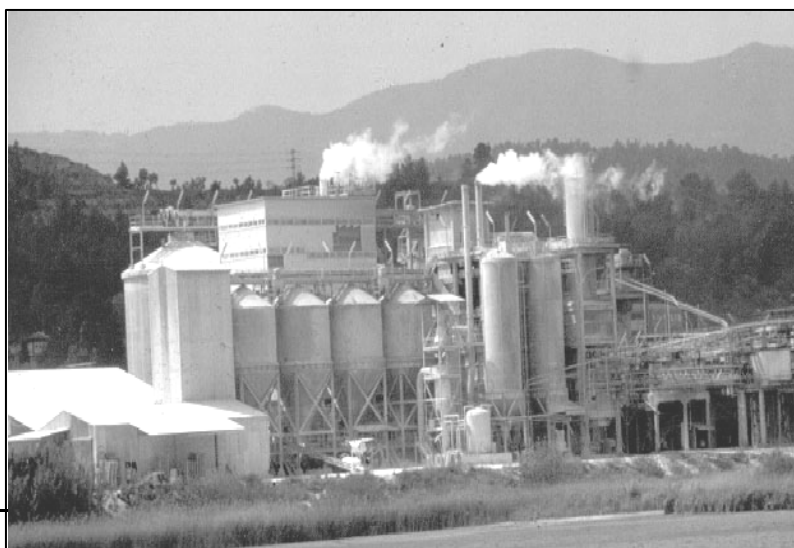
La fàbrica de Flix fou la primera a Espanya i la tercera a Europa en dur a terme el canvi tecnològic experimentat per la indústria química tradicional al fer servir l'electricitat en el procés de fabricació del clor.

A l'actualitat l'activitat productiva de la fàbrica es centra principalment en la fabricació de clor i de sosa, derivats del clor, dissolvents clorats i fosfat bicàlcic.

Des dels seus inicis la fàbrica presenta un producció integrada en el centre de la qual s'hi ubica la planta de producció de clor i de sosa. La resta d'instal·lacions es proveeixen d'aquests productes com a matèries primeres bàsiques en la producció d'hipoclorit sòdic i dissolvents, clorometans, àcid clorhídric i fosfat bicàlcic (www.ercros.es).

Des de 1996 disposa d'una estació depuradora d'aigües residuals on es tracten els efluent líquids procedents de les plantes de clor i fosfat bicàlcic.

Figura 7. L'empresa electroquímica de Flix



c. FLIX: La Recerca En Epidemiologia Ambiental

L'any 1989 a Flix es van detectar nivells inusualment elevats d'un compost químic anomenat hexaclorobenzè (HCB) en l'atmosfera. L'estudi elaborat pel departament de Química Ambiental del Consell Superior d'Investigacions Científiques va descriure uns nivells mitjans d'HCB per períodes de 24 hores unes 100 vegades superiors als obtinguts en una estació de control situada a Barcelona (Grimalt et al., 1994).

Per les característiques d'aquesta contaminació i l'interès de la comunitat científica pels organoclorats en general es va creure convenient comunicar aquestes troballes a la Unitat de Recerca Respiratòria i Ambiental de l'Institut Municipal d'Investigació Mèdica. En el marc d'aquesta unitat es va realitzar un estudi de la mortalitat del període 1984-1991 i de la incidència del càncer del període 1980-89 d'aquesta població. Els resultats van mostrar un augment de la incidència de càncer de tiroide (RIE=6.7; IC=1.6-28) i de sarcoma de teixits tous (RIE=5.5; IC=1.7-17.5) (Grimalt et al., 1994).

Amb aquests antecedents es va preparar un projecte de recerca per estudiar en profunditat l'exposició a HCB i a altres compostos organoclorats de la població de Flix i dels seus efectes sobre la salut en els seus habitants. La població adulta presentava l'any 1994 els nivells en sèrum més alts dels mai descrits en la literatura (mitjana: 17 ng/ml; rang: 1.1-222) entre la població no exposada laboralment. Els nivells en els treballadors arribaven a valors de 280 ng/ml (Sala et al., 1999). Les conclusions que se'n van derivar van ser que la població general de Flix tenia els nivells d'HCB en sèrum més alts descrits mai i que aquesta exposició s'associava fortament a l'ocupació a l'empresa electroquímica; i que la salut percebuda i el nombre de trastorns crònics declarats a la població de Flix no diferien de l'observat a la població general d'altres àrees (Tesi Maria Sala, 1997). Entre els efectes sobre la salut dels habitants va destacar una manca de relació amb els nivells de porfirines urinàries (Herrero et al., 1999) i una moderada afectació metabòlica de la funció tiroïdea (disminució de 0.32 µg/dl de T4 per cada unitat, ln ng/ml, d'augment d'HCB)

i de l'activitat en la inducció enzimàtica (augment del 10% de nivells de GGT per cada unitat logarítmica d'augment d'HCB) (Sala et al., 2001).

Aquest estudis, però, deixaven un gran buit en el paper que aquests contaminants jugaven sobre la salut dels infants.

3. COMPOSTOS ORGANOCLORATS, MARE I NEN

“Una mujer morena,
resuelta en luna,
se derrama hilo a hilo
sobre la cuna.
Ríete, niño,
que te tragas la luna
cuando es preciso.”

Miguel Hernández. Fragment de *Nanas de cebolla*

a. COMPOSTOS ORGANOCLORATS, Mare i Nen: Història Dels OCs

Els compostos organoclorats (OCs) formen part dels compostos tòxics persistents (CTPs) per la seva presència en tot el planeta, la seva bioestabilitat i lenta biodegradació, la seva acumulació en teixits grassos i la seva vida mitja llarga (Jensen, 1987). Com el seu nom indica els OCs són compostos químics orgànics on alguns o la totalitat dels àtoms d'hidrogen es substitueixen per clor. La producció i l'ús intensiu d'aquests compostos es va iniciar als anys 30 en processos industrials (producció d'aïllants) i en processos agrícoles (pesticides). Molts d'aquests compostos estan actualment prohibits, però segueixen estant presents en tots els éssers humans degut al seu ús en països del tercer món, la seva lenta biodegradació i la seva formació actual com a productes dins la síntesi de dissolvents clorats.

Els beneficis d'aquests compostos com a pesticides sintètics són innegables, però la preocupació pels seus possibles efectes adversos sobre la salut a llarg termini ha anat en augment en els darrers temps.

L'hexaclorobenzè (HCB) és un compost clorat format a partir del benzè per substitució dels seus àtoms d'hidrogen per clor (C6 Cl6) (Figura 8). Aquesta configuració li dona una gran estabilitat química i degut a aquesta estabilitat i la seva baixa solubilitat en aigua (6.2 ng/L a 25°C) fa que s'acumuli a les cadenes tròfiques i es distribueixi per tot el planeta (Morris et al., 1986).

L'HCB és un fungicida que s'usava principalment per evitar que el blat fos atacat per la càries del blat (*trilletia tritici*) i pel tractament dels sòls (Morris et al., 1986). Molts països en vies de desenvolupament encara ara l'utilitzen per fumigar el blat. També ha trobat aplicacions com a

ignífug i com a plastificant. És un producte bàsic per la síntesi de diferents compostos orgànics clorats. L'HCB és la base per la producció de pentaclorofenol (PCP). L'HCB pot aparèixer com a subproducte de la cloració industrial d'hidrocarburs. Ingressa al medi ambient per combustió de productes que contenen clor (p.e. per la incineració de residus) o a través de l'ús de pesticides contaminats amb aquesta substància (Jacoff et al., 1996). La seva producció industrial es va iniciar a principis dels anys 30 i es va prohibir en la majoria de països occidentals a la dècada dels anys 70. De totes maneres, a Espanya el seu ús va continuar fins a principis de 1986 (Barbera,1989).

A Espanya el DDT es va utilitzar àmpliament com a plaguicida des de meitats dels anys 50 fins la meitat de la dècada dels 70. Tot i que aquest compost es va prohibir a finals dels anys 70 no queda molt clar quan va acabar realment el seu ús (Camps et al., 1989; Hernandez et al., 1993). A països en vies de desenvolupament el DDT es va fer servir extensivament en el passat per controlar la malària i el tifus. El DDT es metabolitza molt ràpidament a p,p'-diclorodifenildicloroetilè (p,p'DDE) (figura 9) i, per tant, en el medi ambient trobem més concentracions de p,p'DDE que de DDT.

Els bifenils policlorats (PCBs) engloben uns 209 congèneres possibles amb diferent nombre de molècules de clor i planaritat variable (figura 10) (Giesy et al., 1998). Els PCBs s'han utilitzat àmpliament des de finals dels anys 20 en els transformadors elèctrics, com a estabilitzants de pintures, polímers i adhesius o com a lubricants en diversos processos industrials entre d'altres (IPCS, 1993). Actualment la seva producció està prohibida a la majoria de països industrialitzats i el seu ús es restringeix als sistemes elèctrics tancats, transformadors i condensadors de les indústries. Malgrat tot, el seu ús en països en vies de desenvolupament, els abocaments incontrolats o accidentals fan que els PCBs es continuïn incorporant al medi ambient (Dewailly et al., 1996).

L'hexaclorociclohexà (HCH) és un pesticida que es va introduir durant els anys 40. Dels diferents isòmers existents l' α -HCH i el γ -HCH (conegut com a lindà) són els que més s'han usat com a pesticides. De totes maneres, són els isòmers α - i β - els que han mostrat una toxicitat més

elevada en humans. D'aquests isòmers, el $\beta\beta$ -HCH és el que es bioacumula més fàcilment i és més resistent a la metabolització (Li et al., 2002).

Degut a la seva gran inèrcia química, els OCs s'han dispersat i distribuït per tot el planeta. En humans la via d'exposició majoritària és a través de la dieta i diversos estudis han mostrat que a Espanya molts dels aliments de consum habitual (carns, peixos, ous, llets i derivats làctics) contenen traces de p,p'DDE, PCBs, HCB i isòmers de l'hexaclorociclohexà (Hernandez et al., 1994). A més, un estudi recent mostra que Espanya és un dels països on la presència d'aquests compostos en aliments com la mantega és més elevada (Kalantzi et al. 2001).

Figura 8. HCB

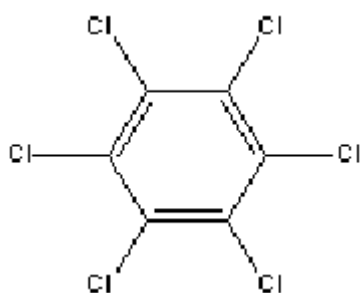


Figura 9. p,p'DDE

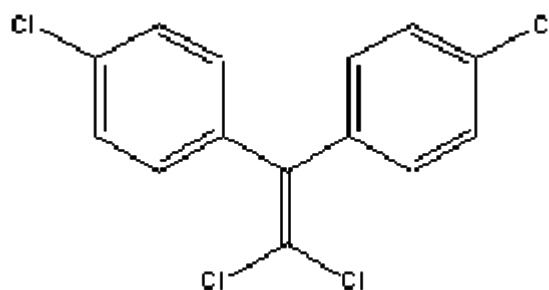
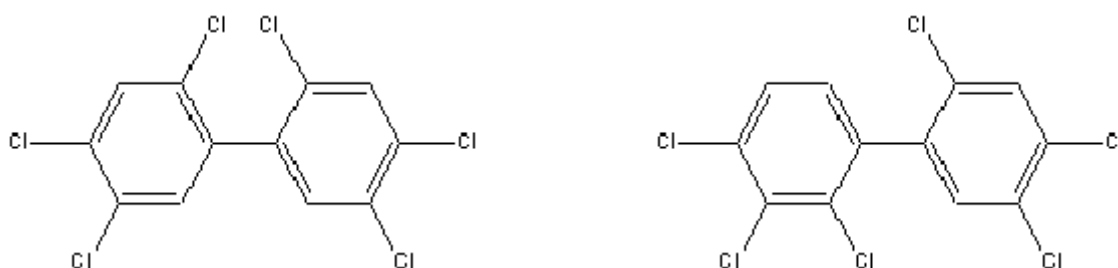


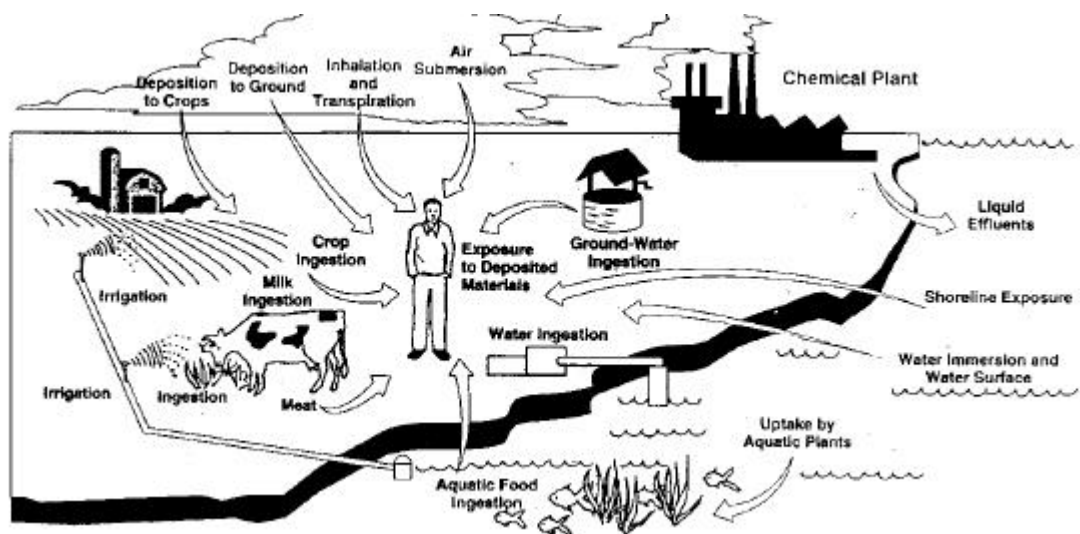
Figura 10. PCBs



b. Compostos Organoclorats, MARE i Nen: La Mare Com A Font Contaminant

Les vies actuals d'exposició als compostos organoclorats en humans són principalment a través de la dieta de productes amb un alt contingut lipídic degut a la seva presència en aigua i aliments (MacIntosh et al., 1996), tot i que existeixen altres vies d'exposició (figura 11) com en el cas de l'hexaclorobenzè a Flix on l'exposició és majoritàriament a través de l'aire. En l'home, aquests compostos s'emmagatzemen en òrgans rics en greix, com el fetge i el cervell (Dewailly et al., 1999). També els trobem en el sèrum i en la llet materna. Degut a la seva capacitat per travessar la barrera transplacentària i la seva presència en la llet materna, els podem detectar en teixits humans des del naixement (Huisman et al., 1995a, Rhainds et al., 1999). S'estima que els lactants incorporen 20 vegades més que els adults i que durant els 3 primers mesos de vida s'arriba a acumular el 6% de tot el que s'acumularà durant la resta de la vida (Karmaus et al., 2001a; van Larebeke et al., 2001). En nens s'han descrit, a més, altres vies d'incorporació diferents a les dels adults, com l'ús domèstic de plaguicides (Spann et al., 2000), la contaminació de l'ambient domèstic per plaguicides en els fills dels treballadors exposats (Loewenherz et al., 1997) o l'exposició a les escoles quan es realitzen tractaments periòdics amb aquests productes (www.epa.gov).

Figura 11. Vies d'exposició als contaminants ambientals (adaptat del U.S. Department of Energy materials).



La lactància materna té uns efectes beneficiosos per la mare i pel nen reconeguts per això es recomana per sobre de les lactàncies artificials (Anderson et al., 1999, Jacobson et al., 1999). De totes maneres, per les seves característiques lipídiques, la llet materna acumula molts tòxics ambientals com els compostos clorats i el propi HCB (Sim et al., 1992). En aquests casos la lactància materna pot arribar a estar contraindicada ja que pot ser una via d'incorporació d'altres concentracions d'aquests compostos en el nounat (Boersma, 2001). La lactància, en aquests casos, suposa un mecanisme de detoxificació per la dona, en detriment del nounat. S'ha observat, efectivament, com la lactància disminueix la concentració d'organoclorats de la mare (Mes et al., 1993). A l'accident de Taiwan es va recomanar a totes les dones no exposades que no donessin el pit als seus fills per evitar la possible transferència de PCBs a través de la lactància. A Turquia es va descriure que l'abandonament de la lactància es va associar a un deteriorament menys ràpid dels infants amb la síndrome de la Pembe Yara.

Per altra banda es coneix que la llet materna és molt rica en àcids grassos poliinsaturats de cadena llarga i que aquests àcids grassos són components estructurals essencials de tots els teixits del cos humà (Lucas et al., 1999). El sistema nerviós és particularment ric en aquests àcids grassos de cadena llarga (Uauy et al., 2001). El paper d'aquests àcids grassos, com l'omega 3, podria explicar perquè la lactància materna promou un millor desenvolupament i creixement del fetus (Olsen, 1993), tot i que hi ha altres explicacions a aquesta troballa, com ara les hipòtesis genètiques que afirmen que les dones que donen el pit tenen un quocient intel·lectual més alt o el major grau d'estimulació ambiental i afectiva en l'al·letament matern (Olsen, 2000) .

Tot i això, el cost/benefici de la lactància materna és un debat que continua obert (Horwood et al., 1998; Mortensen et al., 2002). Per això, la Organització Mundial de la Salut (OMS), en l'avaluació dels riscos de IHCB, va recomanar estudiar adequadament el risc per la salut infantil que suposa l'exposició a l'HCB i a altres compostos relacionats a través de la llet materna (IPCS, 1996).

c. Els Compostos Organoclorats, la Mare i EL NEN: Toxicitat Dels OCs Al Nen

El grau d'impacte d'aquests compostos sobre la salut dels infants és un tema de preocupació mundial (Ross et al., 2000). Entre els diferents compostos organoclorats, els PCBs han estat els més estudiats, degut als dos episodis de contaminació prèviament descrits. En adults, l'exposició a OCs s'ha vist associada amb càncer, malaltia cardiovascular i alteracions endocrines (Kogevinas, 2001). L'exposició als OCs a través de la placenta s'ha relacionat amb una reducció de la intel·ligència i amb alteracions del comportament durant la infància en diferents estudis longitudinals realitzats a Àsia, Europa i Estats Units (Harada, 1976; Rogan et al., 1988, Yu et al., 1991; Chen et al., 1992; Chen et al., 1994a; Lai et al., 1994; Chen et al., 1994b; Yu et al., 1994; Guo et al., 1994; Rogan et al., 1986; Gladen et al. 1988; Rogan et al., 1991; Gladen et al., 1991; Jacobson et al., 1984; Jacobson et al., 1985; Jacobson et al., 1990a; Jacobson et al. 1990b; Jacobson et al., 1992; Jacobson et al., 1996; Huisman et al., 1995a; Huisman et al., 1995b; Lanting et al., 1998; Patandin et al., 1999a; Lonky et al., 1996; Stewart et al., 2000; Darvill et al., 2000 ; Winneke et al., 1998; Walkowiak et al., 2001). En conjunt, els estudis de cohorts en noutats han demostrat una lleugera associació entre l'exposició prenatal a PCBs i el desenvolupament motor i cognitiu posterior dels nens estudiats. De totes maneres, la manca d'estandardització en les mesures d'avaluació del desenvolupament neurològic i en les mesures d'exposició no permeten concloure si l'associació és consistent. Es desconeix si l'efecte dels PCBs pot venir confós per altres exposicions (altres compostos organoclorats, el mercuri o el plom entre d'altres) i també si es tracta de trastorns transitoris o permanents fins l'edat escolar (Vreugdenhil et al., 2002a; Vreugdenhil et al., 2002b). El rol dels altres compostos organoclorats sobre el desenvolupament neuroconductual dels nens no ha estat tan estudiat.

Durant aquests darrers 20 anys s'ha intentat comprendre quines són les possibles bases cel·lulars dels efectes observats, en models animals i en models in-vitro. Els models in-vitro han mostrat que els PCBs produeixen una disminució dels nivells de dopamina intracel·lular (Shain et al., 1991; Chishti et al., 1996). En rates i en primats s'han observat canvis en la funció dopaminèrgica (Seegal et al., 1998), i en l'activitat locomotora (Eriksson, 1996), conductual (Eriksson, 1997) i en les funcions de memòria (Eriksson, 1996; Schantz et al., 1996). El mecanisme pel qual els PCBs tindrien un efecte dopaminèrgic és desconegut.

Per tant, els mecanismes pels quals els tòxics ambientals podrien produir un retard cognitiu són també un enigma (Weiss et al., 2000b). Com s'ha comentat anteriorment, el fet que la finestra de

vulnerabilitat del sistema nerviós és la més llarga de tots els sistemes, sobretot la fase de connexió (sinaptogènesi i mielinització) implica que el temps de vida on es pot produir dany és molt més extens.

Una de les hipòtesis més sòlides es refereix al possible paper de la funció tiroïdea en el desenvolupament neurològic (Porterfield, 2000). En models animals, s'ha demostrat que els compostos organoclorats poden alterar la síntesi i secreció a nivell hipofisari, o competir en el transport i el pas a través de la membrana cel·lular, o fins i tot alterar els gens reguladors de les hormones tiroïdees (Schantz et al., 2001). Estudis epidemiològics recents han observat una afectació de la funció tiroïdea associada als nivells de PCBs (Osius et al., 1999). A la població adulta de Flix es va demostrar una relació dosi-resposta entre els nivells d'HCB i de PCBs amb una disminució de T4 lliure (Sala et al., 2001).

L'evidència científica presentada al voltant de l'efecte dels disruptors endocrins (substàncies capaces d'alterar l'homeostasi hormonal) sobre les etapes embrionària i fetal en espècies animals, en les que s'ha identificat l'agent(s) causal, suggereix que aquesta etapa és crítica tant per la susceptibilitat particular del propi desenvolupament orgànic com pel desenvolupament funcional, que pot manifestar-se de forma més tardana transcorreguts uns anys de vida. Per tant, la complexitat de l'estudi de l'epidemiologia ambiental en les primeres etapes de la vida rau en el fet que l'expressió del dany pot manifestar-se únicament en el fracàs d'una funció específica, i que l'agent causal generalment més que únic és múltiple amb accions sinèrgiques, additives o antagoniques.

L'exposició a diferents compostos tòxics durant la gestació pot induir retards importants en el creixement intrauterí. En el cas dels compostos organoclorats s'ha descrit baix pes al néixer (Rogan et al., 1988; Guo et al., 1995; Patandin et al., 1998) i pels PCBs i el p,p'DDE s'ha observat una relació amb la prematuritat (Fein et al., 1984; Longnecker et al., 2001; Torres-Arreola et al., 2003). Un estudi recent ha observat que el p,p'DDE s'associa amb una talla més baixa en nenes de 9 anys d'edat, però en nens no s'observa aquesta associació (Karmaus et al.,

2002). Els OCs també són tòxics pel desenvolupament reproductiu. En humans s'ha observat que l'exposició a p',p'DDE i a PCBs s'ha associat amb pubertat precoç (Gladden et al., 2000).

L'hexaclorobenzè és potser un dels compostos organoclorats menys estudiats, no existeix cap estudi longitudinal que hagi avaluat l'impacte d'aquest compost sobre la salut dels nens. A part de l'accident a Turquia la informació sobre la toxicitat de l'HCB es basa en estudis experimentals. En animals d'experimentació l'HCB té una ampla gamma d'efectes que van des de la inducció de porfíria, la inducció de carcinogènesi (Cabral et al., 1977; Cabral et al., 1979), l'afectació del teixit reproductiu, l'alteració de la reproducció (Foster et al., 1992a; Foster et al., 1992b), i alteracions en el desenvolupament neuroconductual en les cries exposades via transplacentària i a través de la lactància (Goldey et al., 1992). En models animals exposats a HCB s'han observat també alteracions en el sistema immunitari (Michielsen et al., 1999a; Michielsen et al., 1999b; Michielsen et al., 2000; Michielsen et al., 2001). Entre els efectes del sistema immunitari en humans produïts per xenobiòtics, existeixen evidències que els OCs s'associen amb els nivells de IgE al moment de néixer (Reichrtova et al., 1999) i en edat escolar (Karmaus et al., 2001b) i amb un augment de la patologia infecciosa (Dewailly et al., 2000).

JUSTIFICACIÓ

“Només cal fer una ullada al panorama mundial actual per veure les conseqüències de dècades de fabricació i d’alliberació al medi ambient de mils de nous productes químics sintètics. Pollastres a Bèlgica carregats de dioxines; éssers humans i vida silvestre contaminada pel DDT, PCBs i altres productes químics; pesticides al nostre menjar; comunitats de l’Àrtic amenaçades per productes químics que arriben des de zones agrícoles i industrialitzades dels països desenvolupats, són només una mostra de l’elevada toxicitat de la majoria dels productes químics que la nostra societat ha generat (www.greenpeace.org).”

Tot i la preocupació de l’impacte d’aquests compostos sobre la salut de les persones, existeixen pocs estudis de cohorts que n’avaluïn els seus efectes sobre la salut dels infants. La població de Flix ofereix una oportunitat única per estudiar la transferència de l’HCB a través de la placenta i de la lactància, i avaluar-ne els seus efectes sobre la salut infantil de la població, ja que es tracta d’una població important (5000 habitants) exposada crònicament i específica a uns nivells inusualment elevats d’HCB. Degut a que la taxa de natalitat de Flix era molt baixa (menys de 25 naixements per any) es va ampliar la cohort a les poblacions veïnes pertanyents a l’Àrea Bàsica de Salut de Flix (Ribarroja, Vinebre, La Palma d’Ebre, Ascó i La Torre de l’Espanyol). Aquest estudi ha permès per primera vegada estudiar amb dades de dosi interna els efectes de l’exposició a l’HCB sobre el desenvolupament neuroconductual dels nens. Aquest fet té una aplicació important en la salut pública de la població d’estudi, però també en la comunitat internacional on l’HCB és un dels tòxics ambientals més comuns. Aquest estudi segueix les recomanacions de la OMS on, a més d’investigar el mecanisme carcinogènic de l’HCB i la seva toxicitat sobre la tiroide, el sistema reproductiu i el metabolisme de les porfíries, proposa desenvolupar tècniques adequades per avaluar els defectes de l’HCB sobre el desenvolupament neuroconductual dels nens i valorar el rol de la lactància materna en les zones d’alta exposició.

OBJECTIUS

OBJECTIUS GENERALS

1. Determinar la transferència de l'hexaclorobenzè i d'altres compostos organoclorats a través de la placenta i de lactància materna i determinar el nivell d'exposició i la dosi interna d'aquests compostos als nounats de Flix i de les poblacions veïnes.
2. Determinar l'estat de salut dels infants de la població de Flix, especialment la presència de trastorns relacionats amb l'exposició a l'HCB i als compostos organoclorats en general.

OBJECTIUS ESPECÍFICS

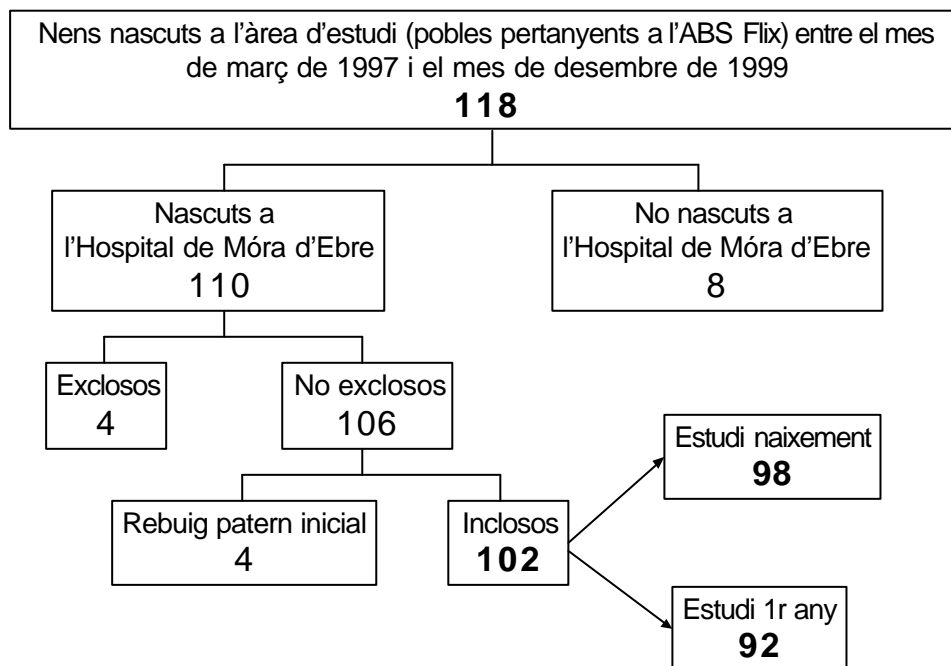
1. Realitzar una revisió sistemàtica sobre l'impacte dels compostos organoclorats sobre el desenvolupament neuroconductual del nen.
2. Analitzar la transferència dels compostos organoclorats a través de la placenta.
3. Analitzar la transferència dels compostos organoclorats a través de la lactància.
4. Analitzar l'associació entre l'exposició prenatal als compostos organoclorats i els nivells d'uroporfirines al néixer.
5. Analitzar l'associació entre l'exposició prenatal als compostos organoclorats i les mides antropomètriques al néixer.
6. Analitzar l'associació entre l'exposició prenatal als compostos organoclorats i els nivells de TSH al naixement.
7. Analitzar l'associació entre l'exposició pre- i postnatal als compostos organoclorats i el desenvolupament neuroconductual del nen al primer any de vida.

METODOLOGIA I RESULTATS

Entre març de 1997 i desembre de 1999 es van reclutar els nounats de famílies residents a la població de Flix i a les cinc poblacions veïnes pertanyents a la mateixa Àrea Bàsica de Salut de Flix (Vinebre, La Torre de l'Espanyol, Ascó, Ribarroja d'Ebre i la Palma d'Ebre). Durant aquests mesos van néixer 118 nens dels quals 8 no van néixer a l'hospital de Móra d'Ebre i quatre van ser exclosos (dos fills de famílies immigrants amb residència variable a la zona i dos bessons) (figura 12).

Dels 106 nens restants, quatre no van participar per rebuig de les famílies. Dels 102 nens inclosos a la cohort, 98 (83% de tots els naixements) van participar a l'estudi sobre els efectes d'aquests compostos en les hormones tiroïdees i en les mesures antropomètriques al moment de néixer. Els quatre nens que no van participar va ser perquè no es va disposar de la informació relacionada amb les variables d'interès.

Figura 12. Esquema participants de l'estudi



92 dels nens inclosos a la cohort van participar en el seguiment fins a l'any d'edat. Dels deu nens que no van participar, en sis casos va ser per rebuig per part dels pares i en quatre casos per emigració de les famílies. L'estudi fou aprovat pel Comitè Ètic de l'Institut Municipal d'Investigació Mèdica.

Es va dissenyar un estudi longitudinal per tal de mesurar els nivells de compostos organoclorats en el sèrum matern al moment del part, el sèrum de cordó, el sèrum del nen a les 8 setmanes de vida i en el calostre i la llet madura de la mare. Al moment de néixer es van prendre les mesures antropomètriques del nen, als tres dies de vida es van mesurar els nivells de TSH i els nivells d'uroporfirines i a l'any de vida es va mesurar el desenvolupament neuroconductual dels nens amb els tests de Bayley i de Griffiths (taula 1).

Taula 1. Disseny de l'estudi

| | Naixement | 8 setmanes | 1 any |
|-----------------------------------|-------------------------|-------------------------------------|-------------------------------------|
| Desenvolupament neurològic | Test de Brazelton | Test de Griffiths Test de Bayley | Test de Griffiths Test de Bayley |
| Antropometria | ☐ | ☐ | ☐ |
| Mostres nens | Sèrum de cordó Orina | Sèrum | Sèrum |
| Mostres mares | Sèrum Calostre | Sèrum Llet (3 setmanes) | — |
| Qüestionaris | General | — | Lactància |
| Altres | — | — | Intel.ligència mare |

Els nivells d'organoclorats es van mesurar en sèrum matern recollit al moment del part i en sèrum de cordó, en sèrum del nen i de la mare a les 8 setmanes de vida, i en calostre i llet materna a les 3 setmanes. El sèrum obtingut per centrifugació es va conservar a -40°C fins el moment de l'anàlisi. Els compostos organoclorats en sèrum foren analitzats per cromatografia de gasos (GC) acoblada a un detector de captura d'electrons i per GC acoblada a un espectòmetre de masses de

ionització negativa (Varian Star 3400 acoblat a Finnigan Mat INCOS XL) (To-Figueras et al., 1997). Totes les mostres foren analitzades al Departament de Química Ambiental (CID-CSIC). Els compostos trobats amb major freqüència en les mostres foren: hexaclorobenzè (HCB), diclorodifenildicloroetilè (pp'DDE), bifenils policlorats (PCBs) que presentem com la suma dels congèneres 28, 52, 101, 118, 138,153, i 180, i el beta-hexaclorociclohexà (β -HCH).

Al moment de néixer es va mesurar a cada infant el pes, la talla i el perímetre cranial. Es va aprofitar la mesura que es fa de manera rutinària al 3r dia de vida de l'hormona estimulante de la glàndula tiroide (TSH) per conèixer el funcionament tiroïdal en el conjunt d'aquests nens. Al 3r dia de vida també es van recollir mostres d'orina fresca que es van conservar a -20 °C. La concentració total de porfirines en orina es va determinar al Departament de Dermatologia de l'Hospital Clínic de Barcelona per espectrofluorometria (model F-2000, Hitachi Ltd., Tokyo, Japó). El patró d'excreció va ser analitzat amb cromatografia líquida d'alta resolució (HPLC) (Blake et al., 1992).

Per l'avaluació de les habilitats cognitives i motores en els nens es van utilitzar els tests de Bayley (Bayley, 1993) i de Griffiths (Griffiths, 1996). Aquests tests estan construïts amb la premissa que un nen ha assolit una habilitat concreta en un moment determinat del seu desenvolupament i la seva puntuació es basa en la puntuació esperada en un nen de la mateixa edat i d'una població general. Les exploracions foren realitzades per la mateixa neuropediatra en totes les visites, que desconeixia el lloc de residència del nen, els seus nivells d'organoclorats i el tipus de lactància realitzada. En el cas que es detectés alguna desviació dels tests més enllà dels límits de la normalitat es feia un informe al pediatre del CAP, perquè iniciés una consulta especialitzada. Els resultats individuals s'informaven a la mare després de cada visita.

L'estudi també va tenir en compte altres variables que podrien estar associades amb el desenvolupament neuroconductual i/o amb l'exposició a compostos organoclorats. Es va mesurar en el moment del part i a l'any de vida la història materna sobre malalties cròniques i medicació durant l'embaràs, l'hàbit tabàquic i el consum d'alcohol, la paritat i les variables perinatals (edat gestacional, tipus de part, Apgar) i el nivell socio-econòmic segons l'educació i l'ocupació. A l'any de vida es va preguntar a la mare sobre el tipus i la durada de la lactància del nen i es va mesurar el coeficient d'intel·ligència de la mare amb el test de Raven (Raven et al., 1996).

A continuació es presenten els 7 articles que constitueixen el treball de la tesi, precedits del resum de la publicació en català. La relació dels diferents treballs publicats amb el seu objectiu principal corresponent es presenten a la taula 2.

Taula 2. Publicacions incloses al treball de la tesi

| ARTICLE | OBJECTIU |
|--|--|
| 1. Ribas-Fitó N, et al. Journal of Epidemiology and Community Health 2001;55:537-46 | Revisió bibliogràfica sobre els PCBs i el desenvolupament neurològic dels nens |
| 2. Sala M, et al. Chemosphere 2001; 43:895-901 | Transferència transplacentària dels compostos organoclorats |
| 3. Ribas-Fitó N, et al. Journal of Epidemiology and Community Health (enviat per publicació) | Transferència dels compostos organoclorats a través de la lactància |
| 4. Ozalla D, et al. Environmental Health Perspectives 2002; 110:205-9 | Impacte dels compostos organoclorats sobre el patró de les porfirines del nounat |
| 5. Ribas-Fitó N, et al. Pediatric Research 2002; 52:163-7 | Impacte dels compostos organoclorats sobre les mides somatomètriques del nounat |
| 6. Ribas-Fitó N, et al. Occupational and Environmental Medicine 2003; 60:301-303 | Impacte dels compostos organoclorats sobre els nivells de TSH en el nounat |
| 7. Ribas-Fitó N, et al. Pediatrics 2003;111:e578-e583 | Impacte dels compostos organoclorats sobre el desenvolupament neurològic al primer any de vida |

ARTICLE 1: N Ribas-Fitó, M Sala, M Kogevinas, J Sunyer. **Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review.** J Epidemiol Community Health 2001;55:537-46

Antecedents: Els bifenils policlorats (PCBs) són una barreja complexa de contaminants persistents que es troben àmpliament distribuïts per tot el planeta. Els nounats s'exposen a aquests compostos a través de la placenta i de l'alletament matern. Els estudis amb animals d'experimentació han mostrat que els PCBs són neuro tòxics, però els efectes neurològics d'aquests compostos en nens no estan del tot clars.

Mètodes: Es va realitzar una revisió sistemàtica de la literatura sobre la relació entre el desenvolupament neurològic dels nens i l'exposició als PCBs.

Resultats: Set estudis longitudinals van avaluar els efectes de l'exposició prenatal a PCBs. Dos d'aquests estudis van avaluar a nens altament exposats. En nounats es va observar un augment dels reflexos patològics en els 4 estudis que ho van avaluar. Durant els primers mesos de vida, es va observar una disminució dels patrons motors en 4 dels 5 estudis que van estudiar el desenvolupament psicomotor; dèficits en l'adquisició de les habilitats cognitives només es van observar en un únic estudi basat en població no altament exposada. Als 4 anys d'edat, es van observar en 4 dels 5 estudis que ho van avaluar efectes en les àrees cognitives. L'exposició postnatal a PCBs a través de la lactància materna no es va associar clarament a cap efecte sobre el desenvolupament neurològic.

Conclusions: Aquest estudis suggereixen un efecte advers subtil de l'exposició prenatal als PCBs sobre el desenvolupament neurològic. Les diferències en el disseny dels estudis, la inconsistència en alguns dels resultats i la manca de dades quantitatives adequades sobre l'exposició no permet concloure quin és el grau de risc dels nivells actuals d'exposició sobre el desenvolupament neurològic

REVIEW

Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review

N Ribas-Fitó, M Sala, M Kogevinas, J Sunyer

Abstract

Background—Polychlorinated biphenyls (PCBs) are complex mixtures of persistent contaminants that are widespread in the environment. Newborns are exposed across the placenta and through breast feeding. Experimental animal studies have indicated that PCBs are neurotoxic. The neurological effects of these compounds on children are not clear. **Methods**—A systematic review of literature on the relation between neurological development in children and exposure to polychlorinated biphenyls.

Results—Seven follow up studies evaluated the effect of prenatal exposure to PCBs. Two of these studies evaluated highly exposed children. In newborns, an increase of the abnormal reflexes was observed in all four studies evaluating it. During the first months of life, a decrease in motor skills was observed in four of the five studies that investigated psychomotor development; deficits in the acquisition of cognitive skills were observed only in one study assessing non-highly exposed populations. At 4 years of age, an effect on the cognitive areas was observed in four of the five studies that evaluated it. Postnatal exposure to PCBs through breast feeding was not clearly related to any effect on neurological development.

Conclusions—These studies suggest a subtle adverse effect of prenatal PCBs exposure on child neurodevelopment. Differences in study design, inconsistency in some of the results, and the lack of adequate quantitative exposure data, do not allow the derivation of the degree of risk associated with neurodevelopmental effects at current levels of exposure.

(J Epidemiol Community Health 2001;55:537–546)

Japan (Yusho)² and Taiwan (Yucheng).³ Public health interest about the potential impact of environmental exposure to organochlorine compounds on child neurodevelopment has recently increased and several observational studies have been conducted.^{4–32} However, the considerable heterogeneity among these studies in the selection of adequate markers of exposure, in the measure of the outcomes and in the control for possible confounder factors complicates the derivation of firm conclusions about the possible neurodevelopmental effects of these compounds.^{33–34}

Properties of PCBs

PCBs are complex mixtures of persistent contaminants that are ubiquitous in the environment. They have been in widespread use, since the 1930s, as dielectric fluids in transformers and capacitors and in a variety of other applications.³⁵ Most, but not all,³⁶ industrialised countries have now banned or severely restricted their production.

PCBs are a family of chemicals including 209 different congeners. However, the number of the congeners present in extracts from biotic samples is much lower than the theoretical numbers.³⁷ The mechanisms of toxicity of these congeners are not similar and the doses at which they are active vary by orders of magnitude.³⁸ The planarity of the molecule is very important in both biostability and toxicology. Planar PCBs bind to the aryl-hydrocarbon (Ah) receptor resulting in some dioxin-like effects.³⁹

Because of their high biostability and lipophilicity and because they are resistant to both chemical and biological degradation they accumulate in food chains and can be found in the tissues of wildlife, domestic animals, and humans worldwide.⁴⁰ Nowadays the sources of human exposure to PCBs are food, particularly fish and fish products and animal fats. PCBs are preferentially stored in adipose tissue and are also present, to a smaller extent, in serum and human milk. The concentrations of PCBs in the different organs depend on the lipid content of such organs, with the exception of the brain, where the concentration is lower than the lipid content would indicate. These lower concentrations are attributable to the nature of brain lipids that are more polar than

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Accepted for publication 15 February 2001

There is growing evidence that some environmental chemicals can interrupt neurodevelopmental processes during critical periods of development, resulting in effects on behaviour, or cognitive function.¹ Awareness of the toxicity of organochlorine compounds was first established after two well documented episodes of accidental exposure to polychlorinated biphenyls (PCBs) via contaminated cooking oil in

adipose tissue lipids.⁴¹ PCBs also pass through the placenta and through human milk.

Experimental studies on neurotoxicity of PCBs

During the past 20 years, there has been an attempt to understand the cellular bases of PCB induced behavioural and neurological effects in animal and in vitro models. In vitro models have shown a decrease of cell dopamine content after PCB exposure.⁴²⁻⁴⁴ Experimental studies with laboratory rodents and non-human primates have indicated changes in dopamine function.⁴⁵⁻⁴⁷ Changes in locomotion activity,^{48, 49} in behaviour^{50, 51} and in learning and memory functions in the adult animal^{48, 52} have also been described after prenatal exposure to PCBs. These effects have been mainly described for non-dioxin-like ortho substituted PCBs congeners⁵³ as well as for the lower chlorinated PCBs.⁵⁴ Recent research also suggests that PCBs can change a number of other physiological processes that may be important for development. For example, PCB induced alteration in thyroid function during development may underline some of the developmental effects of PCBs reported in humans and animal models.^{55, 56}

Assessment of neurodevelopment in newborns and infants

Interpretation of studies on possible adverse developmental effects in infants from perinatal exposure to PCBs, in contrast with experimental models, is hampered by a high number of potential confounding factors and by difficulties in evaluating exposure and outcome measures. In studying human development, besides behaviour and cognition, it is difficult to define what is "normal". Human behaviour and intellect has been assessed by comparison to the "norm", and for this reason, different developmental tests based on general population development have been created. Neonatal tests mainly assess tonic, reflexes, alertness, responsiveness and state regulation of the newborn. Child tests are constructed with the premise that an ability may or may not have been acquired in a given age for the children.⁵⁷

Child tests are divided into mental (that is, language, cognition, memory and social patterns), motor (that is, gross and fine motor functions) and behavioural (that is, activity rating and behaviour) scales. These tests are reliable, well standardised and have specific items for the assessment of motor, mental and behavioural development. Their use facilitates comparison of results from the different studies. All mental and motor standardised tests have a mean full scale score around 100, with the exception of the Fagan Test of Visual Recognition Memory (FTVRM), which calculates the percentage of success. For the behavioural tests a higher score indicates a higher frequency of behavioural or activity problems in the child. The neurodevelopmental assessment tools mentioned in this review are briefly described in table 1.

Epidemiological studies

IDENTIFICATION OF EPIDEMIOLOGICAL STUDIES AND ANALYSIS

All the available studies about the effects of PCBs on children's neurobehavioural development published from 1976 to January 2000 were reviewed. We searched the computerised database of Medline by using "polychlorinated biphenyls", "development", and "neurotoxicology". We also reviewed conference proceedings of related topics as well as citations in the identified reports. We only included papers that had been written in English. A total of 29 publications coming from seven independent populations were identified.

Results have been grouped by effect studied (motor, mental or behavioural) and age at evaluation (newborn (<1 month) and children (>3 months-11 years)). Some studies presented the mean scores of the test used, while others provided a β coefficient of the increment or decrement in the scale score as a function of PCB exposure. To allow comparisons among all studies, we presented 95% confidence intervals of the mean difference or the β coefficients, which were derived from standard deviations or from graphical presentations.^{8, 9, 11} As in one publication³² the scores were reported in a raw form, we transformed them to the standard index score using the *Bayley scales for infant development* (2nd ed).⁵⁷

Table 1 Standardised neurodevelopmental tests mentioned in the publications reviewed

| Test | Age | Assessment | Reference |
|---|----------------|--|-----------|
| <i>Neonatal tests</i> | | | |
| Brazelton neonatal scales (BNBAS) | <3 days | Reflexes, responsiveness, state regulation | 67 |
| Newborn neurological exam (Prechtl) | 10-21 days | Age-appropriated neurodevelopment | 24 |
| <i>Children tests</i> | | | |
| <i>Mental</i> | | | |
| Fagan test of visual recognition memory (FTVRM) | 3-12 months | Novelty preference; short-term memory; "infant intelligence" | 80 |
| Bayley scales of infant development (MDI) | <2.5 years | Age-appropriate cognitive ability | 57 |
| Stanford-Binet (SB) | 2.5-6 years | General intelligence | 66 |
| Kaufman assessment battery for children (KABC) | 2.5-12.5 years | Age-appropriate cognitive ability | 66 |
| McCarthy scales of children's abilities (MCSCA) | >3 years | Age-appropriate cognitive ability | 66 |
| Wechsler Intelligence Scale for children (WISC) | >6 years | General intelligence | 66 |
| Reynell developmental scales (RDS) | — | Age-appropriate development of language | 28 |
| Chinese Child Developmental Inventory (CCDI)* | 6m-6y | General developmental status† | 12 |
| <i>Motor</i> | | | |
| The Neurological exam for toddler age (Hempel) | Toddlers | Age-appropriate motor ability | 27 |
| Bayley scales of infant development (PDI) | <2.5 years | Age-appropriate motor ability | 57 |
| <i>Behavioural</i> | | | |
| Rutter's child behaviour scale (RCBS-A) | 0-12 years | Child behavioural problems, hyperactivity | 66 |
| Werry-Weiss-Peters activity scale (WWPAS) | 3-11 years | Children's activity level | 66 |

*Adopted and modified in 1978 from Minnesota Child Developmental Inventory. †CCDI has seven subscales: two for motor development and five for mental development.

Table 2 Studies about the effects of PCBs on neurodevelopment in children included in this review

| Location | Type of study | Year of start | Study period | Population | Measurement of PCBs exposure | Mean PCBs concentration (biological sample) | Min-max PCBs concentration (biological sample) | Other detected organochlorine compounds | Effect studied |
|---|----------------------------|---------------|-------------------------------------|--|--|---|--|---|-------------------------------|
| Japan ¹ | Prospective | 1968 | 7 years | Children born to poisoned women in Japan in 1968 | Location | 6.0 ppb (exposed children's blood) ^(#) | ? | — | Mental, motor, behavioural |
| Taiwan ⁵⁻¹² | Matched pair cohort study* | 1985-87 | 6 years | Children born to poisoned women in Taiwan in 1979 and their controls | Questionnaire on location and diet | 0.99 ppb (exposed children's blood) [‡] | 0-77.8 ppb (exposed children's blood) | Polychlorinated dibenzofurans (PCDFs) | Mental, motor, behavioural |
| North Carolina, USA ¹³⁻¹⁶ | Prospective | 1978-82 | 5 years | General community | PCB [†] and DDE in biological samples | 1.5-2 ppm (colostrum) | 0->4 ppm (colostrum) | Dichlorodiphenyl dichloroethene (p,p'DDE) | Neonatal, mental, motor |
| Michigan, USA ¹⁷⁻²³ | Prospective | 1980-81 | 11 years | Children born to Lake's Michigan fish eater women | PCB [†] in biological samples/questionnaire on diet | 2.5 ng/ml (cord blood) [#] | 0-12.3 ng/ml (cord blood) | Polybrominated biphenyls (PBBs), DDT | Neonatal, mental, behavioural |
| Rotterdam-Groningen, the Netherlands ²⁴⁻²⁹ | Prospective | 1990-92 | 4 years | General community | PCB _{1,18,1,18,1,15,1,80} and PCDDs in biological samples | 0.38 ng/ml (cord blood) [‡] | 0.18-0.86 ng/ml (cord blood) [§] | PCDFs, polychlorinated dibenzo-p-dioxins (PCDDs) | Neonatal, mental, motor |
| Oswego, USA ³⁰⁻³¹ | Prospective | 1991-94 | 4 years (in course ^(#)) | Children born to Lake's Ontario fish eater women | 69 PCB congeners in biological samples/questionnaire on diet | 0.52 ng/g wet (cord blood) [‡] | Not reported | Hexachlorobenzene, dieldrin, lindane, chlordane, PCDDs, mirex | Neonatal |
| Düsseldorf, Germany ³² | Prospective | Not reported | 7 months | General community (middle and upper class families) | PCB _{3,8,1,15,1,80} in biological samples | 0.55 ng/ml (cord blood) | Not reported | — | Mental, motor |

*Cross sectional in reference 5. †Congeners of PCB not reported. ‡Median. #Detection limit: 3 ng/ml. §p5-p95. — Not applicable. ? Not known.

CHARACTERISTICS OF THE STUDIES

Table 2 summarises the design and reporting of the seven studies considered in this review. All studies were prospective. The Japanese and the Taiwanese studies reported data from children highly exposed to PCBs. In Taiwan, for every exposed child, two control children matched on neighbourhood, age, sex, mother's age and parent's combined education level were used. The studies in Michigan and Oswego were based on children born to women who ate contaminated fish. The other three studies were based on children from general populations. The measure of exposure varied significantly between studies. In the Japanese and Taiwanese studies, information of children's exposure was only based on place of residence and on a mother's questionnaire on diet, respectively. However, in both studies, levels of PCBs in exposed children's sera were analysed in a sub-sample. In the rest of the studies information of children's exposure was specifically based on levels of PCBs measured in different biological samples. In Michigan and in Oswego, a questionnaire was also used to assess exposure to PCBs. Prenatal exposure was estimated through the measure of PCBs in cord blood and maternal plasma close to delivery or colostrum. Postnatal exposure was measured through the quantification of PCBs in milk samples or in child sera. Specific information about the congeners analysed was only given in the Netherlands, German and Oswego studies. The North Carolina and the Netherlands cohorts also studied the effects of other organochlorine compounds on neurodevelopment in children. The reported PCBs concentrations among children (sera or cord blood) in the highly exposed cohorts (Japan, Taiwan) seem to be orders of magnitude higher than among children from general populations (the Netherlands and Germany). Levels of PCBs in the sera of Yusho children are the highest.

NEURODEVELOPMENTAL EFFECTS OF PCB EXPOSURE AMONG NEWBORNS

A neonatal effect was studied in North Carolina, Michigan, the Netherlands and Oswego. Results from all the four studies suggest some negative effects on neonatal development. In North Carolina, higher PCB levels in maternal milk at birth were associated with hypotonicity and hyporeflexia.¹³ Maternal consumption of contaminated fish in Michigan was predictive of newborn motor immaturity, poorer lability of states, a greater amount of startle, and more abnormally hypoactive reflexes in the Neonatal Behavioral Assessment Scale (NBAS)¹⁷ but higher cord serum PCB levels were not related to any adverse outcomes. In the Netherlands, exposure through breast milk to PCBs, polychlorodibenzo-p-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) was associated with a reduced neonatal neurological optimality, but levels of PCB in cord blood were not related to nervous system dysfunction.²⁴ The Oswego study found that newborns highly exposed to highly chlorinated PCBs scored more poorly on the autonomic and habituation

Table 3 Main characteristics of the reports in the different publications of prenatal exposure to PCBs and mental, motor and behavioural development at different ages

| Effect studied, study and publication | Code* | Age at evaluation** | Test used | Number‡ | Age adjusted mean full scale score of all children | Covariates# |
|---|-------|---------------------|-----------|---------|--|--|
| <i>Mental</i> | | | | | | |
| <i>Taiwan</i> | | | | | | |
| Rogan, 88 | 5a | <2.5y | MDI | 45/45 | 103 | Socioeconomic status (2), mother's age, gender, age |
| | 5b | 2.5–6y | SB | 52/52 | 87 | Socioeconomic status (2), mother's age, gender, age |
| | 5c | >6y | WISC | 21/21 | 86 | Socioeconomic status (2), mother's age, gender, age |
| | 6a | <2.5y | MDI† | 28/28 | 101.1 | Socioeconomic status (2), mother's age, gender, age |
| | 6b | <2.5y | MDI† | 19/19 | 101.9 | Socioeconomic status (2), mother's age, gender, age |
| | 6c | 2.5–6y | SB† | 25/25 | 101.7 | Socioeconomic status (2), mother's age, gender, age |
| | 6d | 2.5–6y | SB† | 69/69 | 94.1 | Socioeconomic status (2), mother's age, gender, age |
| | 6e | >6y | WISC | 30/30 | 91 | Socioeconomic status (2), mother's age, gender, age |
| | 9a | 6m | MDI | 6/6 | 107.5 | Socioeconomic status (2), mother's age, gender, age |
| | 9b | 1y | MDI | 15/15 | 113 | Socioeconomic status (2), mother's age, gender, age |
| | 9c | 1.5y | MDI | 24/24 | 101 | Socioeconomic status (2), mother's age, gender, age |
| | 9d | 2y | MDI | 40/40 | 102 | Socioeconomic status (2), mother's age, gender, age |
| | 9e§ | 4y | SB | 86/86 | 94.5 | Socioeconomic status (2), mother's age, gender, age |
| | 9f§ | 7y | WISC | 101/101 | 94.5 | Socioeconomic status (2), mother's age, gender, age |
| | 9g | 11y | WISC | 30/30 | 105 | Socioeconomic status (2), mother's age, gender, age |
| | 10 | 7–12y | WISC | 27/27 | 96.3 | Socioeconomic status (2), mother's age, gender, age |
| | 12 | 6m–6y | CCDI | 66/66 | 103.1 | Socioeconomic status (2), mother's age, gender, age |
| Chen, 94 | 14a | 6m | MDI | 787 | 114.6 | Socioeconomic status (2), parental factors (5), perinatal factors (5), gender, age, duration of breast feeding, examiner |
| Guo, 94 | 14b | 1y | MDI | 720 | 108.9 | Socioeconomic status (2), parental factors (5), perinatal factors (5), gender, age, duration of breast feeding, examiner |
| <i>North Carolina, USA</i> | | | | | | |
| Gladden, 88 | 14a | 6m | MDI | 787 | 114.6 | Socioeconomic status (2), parental factors (5), perinatal factors (5), gender, age, duration of breast feeding, examiner |
| | 14b | 1y | MDI | 720 | 108.9 | Socioeconomic status (2), parental factors (5), perinatal factors (5), gender, age, duration of breast feeding, examiner |
| <i>Michigan, USA</i> | | | | | | |
| Jacobson, 85 | 18a | 7m | FTVRM | 123 | 57% | Socioeconomic status, parental factors (2) |
| | 18b | 7m | FTVRM | 123 | 57% | Perinatal factors (3) |
| | 20 | 4y | MCSA-GCI | 146 | na | Socioeconomic status (7), parental factors (6), perinatal factors (2), gender, age, examiner, PBBs, DDT, lead |
| Jacobson, 90 | 22 | 11y | WISC | 178 | 107 | Socioeconomic status (2), parental factors (2) |
| Jacobson, 96 | 26 | 7m | MDI | 206 | 113 | Socioeconomic status (3), parental factors (3), perinatal factors (3), gender, duration of breast feeding |
| <i>Rotterdam-Groningen, the Netherlands</i> | | | | | | |
| Koopman-Esseboom, 96 | 28a | 4y | K-ABC | 373 | 111 | Socioeconomic status (2), parental factors (4), perinatal factors (2), gender, duration of breast feeding |
| | 28b | 4y | RDS | 190 | 105 | Socioeconomic status (2), parental factors (4), perinatal factors (2), gender, duration of breast feeding |
| Patandin, 99 | 32a | 7m | MDI | 131 | 94.5 | Socioeconomic status (3), parental factors (3), perinatal factors (3), age, duration of breast feeding, lead |
| | 32b | 7m | FTVRM | 131 | 59% | Socioeconomic status (3), parental factors (3), perinatal factors (3), age, duration of breast feeding, lead |
| <i>Düsseldorf, Germany</i> | | | | | | |
| Winneke, 98 | 32a | 7m | MDI | 131 | 94.5 | Socioeconomic status (3), parental factors (3), perinatal factors (3), age, duration of breast feeding, lead |
| | 32b | 7m | FTVRM | 131 | 59% | Socioeconomic status (3), parental factors (3), perinatal factors (3), age, duration of breast feeding, lead |

*Useful for interpreting the figures (the number coincides with the reference). **In the Taiwanese cohort, we only included <2y, 4, 7 and 11 years. †Different visits. ‡In matched paired cohort studies, number of subjects exposed/non-exposed. #In parentheses, number of covariates. §Also reported in reference 4. ||Also reported in reference 8. || General developmental score (motor, mental and social), na: Not available.

Table 3 Continued

| Effect studied, study and publication | Code* | Age at evaluation** | Test used | Number‡ | Age adjusted mean full scale score of all children | Covariates# |
|---------------------------------------|-------|---------------------|-----------|---------|--|--|
| Motor | | | | | | |
| <i>Taiwan</i> | | | | | | |
| Rogan, 88 | 5 | <2.5 | PDI | 45/45 | 104.5 | Socioeconomic status (2), mother's age, gender, age |
| Yu, 91 | 6a | <2.5 | PDI† | 28/28 | 105.2 | Socioeconomic status (2), mother's age, gender, age |
| | 6b | <2.5 | PDI† | 19/19 | 107.6 | Socioeconomic status (2), mother's age, gender, age |
| Lai, 94 | 9a | 6m | PDI | 6/6 | 110 | Socioeconomic status (2), mother's age, gender, age |
| | 9b | 1y | PDI | 15/15 | 110 | Socioeconomic status (2), mother's age, gender, age |
| | 9c | 1.5y | PDI | 24/24 | 108 | Socioeconomic status (2), mother's age, gender, age |
| | 9d | 2y | PDI | 40/40 | 99 | Socioeconomic status (2), mother's age, gender, age |
| <i>North Carolina, USA</i> | | | | | | |
| Gladen, 88 | 14a | 6m | PDI | 787 | 114.7 | Socioeconomic status (2), parental factors (5), perinatal factors (5), gender, age, duration of breast feeding, examiner |
| | 14b | 1.2m | PDI | 787 | 108.6 | Socioeconomic status (2), parental factors (5), perinatal factors (5), gender, age, duration of breast feeding, examiner |
| Rogan, 91 | 15a | 1.5y | PDI | 676 | 108 | Socioeconomic status (2), parental factors (5), gender, age, examiner |
| | 15b | 2y | PDI | 670 | 11.4 | Socioeconomic status (2), parental factors (5), gender, age, examiner |
| <i>the Netherlands</i> | | | | | | |
| Huisman, 95 | 25 | 18m | Hempel | 373 | na | Socioeconomic factors (1), study centre |
| Koopman-Esseboom, 96 | 26 | 3m | PDI | 198 | 117 | Socioeconomic factors (3), parental factors (2), perinatal factors (4), gender, duration of breast feeding |
| <i>Germany</i> | | | | | | |
| Winneke, 98 | 32 | 7m | PDI | 131 | 83 | Socioeconomic status (3), parental factors (3), perinatal factors (3), age, duration of breast feeding, lead |
| <i>Behavioural</i> | | | | | | |
| <i>Taiwan</i> | | | | | | |
| Rogan, 88 | 5 | 3y-12y | RCBS-A | 117/119 | 10.2 | Socioeconomic status (2), mother's age, gender, age |
| Chen, 94 | 8a¶ | 4y | RCBS-A | na | 14.8 | Socioeconomic status (2), mother's age, gender, age |
| | 8b¶ | 7y | RCBS-A | na | 12.2 | Socioeconomic status (2), mother's age, gender, age |
| | 8c¶ | 11y | RCBS-A | na | 10.1 | Socioeconomic status (2), mother's age, gender, age |
| | 8d | 4y | WWPAS | na | 48 | Socioeconomic status (2), mother's age, gender, age |
| | 8e | 7y | WWPAS | na | 37 | Socioeconomic status (2), mother's age, gender, age |
| | 8f | 11y | WWPAS | na | 25 | Socioeconomic status (2), mother's age, gender, age |
| Guo, 94 | 12 | 3y-6y | RCBS-A | 40/40 | 12.9 | Socioeconomic status (2), mother's age, gender, age |

*Useful for interpreting the figures (the number coincides with the reference). **In the Taiwanese cohort, we only included <2y, 4, 7 and 11 years. †Different visits. ‡In matched paired cohort studies, number of subjects exposed/non-exposed. #In parentheses, number of covariates. §Also reported in reference 4. ¶Also reported in reference 8. || General developmental score (motor, mental and social). na. Not available.

KEY POINTS

- Health risk of the human intake of persistent organochlorine compounds to child neurodevelopment is a matter of worldwide concern.
- The existing research suggests that polychlorinated biphenyls (PCBs) hinder neurodevelopment to children exposed early in life.
- Considerable heterogeneity between study designs does not permit a joint quantitative measure of the association.
- The universal exposure to organochlorine compounds makes necessary further research of populations exposed to the current levels of these contaminants.

clusters of the NBAS and, although not significantly, had more abnormal reflexes.^{30 31}

NEURODEVELOPMENTAL EFFECTS OF PCB EXPOSURE AMONG CHILDREN

Prenatal exposure to PCBs

Original quantitative data of the effects of prenatal exposure to PCBs on children from 6 months to 11 years were reported in 15 publications from Taiwan, North Carolina, Michigan, the Netherlands and Germany (table 3).

A negative association between PCBs and mental development was found in children born to mothers who had consumed contaminated oil in 1979 in Taiwan (fig 1A). In most of the evaluations at different ages, the average difference between exposed and non-exposed was around -4 to -6 points. In the studies based on non-highly exposed populations a negative effect on the acquisition of cognitive skills during the first months of life was only observed in the Michigan study (fig 1B). In addition, a significant association between prenatal PCBs exposure and mental impairment was observed at 4 years of age in the Netherlands and 11 years in Michigan (fig 1B). When memory was specifically assessed, an inverse association could also be observed in Michigan at 4 years of age.^{20 21} In contrast, the North Carolina study reported that no effects were observed at 3, 4 or 5 years.¹⁶

An inverse association with the psychomotor scale scores was also observed both in children prenatally exposed in Taiwan and in children highly exposed in North Carolina compared with the non-exposed and the less exposed, respectively (fig 2A). In North Carolina and in the Netherlands, negative associations with psychomotor scales were observed during the first year of life, particularly at 3 months (fig 2B). In the Netherlands study the authors also reported that cord blood PCB levels did not predict poorer scores in motor development at either 7 months²⁶ or at 42 months.²⁷

Behavioural scales were only studied in Taiwan and Michigan. In all the publications from the Taiwanese study there was a negative association between children born to women who had consumed contaminated oil and higher scores in the behavioural scales (fig 3). In Michigan, prenatal PCB exposure was not

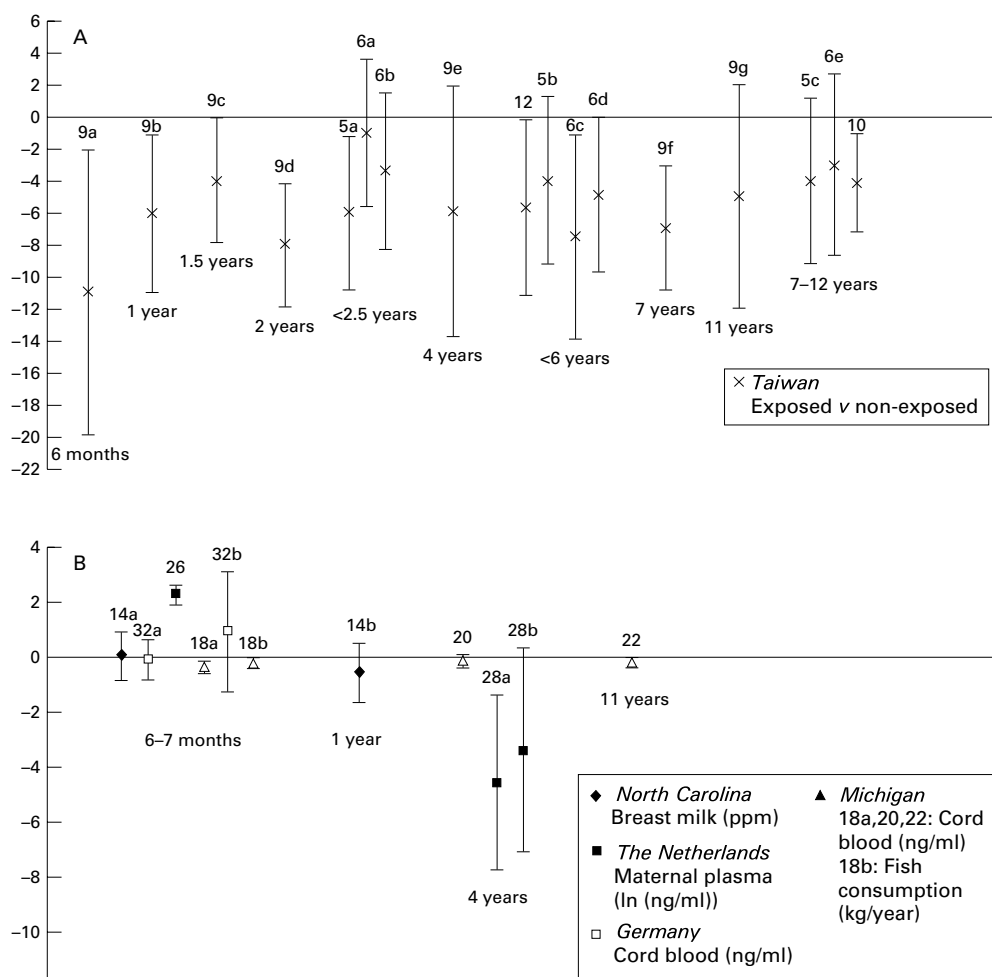


Figure 1 Mean difference in full mental scales (and 95% confidence intervals): (A) between prenatally exposed and non-exposed children to PCBs and (B) for a change in one unit of prenatal exposure to PCBs; ordered by age of the infant and type of test of the different studies.

associated with worse behavioural score in either the composite activity rating¹⁹ or sustained attention rating²¹ at 4 years of age.

The Japanese study did not use any specific standardised test and did not report quantitative data. However, a clinical examination of 127 exposed children showed that they had a mean IQ of 70, had hypotonia and appeared to be sullen, expressionless and hypoactive.⁴ Overall, in addition to the effects found in the Japanese and the Taiwanese accidents, prenatal PCB exposure seems to be related to a decrease in motor skills during the first months of life and to an effect on the cognitive areas among children older than 4 years.

POSTNATAL EXPOSURE TO PCBs

Postnatal exposure was studied in North Carolina, Michigan, the Netherlands and Germany. Unlike prenatal exposures, postnatal exposures have rarely been found to be associated with any neurodevelopmental effect. In North Carolina, postnatal exposure to PCBs, based on a function of the concentration of the chemical in milk and the duration of breast feeding, was not related to worse performance in any of the tests and ages studied.¹⁴⁻¹⁶ In Michigan, levels of PCBs in maternal milk were unrelated to recognition

memory performance at 7 months,¹⁸ cognitive performance at 4 years,^{20, 21} and at 11 years.²² Only the composite activity rating at 4 years was negatively related to both the maternal milk PCBs level and the four year serum PCBs level.¹⁹ In the Netherlands, postnatal exposure to PCBs was analysed together with the effects of dioxins. According to the toxic equivalent factor of each congener, a total of 17 dioxins and three PCBs were added and summarised as a total PCB-dioxin toxic equivalent concept (TEQ). Examination of the postnatal exposure revealed no significant effect of the total PCB-dioxin TEQ exposure at 3 months of age. At 7 months higher amounts of PCBs and dioxin exposure through breastfeeding had a significant adverse effect on the psychomotor outcome among breast feeders.²⁶ At 18 months^{25, 26} and at 24 months,^{27, 28} an effect of lactational exposure to these compounds could not be detected.^{25, 26} In Germany, only the Bayley mental developmental index exhibited a significant negative association with PCBs levels in breast milk at 7 months.³²

Discussion

The reviewed studies have shown that PCBs exposure through the mother might be related

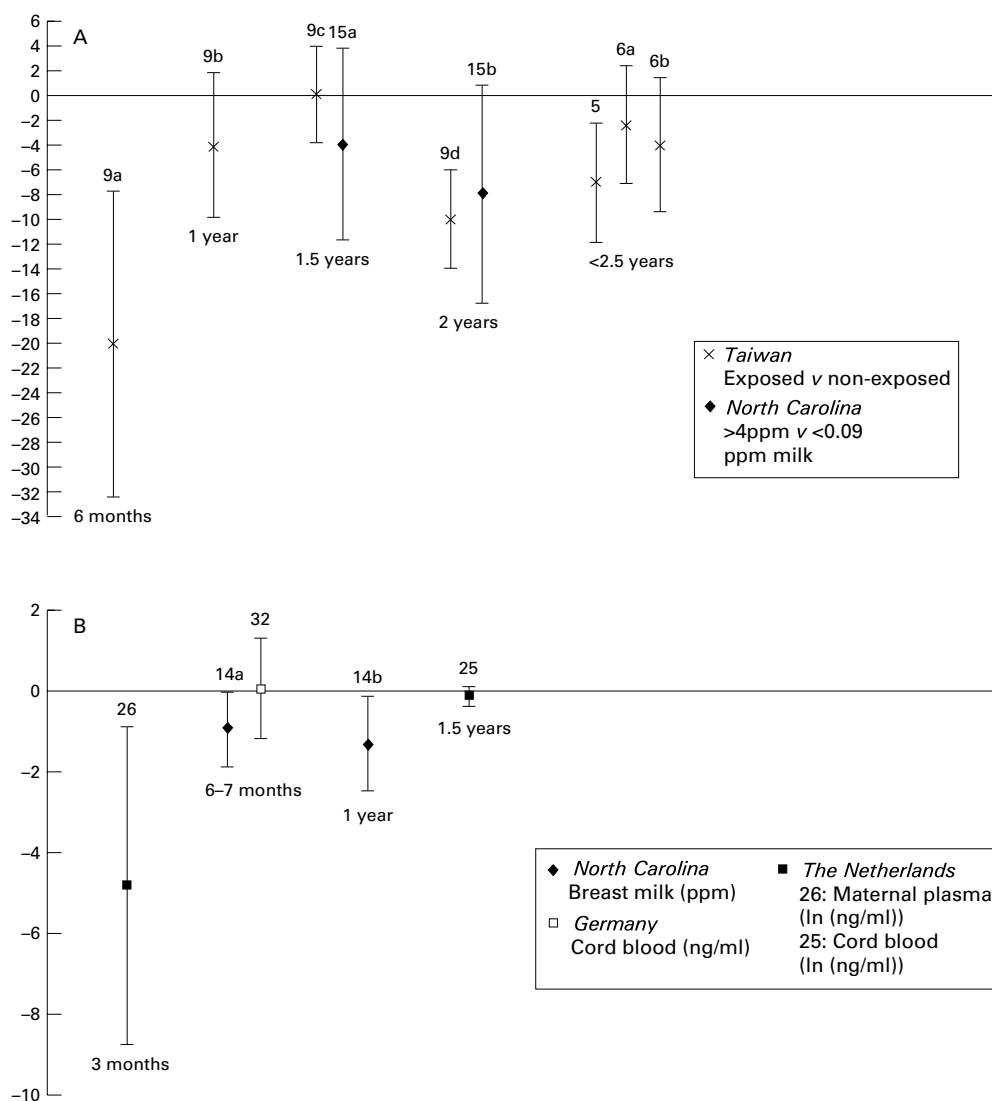


Figure 2 Mean difference in full motor scales (and 95% confidence intervals): (A) between prenatally exposed and non-exposed children to PCBs and (B) for a change in one unit of prenatal exposure to PCBs; ordered by age of the infant and type of test of the different studies.

to some small adverse effects on the neurodevelopment and behaviour of children. Seven follow up studies evaluated the effect of prenatal exposure to PCBs. Two of these studies evaluated highly exposed children. In newborns, an increase of the abnormal reflexes was observed in all four studies evaluating it. During the first months of life, a decrease on the motor skills was observed in four of the five studies that investigated psychomotor development; deficits in the acquisition of cognitive skills were observed only in one study assessing non-highly exposed populations. The studies based on general populations mainly evaluated motor development in infants up to 2 years, so it cannot be excluded that the observed psychomotor effects during the neonatal period and early infancy might disappear later in life. At 4 years of age, an effect on the cognitive areas was observed in four of the five studies that evaluated it. The strongest effects on motor and mental development were observed in Taiwan, in the reports at the age of 6 months. A possible explanation could be related to the

fact that exposed mothers were encouraged not to breast feed their infants,⁵⁸ impeding the possible neurodevelopmental benefits of breast feeding to their newborn.^{59 60} Effects on neurological development of postnatal exposure to PCBs through breast feeding were inconsistent among the studies and preponderantly non-significant. PCBs exposure is higher through breast feeding than in utero,^{58 61} but intrauterine exposure seems to pose a greater threat to the infant than postnatal exposure as has been observed for other chemicals.⁶²

Exposure to PCBs was assessed through biological samples or questionnaires on residence or diet. Questionnaires did not specifically discriminate between prenatal or postnatal exposure and were not sufficiently sensitive to detect either exposure to specific compounds or the magnitude of the exposure. In Taiwan, the authors tried to improve the exposure measurement provided by the questionnaire by using the physical signs observed in the children after PCBs intoxication, such as presence of nail abnormalities. No relation

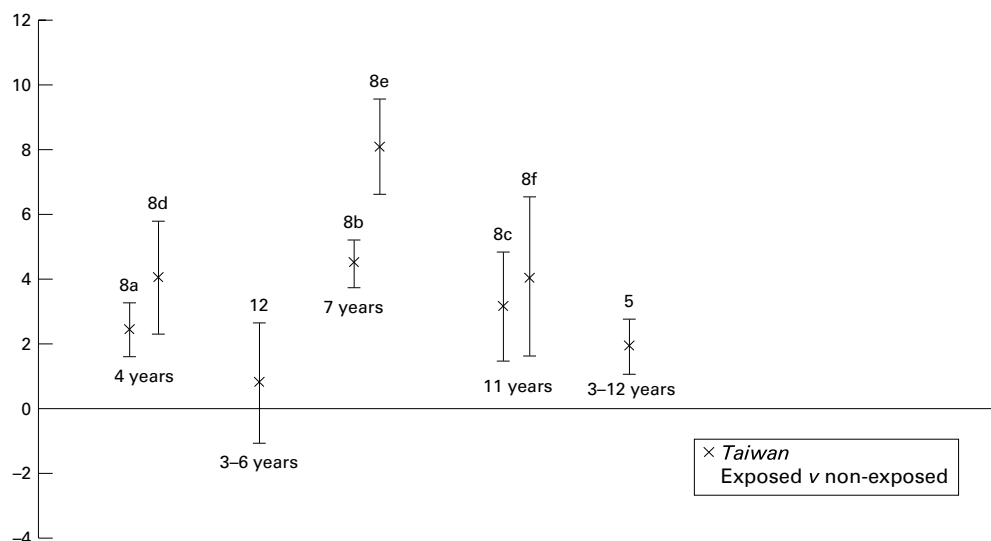


Figure 3 Mean difference in full behavioural scales (and 95% confidence intervals) between prenatally exposed and non-exposed children to PCBs; ordered by age of the infant and type of test of the different studies.

between severity of the external clinical examination and the neurodevelopmental effects could be observed.⁶ However, the measurement of exposure through an index of physical signs could be less valid than the questionnaire as individual susceptibility was not taken into account. Problems with biological measures of exposure were of a different nature. Some studies measured prenatal exposure of newborns through assays in maternal blood or milk close to delivery rather than cord blood. Although there is a correlation between maternal and child levels,⁶³ a within individual variability can be observed in the correlation between levels of specific PCBs' congeners in maternal milk close to delivery and levels in cord blood.⁶⁴ Differences in the analytical methods used could also explain some of the differences observed among studies. In Michigan and in North Carolina exposure was defined from total PCBs levels by summing different Webb-McCall peaks.⁶⁵ This method may have hampered the identification of specific PCB congeners, such as the non-dioxin like PCBs or the less chlorinated, which have been described to be the most neurotoxic in animals.⁵⁴

The measure of the outcome was also significantly different among the studies. The age at evaluation and the specific use of the available standardised tests differed in each report. Mental development was assessed in all studies at 6-7 months of age. Only some of these studies followed up their children up to school ages. Motor development was mainly evaluated in children aged less than 2 years. The behavioural and mental repertoires of the younger child are more vulnerable to acute external influences difficult to control for than the motor skills, and therefore less reliable.⁶⁶ Clusters like mobility or reflexes, which predict better future motor function, tend to respond less to the environmental stimuli during the examination.⁶⁷ In addition, the mental skills early in life are less predictive of later functioning than motor skills.⁶⁸

The major differences between studies laid in the methods of controlling for confounders. Socioeconomic status, prenatal maternal alcohol consumption, maternal smoking during pregnancy, maternal age, dietary habits, gender, the caregiving environment as measured by the HOME inventory⁶⁹ and breast feeding itself are strongly associated with neurodevelopment,⁷⁰ and adjustment for these factors was heterogeneous among studies and not comparable. Besides, humans are exposed to PCBs that are quite frequently present in mixtures with other organochlorine compounds or other chemical products, but only a few of the studies measured other compounds. Other organochlorine compounds were studied in North Carolina, Michigan and Oswego. The studies in Michigan, Germany and Oswego took into account levels of lead. Only the study in Oswego controlled for methylmercury, which has been reported to act synergistically with polychlorinated biphenyls in *in vitro* models,⁷¹ and has been shown to be associated with deficits in language, attention and memory in children.⁷² In a recent study in the Faroe Islands, an association with PCBs exposure disappeared after adjustment for methyl mercury.⁷³

Systematic reviews are prone to publication bias⁷⁴ as those studies with negative findings are less likely to be published. However, it seems unlikely that any large study might have not been published because of negative findings. We are quite confident that all published studies about the effects of PCBs on child neurodevelopment have been considered in this review, but publication bias cannot be absolutely discarded.

Despite the relatively consistent results due to prenatal exposures, the lack of homogeneity between study designs does not allow having a joint quantitative measure of the association. The present studies do not provide enough information on type of dose-response relation and the presence of a threshold level. Taiwan and Japan, which had the highest levels of PCBs and PCDFs, did not use levels of

internal dose of PCBs to assess the association with neurodevelopmental tests and only quantified exposure in two levels (exposed/non-exposed). The critical temporal period during the brain growth spurt where the potential damage might occur is also not known. Measurements at birth averaged all prenatal exposures and could not differentiate acute exposures during pregnancy. Moreover, information on the possible transience of the effects, or the specific cognitive skills that might remain permanently affected in later childhood cannot be derived from the present studies. As was fully assessed in the case of lead, it is important to clarify the possible reversibility of the observed effects.⁷⁵

Another important issue of concern is whether the small group average effects observed have a clinical significance at the individual level.⁷⁶ At current levels in the Great Lakes region, the effects of intrauterine PCBs exposure appeared to be so subtle that they would not have been evident in a routine clinical examination.⁷⁷ Studies on lead exposure have shown that a decrease of 4 points in average population intelligence increases the number of children with IQs of <80 by threefold and decreases the percentage of children with IQ scores of >125.⁷⁸ In a similar way, exposure to these ubiquitous environmental pollutants may have a seemingly small effect at individual level, but probably have a large impact at population level.⁷⁹

We conclude that the available studies suggest an adverse effect of prenatal PCBs exposure on neurological development in children. However, differences in study design, some inconsistencies in the results, and the lack of homogeneous quantitative exposure data, do not allow the derivation of the degree of risk associated with neurodevelopmental effects at current levels of exposure. Future investigation, as well as ongoing cohort studies,⁸⁰⁻⁸⁴ should guarantee standard evaluation of specific organochlorine exposure using biological samples, homogeneous outcome assessment through standardised tests and measurement of important covariates such as socioeconomic factors and other neurotoxic chemicals.⁸⁵ The universal exposure to these compounds and the importance of early assessment of signs of dysfunction to provide the best possible basis for decisions on prevention,⁸⁶ makes necessary the setting up and continuation of large follow up studies of populations exposed to the current levels of these contaminants.

We are grateful to Dave Macfarlane for his help in editing this manuscript.

Funding: supported in part by grants from "Fundació La Caixa" 97/009.00, and Generalitat de Catalunya-CIRIT 1999SGR 00241.

Conflicts of interest: none.

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ARTICLE 2: M Sala, N Ribas-Fitó, E Cardo, ME de Muga, E Marco, C Mazón, A Verdú, JO Grimalt, J Sunyer. **Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta.** Chemosphere 2001;43:895-901

L'hexaclorobenzè (HCB) és un compost organoclorat àmpliament distribuït per tot el planeta, altament lipofílic i que s'acumula als sistemes biològics. Els nounats s'exposen a aquests compostos a través de la placenta i de la lactància materna. Tot i que l'HCB és un dels compostos organoclorats més comuns, la transferència a través de la placenta de l'HCB de la mare al fetus durant la gestació està poc documentada. Aquest estudi presenta els nivells d'HCB, de DDT i el seu metabòlit p,p'DDE, PCBs i β -HCH en 72 mostres de sang materna al moment del part i 69 mostres de sang de cordó, de les quals 62 corresponen a parelles de mare-nen nascuts entre maig de 1997 i setembre de 1999 en una àrea rural altament exposada a HCB. Els resultats mostren que tots els nounats presenten nivells detectables d'HCB, PCBs i p,p'DDE, i que, en menor nivell, de β -HCH. Les concentracions d'HCB són les més elevades. La mitjana geomètrica de l'HCB va ser de 1.1 ng/ml, amb un rang de 0.3 a 5.7 ng/ml. Les concentracions d'HCB en sang de cordó (log ng/ml) es van associar positivament a les concentracions en la sang materna (log ng/ml) (coeficient=0.45 p<0.01). L'edat gestacional no es va associar amb la transferència transplacentària de l'HCB. Els nivells de p,p'DDE i de β -HCH de la mare també es van associar als nivells del nounat, però no els nivells de PCBs. Es conclou que l'HCB, de la mateixa manera que altres compostos organoclorats, es transfereix a través de la placenta.



Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta

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Abstract

Hexachlorobenzene (HCB) is an organochlorine compound widespread in the environment, highly lipophilic, that accumulates in biological systems. It has been suggested that it should be classified as a dioxin-like compound. Newborns are exposed to organochlorine compounds across the placenta and through breastfeeding. Although HCB is one of the most common organochlorine compounds, the transplacental transference of HCB from mother to fetus during pregnancy has been scarcely documented. This study reports the levels of HCB, dichlorodiphenyl trichloroethane (DDT) and its metabolite *p,p'*DDE, polychlorinated biphenyls (PCBs), and beta-hexachlorocyclohexane (β -HCH) in 72 maternal blood samples at delivery and in 69 cord blood samples, from which 62 corresponded to mother–infant pairs born between May 1997 and September 1999 in a rural area highly exposed to HCB. Results show that all newborns presented detectable levels of HCB, PCBs, and *p,p'*DDE, and, to a lesser extent, of β -HCH, the HCB levels being the highest. The geometric mean of HCB was 1.1 ng/ml, ranging from 0.3 to 5.7 ng/ml. Concentrations of HCB levels in cord blood (log ng/ml) were positively associated with concentrations in maternal blood (log ng/ml) (coefficient = 0.45, $P < 0.01$). Gestational age was not associated with the transplacental transfer of HCB. Maternal *p,p'*DDE and β -HCH levels were also associated with newborn levels, but levels of PCBs were not. We conclude that HCB, similar to other organochlorinated compounds, has a transplacental transfer. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Hexachlorobenzene; Organochlorine compounds; Cord blood; Transplacental

1. Background and objectives

Hexachlorobenzene (HCB) is an organochlorine compound widespread in the environment, formerly used as a fungicide. Nowadays, the major source of HCB is industrial emission as a side-product related to

the manufacture of chlorinated solvents and pesticides. HCB is highly lipophilic and accumulates in biological systems (Morris and Cabral, 1986). Recently, it has been suggested that HCB should be classified as a dioxin like compound because it binds to the aryl-hydrocarbon (Ah) receptor, resulting in some dioxin like effects (van Birgelen, 1998). Polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane (DDT) and its metabolite *p,p'*DDE, and beta-hexachlorocyclohexane are also persistent, and lipophilic chlorinated organic compounds that can be found in all ecosystems. Exposure to

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organochlorine compounds has been the subject of interest during recent years given their potential toxicity. A wide range of toxic effects in experimental animals, including carcinogenicity (Woodruff et al., 1994), immunotoxicity (Hattula et al., 1976), neurotoxicity and effects on the reproductive system (Kelce et al., 1995) have been reported for most of them. In humans, several epidemiologic studies have reported associations between organochlorine compounds' exposure, mainly DDT and PCBs, and health effects such as cancer (Saracci et al., 1991; Garabrant et al., 1992; Wolff et al., 1993; Wrensch et al., 1993), adverse reproductive outcomes (Karmaus and Wolf, 1995; Savitz et al., 1996), Parkinson's disease (Semchuk et al., 1993), and developmental disorders among infants (Rogan et al., 1986; Jacobson et al., 1990; Huisman et al., 1995). The main intake of organochlorinated compounds in humans is from diet and, exceptionally, by inhalation. Newborns are exposed across the placenta and through breastfeeding (Huisman et al., 1995; Rhoads et al., 1999). The exposure to HCB across the placenta has scarcely been documented in newborns.

Complaints of odor by the population of a rural village of 5000 inhabitants located in the vicinity of an organochlorine-compound factory (Flix, Catalonia, Spain) led to the detection of unusually high atmospheric levels of HCB. Inhabitants of Flix presented the highest serum HCB levels ever found (Sala et al., 1998). The aim of this study is to describe the internal dose levels of HCB and other organochlorine compounds (*p,p'*DDE, β -HCH and PCBs) in maternal blood at delivery and in cord blood of newborns from Flix and nearby villages. The present study was carried out in the framework of a larger project whose main objective is to evaluate the effect of HCB exposure on the neurological development of infants.

2. Population and methods

Between May 1997 and September 1999 we examined 62 pairs of mothers and their healthy full-term newborns (71% of all eligible births). In addition, we included 10 samples of maternal blood at delivery for which we could not obtain corresponding infant cord blood, and seven cord blood samples of newborns for which no maternal blood samples were available. Levels of organochlorine compounds were measured in maternal blood at delivery and in cord blood. Serum obtained by centrifuging the blood sample was stored at -40°C until analysis. Organochlorine compounds in sera were analyzed by gas chromatography (GC) coupled to electron capture detection and GC coupled to chemical ionization negative-ion mass spectrometry. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL was used for the analyses. All the analyses were carried out in the

Department of Environmental Chemistry (CID-CSIC). Details of the methodology have been reported elsewhere (To-Figueras et al., 1997).

2.1. Gas chromatography analysis

The chromatographic analysis consisted of GC with data output connected through a PE-Nelson interface to a PC for acquisition, analysis and storage of GC runs using the Nelson 2600 data system. The GC system consisted of a Hewlett-Packard model 5890 equipped with EC detector, a Hewlett-Packard model 7673 autosampler, and split/splitless injector. The analysis was performed using a 30 m DB-5 (5% phenyl-95% methylsiloxane) capillary column (0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The initial oven temperature was 80°C , held for 2 min and programmed to 300°C at $6^{\circ}\text{C}/\text{min}$. The final temperature was held for 10 min. The injector temperature was kept at 270°C , the detector at 310°C . The carrier gas was ultrapure helium at 18 psi head column pressure and linear velocity of 30 cm/s and the make-up gas was ultrapure nitrogen at a flow rate of 60 ml/min. The samples were injected by the autosampler in the split/splitless mode. The data from the GC were acquired and stored directly in the computer by the Nelson 2600 data system. The analytes were identified by their retention time relative to the surrogate standard. Quantification was performed by external standard. The concentrations were calculated by interpolation on the linear curve corresponding to each compound. In the compounds eluting near HCB, these concentrations were corrected for recovery calculated from the surrogate standard TBB and PCB 209.

We present the results for the most prevalent compounds found in sera samples: hexachlorobenzene (HCB), dichlorodiphenyl dichloroethene (*p,p'*DDE), polychlorinated biphenyls (PCBs) which we present as the summation of the individual congeners 28, 52, 101, 118, 138, 153, and 180, and beta-hexachlorocyclohexane (β -HCH). Because the sum of PCBs 118, 138, 153 and 180 represented 91% and 98% of total PCBs in cord blood and in maternal blood, respectively, we provide the results of these four congeners. Detection limits for HCB, β -HCH and *p,p'*DDE were 0.03, 0.15 and 0.09, respectively, and for the individual congeners of PCBs were as follows: 0.17, 0.15, 0.09, 0.11, 0.15, 0.12, 0.10.

Information on age, general lifestyle, occupation and residence of the parents as well as on characteristics of the newborns was obtained through a questionnaire. Written consent was obtained from all parents.

3. Statistical analysis

Initial descriptive statistical parameters were computed. Because organochlorine compound distributions

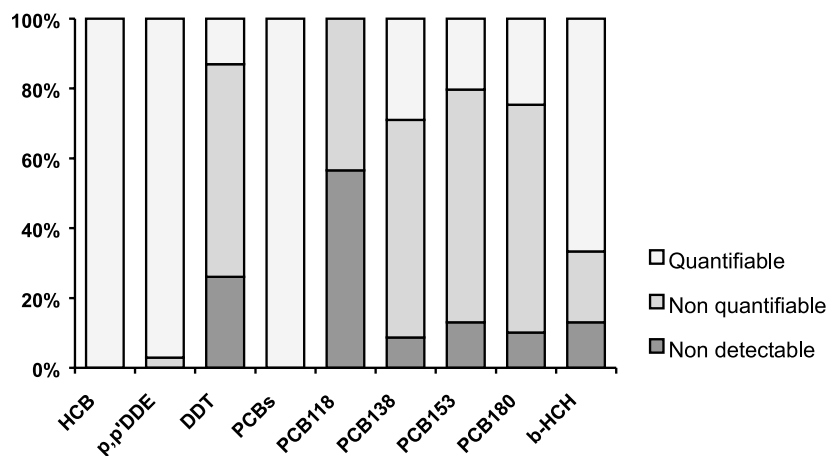


Fig. 1. Detectable and quantifiable levels of HCB, *p,p'*DDE, PCBs and β -HCH in cord blood of all newborns included in the study ($n = 69$).

in serum were skewed to the right, we present medians and geometric means. When necessary, the natural logarithmic transformation was used in the analysis. In all statistical analysis, the concentrations of organochlorine compounds below the detection limit were set at half the limit of detection. Multiple linear regression analysis was conducted to model the adjusted association between levels of organochlorine compounds in cord blood and maternal capillary blood at delivery. All statistical analyses were conducted with the SPSS and STATA packages.

4. Results

We analyzed 72 maternal blood samples and 69 cord blood samples. Of these, 62 were mother–infant pairs, 31 from Flix and 31 from nearby villages. The mean age of the mothers was 30.3 and 31.5 (range 17–41), the gestational age was 39.9 and 39.8 weeks, and the weight of newborns was 3236.1 and 3348.9 g, respectively, none of these differences being statistically significant. Levels of HCB and PCBs in cord blood were detected and quantifiable in 100% of the samples, and levels of *p,p'*DDE and β -HCH in 97.1% and 66.7%, respectively (Fig. 1). In mothers, blood levels of HCB and PCBs were also detected and quantifiable in 100% of the samples. In Fig. 2 we show the chromatograms of maternal blood and cord blood from mothers and newborns from Flix and from nearby villages. The highest levels found were for HCB in mothers and newborns from Flix (Table 1), with a geometric mean of 3.98 and 1.40 ng/ml, respectively compared to 2.51 ng/ml and 0.85 in the nearby villages, the difference in both cases being statistically significant ($P < 0.05$). The second highest level was that

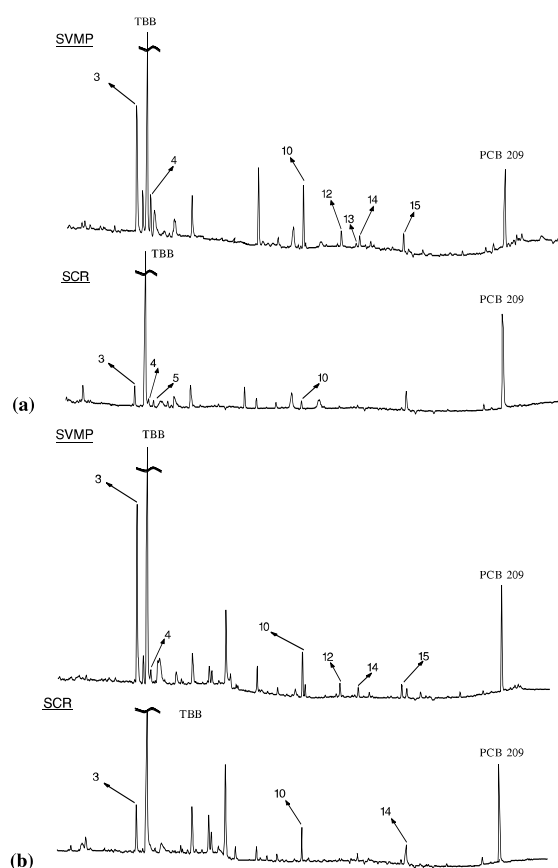


Fig. 2. Characteristic chromatogram of a maternal serum (SVMP) and umbilical cord (SCR) of mothers from Flix (a) and nearby villages (b). Compounds detected: 3 – HCB, 4 – β -HCH, 5 – γ -HCH, 10 – *pp'*DDE, 12 – PCB 153, 13 – *pp'*DDT, 14 – PCB 138, 15 – PCB 180.

Table 1

Geometric mean (GM) and range of HCB, *p,p'*DDE, DDT, PCBs and β -HCH levels in cord blood and in maternal blood at delivery, by residence

| Maternal blood | Total (<i>n</i> = 72) ^a | | Flix (<i>n</i> = 37) | | Nearby villages (<i>n</i> = 34) | |
|----------------------------|--|------------|--------------------------|------------|-------------------------------------|------------|
| | GM | Range | GM | Range | GM | Range |
| HCB (ng/ml) | 3.19 | 0.36–20.78 | 3.98 | 0.50–20.78 | 2.51 | 0.36–7.46 |
| <i>p,p'</i> DDE (ng/ml) | 2.24 | 0.36–24.30 | 1.96 | 0.36–9.93 | 2.59 | 0.41–24.30 |
| DDT (ng/ml) | 0.10 | 0.01–3.25 | 0.08 | 0.01–0.78 | 0.12 | 0.01–3.25 |
| PCBs (ng/ml) | 1.64 | 0.23–7.74 | 1.59 | 0.23–6.96 | 1.71 | 0.46–7.74 |
| PCB ₁₁₈ (ng/ml) | 0.04 | 0.01–0.33 | 0.03 | 0.01–0.20 | 0.05 | 0.01–0.33 |
| PCB ₁₃₈ (ng/ml) | 0.44 | 0.05–3.70 | 0.42 | 0.05–1.81 | 0.46 | 0.05–3.70 |
| PCB ₁₅₃ (ng/ml) | 0.48 | 0.01–2.97 | 0.44 | 0.01–2.97 | 0.54 | 0.01–2.34 |
| PCB ₁₈₀ (ng/ml) | 0.46 | 0.05–3.34 | 0.46 | 0.05–2.58 | 0.46 | 0.05–3.34 |
| β -HCH (ng/ml) | 1.06 | 0.01–11.09 | 1.00 | 0.01–11.09 | 1.14 | 0.01–9.31 |
| Cord blood | (<i>n</i> = 69) ^b | | (<i>n</i> = 35) | | (<i>n</i> = 32) | |
| | GM | Range | GM | Range | GM | Range |
| HCB (ng/ml) | 1.11 | 0.13–5.77 | 1.40 | 0.30–5.77 | 0.85 | 0.13–2.45 |
| <i>p,p'</i> DDE (ng/ml) | 0.83 | 0.05–7.11 | 0.86 | 0.05–7.11 | 0.81 | 0.05–3.06 |
| DDT (ng/ml) | 0.05 | 0.01–1.87 | 0.04 | 0.01–1.30 | 0.06 | 0.01–1.87 |
| PCBs (ng/ml) | 0.36 | 0.07–3.85 | 0.39 | 0.07–3.85 | 0.32 | 0.11–1.96 |
| PCB ₁₁₈ (ng/ml) | 0.02 | 0.01–0.05 | 0.02 | 0.01–0.05 | 0.02 | 0.01–0.05 |
| PCB ₁₃₈ (ng/ml) | 0.08 | 0.01–1.86 | 0.08 | 0.01–0.81 | 0.08 | 0.01–1.86 |
| PCB ₁₅₃ (ng/ml) | 0.06 | 0.01–1.33 | 0.06 | 0.01–1.33 | 0.06 | 0.01–0.48 |
| PCB ₁₈₀ (ng/ml) | 0.07 | 0.01–1.59 | 0.10 | 0.01–1.59 | 0.04 | 0.01–0.34 |
| β -HCH (ng/ml) | 0.26 | 0.01–3.20 | 0.25 | 0.01–2.61 | 0.29 | 0.01–3.20 |

^a Residence not available in one mother.

^b Residence not available in two newborns.

for *p,p'*DDE followed by the PCBs. Among the PCB congeners, the least prevalent was PCB118 in both mothers and newborns. No significant statistical differences were found between organochlorinated compounds other than HCB between mothers and newborns from Flix and from nearby villages. The crude Pearson correlation coefficient between HCB, *p,p'*DDE, PCBs and β -HCH in cord blood and the same compounds in maternal blood of the 62 pairs were 0.63, 0.58, 0.005 and 0.40, respectively, all except PCBs being statistically significant ($P < 0.01$). The associations between the organochlorinated compounds in cord blood and maternal blood were derived from multiple linear regression analysis (Table 2). A positive statistically significant association was observed between HCB, *p,p'*DDE and β -HCH levels in cord blood and levels in maternal blood (β coefficients were 0.45, 0.38 and 0.36, respectively). However, total PCBs presented a negative association that was not statistically significant. Only PCB153 and PCB180 presented a positive association between maternal blood levels and cord blood ($P > 0.1$). No statistically significant association was found between any organochlorinated compound analyzed in cord blood and gestational age. Maternal age was only associated with *p,p'*DDE cord blood levels (β coefficient 0.07, $P = 0.01$).

5. Discussion

All newborns studied presented detectable levels of organochlorine compounds, with the highest levels for HCB, followed by *p,p'*DDE and PCBs. Levels of HCB were higher in mothers and newborns from Flix than nearby villages with a statistically significant difference. There was an adjusted positive association between HCB levels in maternal blood and in cord blood. A positive association was also observed for *p,p'*DDE and β -HCH but not for PCBs.

In the present study, the geometric mean of blood samples of mothers at delivery was 3.98 ng/ml, which was similar to the GM observed among women of the same age range in a previous study on a general population sample from Flix. In this previous study (Sala et al., 1998), the geometric mean of HCB in the general population was much higher (16.5 ng/ml) because it included current workers of an electrochemical factory (10% of studied population), the mean age being 46.3 years. The observed levels of HCB in maternal blood at delivery, however, are still higher than those reported in other populations. In a study conducted in Norway in 1988 (Skaare et al., 1988) levels of maternal blood at delivery were 2 ppm. In our study, the arithmetic mean was 4.1 ng/ml, which is approximately double. Other

Table 2

Maternal age adjusted association between maternal and cord blood levels of organochlorine compounds ($n = 62$ mother–infant pairs)

| Maternal blood levels (ln ng/ml) | Log transformed levels in cord blood (ng/ml) | | | | | | | | | | | | | |
|----------------------------------|--|------|-----------------|------|-------|------|--------------------|------|--------------------|------|--------------------|------|--------|------|
| | HCB | | <i>p,p'</i> DDE | | PCBs | | PCB ₁₃₈ | | PCB ₁₅₃ | | PCB ₁₈₀ | | β-HCH | |
| | β | SE | β | SE | β | SE | β | SE | β | SE | β | SE | β | SE |
| HCB | 0.45* | 0.11 | | | | | | | | | | | | |
| <i>p,p'</i> DDE ^a | | | 0.38* | 0.14 | | | | | | | | | | |
| PCBs | | | | | -0.29 | 0.18 | | | | | | | | |
| PCB ₁₃₈ | | | | | | | -0.30 | 0.21 | | | | | | |
| PCB ₁₅₃ | | | | | | | | | 0.02 | 0.16 | | | | |
| PCB ₁₈₀ | | | | | | | | | | | 0.14 | 0.17 | | |
| β-HCH | | | | | | | | | | | | | 0.36** | 0.18 |

^a β–mother's age: 0.067 (SE: 0.026, $P = 0.012$).* $P < 0.01$.** $P < 0.05$.

Table 3

Mean concentration of organochlorinated compounds in cord blood in different countries and periods

| Author | Country | Year(s) of study | <i>n</i> | Mean (ng/ml) | | |
|-------------------------|-------------------|------------------|----------|---------------------|-------------------|-------------------|
| | | | | PCBs | DDE | HCB |
| Schwartz et al. (1983) | USA (Michigan) | 1980 and 81 | 198 | 2.5 ^a | – | – |
| Skaare et al. (1988) | Norway | 1981 and 82 | 20 | 3.0 ^b | 3.0 | 1.0 |
| Grandjean et al. (1997) | Faroe Islands | 1986 and 87 | 435 | 1.10 ^c | – | – |
| Huisman et al. (1995) | Netherlands | 1990–92 | 373 | 0.38 ^d | – | – |
| Lackmann et al. (1996) | Germany | 1984 and 85 | 80 | 1.37 ^{c,e} | – | 2.03 ^e |
| | | 1994 and 95 | 80 | 0.96 ^{c,e} | – | 0.61 ^e |
| Rhainds et al. (1999) | Canada (Quebec) | 1993–95 | 1109 | 0.50 ^a | 0.4 | 0.04 ^f |
| Present study (2000) | Catalonia (Spain) | 1997–99 | 69 | 0.36 ^e | 0.83 ^e | 1.20 ^e |

^a Aroclor 1260.^b Aroclor 1254.^c Total PCBs ($[\sum \text{congeners } 138 + 153 + 180] \times 2$).^d Median of Total PCBs ($\sum \text{congeners } 118 + 138 + 153 + 180$).^e Median.^f Geometric mean.

studies conducted in general populations in the 1990s reported levels usually below 1 ng/ml (Needham et al., 1990; Krauthacker, 1991; Jarrell et al., 1993). Levels of PCBs, *p,p'*DDE and β-HCB, on the other hand, are in the range observed in other populations (Sauer et al., 1994; Greizerstein et al., 1999; Patandin et al., 1999).

Very little information is available on levels of HCB in cord blood (Table 3). In a study conducted in Germany, Lackmann et al. (1996) reported a decrease in HCB and PCB levels between 1985 and 1995. Medians of HCB in cord blood in these two periods were 2.03 and 0.61 ng/ml, respectively. The median observed in our study was 1.17 ng/ml, being higher in newborns from Flix than in newborns from nearby villages (1.45 and 0.97, respectively), which can be explained by the presence of the electrochemical plant in Flix (Girmalt et al., 1994). Levels much lower have been reported by Rhainds et al. (1999) in a study conducted in Canada, with a geometric mean of HCB in cord blood of 0.04 ng/ml. Regarding PCBs, our results showed levels similar to

those reported in the study conducted in the Netherlands (Huisman et al., 1995). These levels are lower than those reported in Germany (Lackmann et al., 1996) or Canada (Rhainds et al., 1999) for similar periods. For DDE, our levels were higher than those reported by Rhainds et al. (1999) in Canada. However, methodological problems such as differences in analytical methods used and small sample size, restrict comparisons with these studies.

The transplacental transference of organochlorine compounds from mother to infant had been reported previously (Skaare et al., 1988; Lackmann et al., 1999), but few studies have provided information specifically on HCB. In our study, levels of HCB in maternal blood were associated with levels in cord blood. Maternal age and gestational age have also been described as predictors of levels of DDE, PCBs and HCB in cord blood (Rhainds et al., 1999; Lackmann et al., 1999). In our study, no statistically significant association was found for gestational age although newborns of 40 weeks or

more presented higher levels of HCB. Maternal age was not associated with HCB levels in cord blood levels. Only levels of DDE in cord blood presented an association with maternal age. Regarding PCBs, in our study no association was observed between maternal levels and cord blood levels and the relationship between PCB concentrations in maternal blood and cord blood has been found to differ for each PCB congener. This has also been observed in other studies (Skaare et al., 1988).

In conclusion, results of this study, in agreement with what has been reported previously, clearly demonstrate a prenatal uptake of organochlorine compounds, and provide more information on the transplacental transference of HCB, one of the most common organochlorine compounds. Levels of HCB in this population are among the highest ever reported in the 1990s in western countries, while levels of other organochlorine compounds are in the range observed elsewhere. Because it has been suggested that prenatal exposure to these compounds may play a more important role in the neurodevelopment of infants than postnatal exposure, more effort should be made to reduce organochlorine compounds' accumulation.

Acknowledgements

We thank all the participants for their generous collaboration and all the personnel of the pediatric department of the Hospital de Mora d'Ebre and Primary Health Care Center of Flix for their help in obtaining the data. The study was funded by grants from the Spanish Ministry of Health (FIS-97/1102) and by the "Fundació La Caixa" (97/009-00).

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ARTICLE 3: N Ribas-Fitó, E Marco, M Sala, A Verdú, JO Grimalt, J Sunyer. **Exposure to organochlorine compounds through breastfeeding in a population exposed to airborne hexachlorobenzene**. J Epidemiol Community Health (enviat per publicació)

Objectiu: L'exposició a OCs es produeix a través de l'úter i de la lactància materna. Els nivells d'HCB en el sèrum de cordó dels nounats nascuts en una població veïna a una fàbrica electroquímica són dels més alts mai descrits. L'objectiu del treball és avaluar el grau de contaminació de la llet materna en aquesta població i la subseqüent exposició dels nens a través de l'alletament matern. **Mètodes:** Es va reclutar una cohort de 92 parelles de mare i nen (84% de tots els nounats nascuts a l'hospital de referència) entre 1997 i 1999 en 5 poblacions veïnes. Els OCs es van mesurar en sèrum de cordó, calostre, llet i sèrum del nen a les 8 setmanes de vida. **Resultats:** Els nivells d'OCs es van detectar i quantificar a totes les mostres de calostre i de llet (medianes en calostre d'HCB, p,p'DDE i PCBs van ser de 0.90, 1.03 i 0.61 µg/g, respectivament). Les concentracions d'aquests compostos en calostre es van correlacionar significativament amb les concentracions del nen a les 8 setmanes de vida. Els nens que van fer lactància materna van presentar unes concentracions més elevades d'OCs a les 8 setmanes que els que van fer lactància artificial (2.84 ng/ml vs. 2.00 ng/ml per l'HCB, 4.55 vs. 0.98 pel p,p'DDE ($p < 0.05$), i 1.62 vs. 0.65 pels PCBs ($p < 0.05$)). Després d'ajustar per l'edat de la mare, la paritat, l'índex de massa corporal, la proximitat a l'empresa electroquímica i les concentracions de sèrum de cordó les diferències van ser significatives pel p,p'DDE i els PCBs. Per l'HCB el fet de viure a la zona de major exposició va implicar un augment de les concentracions durant les 8 primeres setmanes de vida. **Conclusions:** Els nivells d'HCB i de p,p'DDE en el calostre de les mares d'aquesta població són dels més alts mai descrits en poblacions occidentals. La lactància materna implica un canvi d'aquests contaminants al cos i incrementa el grau de contaminació dels lactants durant les primeres setmanes de vida, majoritàriament a través del calostre.

EXPOSURE TO ORGANOCHLORINE COMPOUNDS THROUGH BREASTFEEDING IN A POPULATION EXPOSED TO AIRBORNE HEXACHLOROBENZENE

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Supported in part by grants from 'Fundació La Caixa' 97/009.00, the Spanish Ministry of Health (FIS-97/1102) and Generalitat de Catalunya-CIRIT 1999SGR 00241.

Word counts:

Abstract: 302

Manuscript (not including references and tables): 2003

Number of references: 18

Number of tables: 3

Number of figures: 1

Submitted to the *Journal of Epidemiology and Community Health*

ABSTRACT

Objective: Exposure to organochlorine compounds (OCs) occurs both in utero and through breastfeeding. Levels of hexachlorobenzene (HCB) in the cord serum of newborns from a population located in the vicinity of an electrochemical factory in Spain are among the highest ever reported. We aimed to assess the degree of breast milk contamination in this population and the subsequent child's exposure to these chemicals through breastfeeding.

Methods: A birth cohort including 92 mother-infant pairs (84% of all births in the study area) was recruited between 1997-1999 in 5 neighbouring villages. OCs were measured in cord serum, colostrum, breast milk and child's serum at 8 weeks of age.

Results: Levels of OCs were detected and quantified in all colostrum and milk samples (medians in colostrum for HCB, p,p'DDE and PCBs were 0.90, 1.03 and 0.61 µg/g on lipid basis, respectively). Concentrations of these chemicals in colostrum were significantly correlated with the concentrations in child's serum at 8 weeks of life. Those infants who were breastfed had higher concentrations of OCs at 8 weeks of life than those who were formula fed (2.00 ng/ml among formula feeders vs. 2.84 among breast feeders of HCB, 0.98 vs. 4.55 of p,p'DDE ($p<0.05$), and 0.65 vs. 1.62 of PCBs ($p<0.05$)). After adjusting for maternal age, parity, maternal body mass index, vicinity to the electrochemical factory and concentrations in cord serum the differences were statistically significant for p,p'DDE and PCBs. For HCB, residing in the exposed area significantly increased serum levels during the first 8 weeks of life.

Conclusions: Levels of HCB and p,p'DDE in colostrum in this population are among the highest described in the Western countries. Breastfeeding leads to rapid removal of these contaminants from the body and increases the degree of contamination among breast feeders during the first weeks of life, mainly through colostrum.

KEY WORDS

Hexachlorobenzene, dichlorodiphenyl dichloroethylene, polychlorinated biphenyls, breastfeeding

INTRODUCTION

Persistent organic pollutants (POPs) such as hexachlorobenzene (HCB), dichlorodiphenyl dichloroethane (p,p'DDE) and polychlorinated biphenyls (PCBs) find their way into body fat and tend to be eliminated slowly. Therefore, over time, they concentrate in the human body. Due to the relatively high fat content of breast milk, these lipophilic compounds are transferred to the milk. These compounds are excreted from the mother to the child via the placenta and primarily via breast milk. Breastfeeding has been associated with an increase in the whole blood concentration of OCs in children (1). For dioxins, it has been estimated that during 3 months of breast-feeding, newborns uptake 6% of their lifetime dose (2). For most infants, breast milk is the sole source of food initially and bears several advantages. Taking into account the well-known benefits of breastfeeding for developing infants, it is recommended by the World Health Organization (WHO) that breastfeeding should be encouraged and promoted. Although over the past few decades, levels of these contaminants have declined in breast milk in countries where these chemicals have been banned (3), it is still essential to ensure that the degree of contamination is minimised with a continued monitoring of these chemicals in human milk (4).

Unusually high atmospheric concentrations of HCB were found in the population of a rural village of 5000 inhabitants in the vicinity of an electrochemical factory (Flix, Catalonia, Spain) (5). Production of DDT in the factory ended in 1971 and of PCBs in 1987. Adult inhabitants studied in 1994 had the highest serum HCB levels ever found (mean of 36.7 ng/ml) (6) and levels of HCB and p,p'DDE in the cord serum of newborns from this population studied in 1999 were among the highest ever reported (7), while other OCs were at similar levels described in other general populations.

We aimed to study the current extent of OCs contamination in the milk from mothers living in this population and to study the transference of these chemicals to the child through breastfeeding.

METHODS

A birth cohort of 92 mother-infant pairs was set-up including 84% of all singleton children born in the main hospital of the study area during the period March 1997 to December 1999. The study area included the village of Flix (where the electrochemical factory is located) and all other towns of the same administrative health area (12.000 inhabitants) (some as far as 10km from the factory). None of the children had major congenital anomalies or other diseases. This study was approved by the ethics committee of the Institut Municipal d'Investigació Mèdica and all mothers provided a signed informed consent.

Colostrum samples (10-15 mL) were collected in glass vials by manual expression in the hospital within the first 3 days after delivery. Milk samples (10-15 mL) were collected in glass vials after manual expression at home three weeks after delivery. OCs in cord serum, child serum at 8 weeks of life and maternal colostrum and milk were measured by gas chromatography (GC) with electron capture detection and GC coupled to chemical ionisation negative-ion mass spectrometry. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL was used for the analyses. Serum samples were stored at -40°C until analysis. All the analyses were carried out in the Department of Environmental Chemistry (CID-CSIC). We present results for the most prevalent compounds found in sera samples: hexachlorobenzene (HCB), dichlorodiphenyl dichloroethylene (p,p'-DDE), beta-hexachlorocyclohexane (β -HCH), and polychlorinated biphenyls (PCBs) which we present as the summation of the individual congeners 28, 52, 101, 118, 138, 153, and 180. Detection limits for HCB, p,p'-DDE and β -HCH and were 0.03, 0.09 and 0.15 respectively, and around 0.10 for the individual PCB congeners. A value of 0.01 ng/ml was given for the non-detectable levels and a value of 0.05 ng/ml for those detectable but not quantifiable. The between-assay coefficient of variation for the assays was 6.4% for HCB, 11.5% for β -HCH, 8.6% for p,p'-DDE, and between 6 and 11% for the individual PCB congeners.

Levels of OCs in colostrum and milk were adjusted for the lipid content.

Information on socio-economic background, maternal diseases and obstetric history, parity, gender, fetal exposure to alcohol and cigarette smoking, and type and duration of

breastfeeding was obtained through questionnaires administered in-person after delivery and at 13 months.

Descriptive statistical parameters were computed. Because organochlorine compound distributions in serum and colostrum and milk were skewed to the right, the concentrations were natural logarithmic transformed. Confounding variables selected were those described in previous studies such as maternal age, number of siblings and body mass index. Variables with a p-value < 0.10 were retained in the final model. Multiple linear regression analysis was conducted to model the adjusted association between type of feeding and levels of OCs in colostrum with levels in child's serum at 8 weeks of age. All statistical analyses were conducted with the STATA 6.0 statistical software package. Criteria of statistical significance was $p < 0.05$.

RESULTS

Levels of OCs were detected and quantified in all colostrum and milk samples. Table 1 shows the medians and interquartile ranges of HCB, p,p'DDE, PCBs and b-HCH concentrations in colostrum, maternal milk and child's serum at 8 weeks of life. In colostrum and maternal milk the highest levels were for p,p'DDE followed by HCB with a median of 1.03 and 0.9 µg/g respectively in colostrum, and a median of 0.8 and 0.63 µg/g respectively in milk. In child's serum at 8 weeks of age the highest levels were for HCB (median of 2.68 ng/ml) followed by p,p'DDE (median of 1.50 ng/ml).

The crude Spearman correlation coefficients between HCB, p,p'DDE, PCBs and b-HCH in cord serum and the same compounds in colostrum, milk, child's and maternal serum at 8 weeks of life are shown in table 2. The concentrations of HCB, p,p'DDE and PCBs in colostrum were highly correlated with the concentrations in child's serum at 8 weeks of life (0.65 for HCB, 0.70 for p,p'DDE and 0.65 for PCBs ($p < 0.05$)). The concentrations of these chemicals in milk were not correlated with the concentrations in the child's serum at the age of 8 weeks but were significantly correlated with the maternal levels at 8 weeks after childbirth. Only levels of p,p'DDE in cord serum were correlated with the levels of these OC in the child serum at the age of 8 weeks (0.57 ($p < 0.05$)).

Figure 1 shows the concentrations of OCs in child's serum at 8 weeks of life according to type of feeding. Those infants who were breastfed had higher concentrations of OCs at 8 weeks of life than those who were formula fed (2.00 ng/ml among formula feeders vs. 2.84 among breast feeders of HCB, 0.98 vs. 4.55 of p,p'DDE ($p < 0.05$), and 0.65 vs. 1.62 of PCBs ($p < 0.05$)). After adjusting for levels in cord serum, parity, maternal age, maternal body mass index and residence in Flix the associations with levels of p,p'DDE and PCBs remained statistically significant. When we considered the difference between the concentrations in the child's serum at 8 weeks and the concentrations in cord serum, breastfeeding was only associated with an increase in the concentrations of p,p'DDE (an increase of 0.86 ng/ml among formula feeders vs. an increase of 2.72 ng/ml among breast feeders ($p < 0.05$)).

In table 3 the association between concentrations of OCs in child's serum at 8 weeks of life and the combination between type of feeding and concentration of OCs in colostrum is shown. Those infants who were breastfed and whose mother's had higher concentrations of OCs in colostrum (above the median) increased significantly their levels at 8 weeks of life. In the case of HCB, residence in Flix was an important predictor of the increasing concentrations during the first weeks of life.

DISCUSSION

Levels of OCs were detected and quantified in all colostrum and milk samples. Concentrations of these chemicals in colostrum were significantly correlated with the concentrations in child's serum at 8 weeks of life. Those infants who were breastfed had higher concentrations of OCs at 8 weeks of life than those who were formula fed.

Levels of HCB and p,p'DDE in colostrum and milk samples from this population are among the highest reported in any Western country. In a study conducted in Madrid (Spain) in 1991 mean levels of HCB and p,p'DDE in milk were 0.008 $\mu\text{g/g}$ and 0.60, respectively (8). In previous studies conducted in other European countries the median levels in milk reported for HCB and p,p'DDE were 0.08 and 0.24 $\mu\text{g/g}$ in Germany in 1997 (4), and 0.01 and 0.13 in Sweden in 1991 (9). Levels of PCBs were among the range observed in other populations although comparability is difficult due to differences in the number of PCB congeners measured and differences in reporting results (3, 10).

The production of most of these OCs is forbidden in some countries as a result of their known broad-spectrum effects in animals and humans. Their release into the environment, however, persists since some industrialised countries still have not banned or restricted their production, and they may be also emitted as a sub product in the organochlorine industry and incineration (11). Agricultural use of DDT (the precursor of p,p'DDE) is known to have continued after its banning (12). Current levels of these organochlorines in human tissues can be a result of past exposure since the mean life of these compounds is several years, but also from current exposures through diet and air. Although the data that do exist suggest that bans and restrictions in recent decades on the use of many of the POPs have led to a decline in levels of these chemicals in breast milk (12, 13), there is still limited data regarding some countries. In this population high concentrations of HCB are explained because HCB is an airborne pollutant in this population. The origins of high concentrations of pp'DDE in Spain are not known, but current levels of p,p'DDE in Spanish butter are also among the highest ever reported (14).

In this study we attempted to understand which is the critical period for exposure to OCs through breastfeeding. We have observed that the correlations of OCs in child's serum at 8 weeks are higher with colostrum than with milk. This could be explained by a higher transference of these compounds during the early lactation than later on. A recent study in Mexico also observed the same tendency of decreasing levels from colostrum to mature milk (15).

In this study differences in the transference of the different OCs through breastfeeding are notable. For HCB, the major contaminant in the area, it is interesting to observe how breastfeeding is only in part responsible of the increasing levels in the child during the first weeks of life. The fact that residence in Flix is one of the predictors of the increasing levels of HCB could be explained by airborne exposure to this chemical.

For p,p'DDE is surprising to observe that both in mature milk and in colostrum it is the most important contaminant. Among breastfeeders levels of p,p'DDE at 8 weeks of life were also higher than HCB. The fact that the concentration of this chemical is higher in milk and colostrum and that breastfeeding leads to a higher transference of this chemical could be due to a different pattern of excretion.

We did not observe any association between maternal body mass index and transference of OCs compounds through breastfeeding. Only maternal age was positively associated with the child's concentrations at 8 weeks of life.

The advantages of breastfeeding have been documented in the neonatal period and extend throughout childhood and into adulthood (16). There are also clear benefits to the mother (17). In the present population we observed that long-term breastfeeding was found to be beneficial to neurodevelopment, potentially counterbalancing the impact of the exposure to these chemicals through breast milk (18).

Although the weight of the evidence to date indicates that the advantages of breast-feeding outweigh any risks from contaminants in breast milk, it is important to identify contaminant trends, locate disproportionately exposed populations, and take public health measures to decrease and eliminate xenobiotics from breast milk (3).

ACKNOWLEDGMENTS

We thank all of the study participants for their generous collaboration, and all of the personnel in the pediatric department of the Hospital de Móra d'Ebre and the Primary Health Care Center of Flix for their help in obtaining data; in particular thank Mrs. Rosa M. Sabaté. We are grateful to Dave Macfarlane for his help in editing the manuscript. This study was funded by grants from the Spanish Ministry of Health (FIS-97/1102), "Fundació La Caixa" (97/009-00 and 00/077-00) and the Generalitat de Catalunya-CIRIT 1999SGR 00241.

Table 1. Median and interquartile range (IQ range) of HCB, p,p'DDE, PCBs and b-HCH concentrations in colostrum, maternal milk and child's serum at 8 weeks of life*

| | Colostrum ($\mu\text{g/g}$) n=59 | | Maternal milk ($\mu\text{g/g}$) n=57 | | Child's serum at 8 weeks (ng/ml) n=43 | |
|---------|---------------------------------------|-----------|---|-----------|---|-----------|
| | Median | IQ range | Median | IQ range | Median | IQ range |
| HCB | 0.9 | 0.51-1.39 | 0.63 | 0.35-.97 | 2.68 | 0.83-4.16 |
| p,p'DDE | 1.03 | 0.49-1.90 | 0.8 | 0.45-1.23 | 1.5 | 0.73-4.11 |
| PCBs | 0.61 | 0.32-1.05 | 0.39 | 0.28-0.75 | 1.22 | 0.53-2.01 |
| b-HCH | 0.48 | 0.23-0.75 | 0.29 | 0.15-0.51 | 0.47 | 0.01-1.87 |

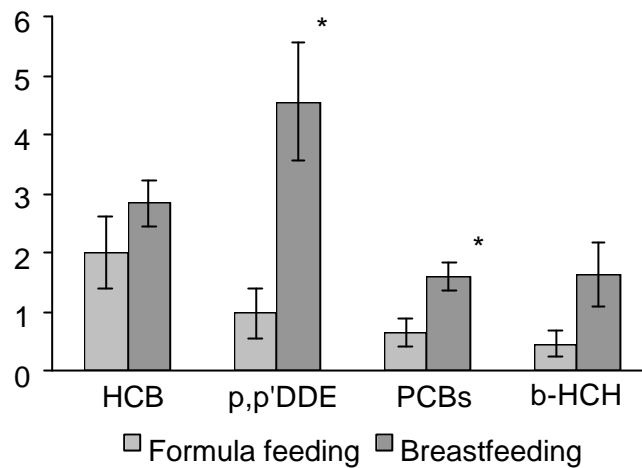
* Median concentrations in cord serum were 1.13 ng/ml for HCB, 0.85 for p,p'DDE, 0.27 for the sum of PCBs and 0.45 for β -HCH

Table 2. Spearman correlations between concentrations of OCs in cord serum, colostrum, maternal milk and child and maternal serum at 8 weeks of life.

| | Colostrum | MatureMilk 3 weeks | Child's serum 8 weeks | Maternal serum 8 weeks |
|--------------------------|-----------|-----------------------|--------------------------|---------------------------|
| Cord serum | HCB | 0.53* | 0.32* | 0.22 |
| | P,p'DDE | 0.47* | 0.33* | 0.57* |
| | PCBs | 0.38* | 0.14 | 0.06 |
| | b-HCH | 0.31 | 0.31* | 0.22 |
| Colostrum | HCB | 0.38* | 0.65* | 0.14 |
| | P,p'DDE | 0.17 | 0.70* | 0.51* |
| | PCBs | 0.21 | 0.65* | 0.14 |
| | b-HCH | 0.35* | 0.13 | 0.56* |
| MatureMilk 3 weeks | HCB | | 0.02 | 0.36* |
| | P,p'DDE | | 0.27 | 0.56* |
| | PCBs | | 0.19 | 0.52* |
| | b-HCH | | 0.05 | 0.68* |
| Child's serum 8 weeks | HCB | | | 0.19 |
| | P,p'DDE | | | 0.48* |
| | PCBs | | | 0.25 |
| | b-HCH | | | 0.20 |

* $p < 0.05$

Figure 1. Concentrations of OCs in child's serum at 8 weeks of life according to type of feeding.



* $p < 0.05$ when adjusted for parity, maternal age, maternal body mass index and residence in Flix. Concentrations of OCs were log-transformed in the multivariate models.

Table 3. Association (betas (b) and standard errors (SE)) between concentrations of OCs in child's serum at 8 weeks of age with the type of breastfeeding and the concentration of OCs in colostrum.

| | HCB | p,p'DDE | PCBs | b-HCH |
|--|--------------|--------------|--------------|--------------|
| | b (SE) | b (SE) | b (SE) | b (SE) |
| BF/ [OCs] in colostrum = median | 0.20 (0.39) | 1.62 (0.43)* | 0.84 (0.39)* | 1.00 (0.83) |
| BF/ [OCs] in colostrum > median | 1.39 (0.40)* | 2.16 (0.45)* | 1.23 (0.35)* | 0.33 (0.94) |
| Parity (per each sibling) | -0.36 (0.24) | - | - | -1.07 (0.56) |
| Maternal age (per year) | - | 0.11 (0.04)* | 0.06 (0.03)* | 0.28 (0.08)* |
| Maternal body mass index (per kg/m^2) | 0.09 (0.05) | - | - | - |
| Residence in Flix | 0.96 (0.34)* | - | 0.50 (0.31) | - |

BF: Breastfeeding; [OCs]: concentrations of OCs.

* $p < 0.05$

Each column derives from a different multivariate model for each specific organochlorine compound. Levels of OCs are log transformed.

Reference value: infant with formula feeding, with no siblings, whose mother's had 30 years of age and a BMI of $26 \text{ kg}/\text{m}^2$, not residing in Flix.

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ARTICLE 4: D Ozalla, C Herrero, N Ribas-Fitó, J To-Figueras, A Toll, M Sala, JO Grimalt, X Basagaña, M Lecha, J Sunyer. **Evaluation of urinary porphyrin excretion in neonates born to mothers exposed to airborne hexachlorobenzene** . Environ Health Perspect 2002;110:205-9

L'existència d'un lligam entre l'HCB i la porfíria cutània tarda es coneix des de fa molt temps. La informació epidemiològica existent sobre els efectes sobre la salut de l'exposició prenatal no ha proporcionat evidències convincentes de que l'HCB alteri el metabolisme de les porfirines. Els objectius d'aquest treball eren els d'analitzar el patró d'excreció de les porfirines urinàries i els nivells d'HCB en sèrum matern i en sang de cordó en els nounats nascuts a un poble (Flix) proper a una empresa de dissolvents clorats, per tal de detectar possibles efectes adversos en el patró d'excreció de porfirines urinàries, i avaluar-ne la seva relació amb els nivells d'HCB. Es va dur a terme un estudi transversal entre la Unitat de Porfirines d'un hospital terciari de Barcelona i la Unitat de Pediatria de l'hospital de Móra d'Ebre (el centre hospitalari de referència de la zona). Es van incloure els nounats nascuts a l'hospital de Móra d'Ebre 1997-1999 i les seves mares. Es van obtenir 68 mostres d'orina dels nounats 3 dies després del naixement per avaluar el patró d'excreció de les porfirines urinàries. Es van obtenir 52 mostres de sang de cordó i 56 mostres de sang de la mare per tal de mesurar els nivells d'HCB. Les uroporfirines totals es van mesurar amb espectrofluorometria. El patró de les porfirines es va mesurar amb HPLC. Els nivells d'HCB en sèrum es van mesurar a través de cromatografia de gasos acoblada a detecció de captura d'electrons. En el conjunt de la població, la mediana de la concentració d'HCB va ser de 1.08 ng/ml en sang de cordó i de 3.31 ng/ml en la sang de la mare. La concentració de porfirines urinàries va ser de 37.87 µmol/mol creatinina. La coproporfirina I i la coproporfirina II van ser les porfirines més excretades. No es va trobar cap associació positiva entre l'excreció de porfirines urinàries i els nivells d'HCB. Es va observar una associació entre el tabaquisme matern i l'excreció de coproporfirines. Tot i que a la població de Flix s'hagin reportat nivells tan elevats d'HCB, no s'ha trobat cap associació amb el patró d'excreció de porfirines urinàries.

Evaluation of Urinary Porphyrin Excretion in Neonates Born to Mothers Exposed to Airborne Hexachlorobenzene

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The existence of a link between hexachlorobenzene (HCB) and porphyria cutanea tarda has been known for a long time. However, the epidemiologic data on effects on health caused by prenatal exposure have not provided convincing evidence that HCB alters porphyrin metabolism. Our objectives were to analyze urinary porphyrin excretion and HCB in maternal serum and fetal cord blood in neonates born in a village (Flix) near a chlorinated solvent factory, to detect possible adverse effects in urinary porphyrin excretion caused by prenatal exposure, and to assess their relationship with HCB blood levels. We conducted a cross-sectional study in the Porphyria Unit at a tertiary care facility in Barcelona, Spain, and the Pediatric Unit of the Móra d'Ebre Hospital, the reference hospital of the study area. We included in the study all neonates ($n = 68$) born in Móra d'Ebre Hospital 1997–1999 and their mothers. We obtained 68 urine specimens of singleton neonates on the third day after birth to test for urinary porphyrin excretion. We obtained 52 fetal cord blood and 56 maternal serum samples for HCB analysis. Total urinary porphyrins were quantified using spectrofluorometry. Porphyrin profile was determined by HPLC. Serum HCB was analyzed by gas chromatography coupled with electron capture detection. In total population, median HCB levels were 1.08 ng/mL in cord blood and 3.31 ng/mL in maternal serum. Total urinary porphyrin concentration was 37.87 $\mu\text{mol/mol}$ creatinine. Coproporphyrin I and coproporphyrin III were the major porphyrins excreted. We found no positive relationship between urinary porphyrin excretion and HCB levels. However, we observed an association between maternal smoking and coproporphyrin excretion. Although high environmental levels of HCB are reported in the town of Flix, we found no alteration in urinary porphyrin excretion. **Key words:** coproporphyrin I, coproporphyrin III, hexachlorobenzene, neonates, porphyria, uroporphyrin. *Environ Health Perspect* 110:205–209 (2002). [Online 18 January 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p205-209ozalla/abstract.html>

Hexachlorobenzene (HCB) is a widespread, highly lipophilic environmental pollutant that accumulates in biologic systems. Nowadays, the major source of HCB is industrial emission as a by-product of the manufacture of organochlorinated products.

Porphyria cutanea tarda (PCT) is the most common of human porphyria. This disease is caused by a partial deficiency of the uroporphyrinogen decarboxylase (UROD) enzyme in the liver, and it is one of the major potential toxic manifestations of this chemical, as several studies in experimental animals have demonstrated (1,2). The disease is characterized biochemically by marked increases in uroporphyrin and heptaporphyrin in urine (3).

Although the existence of a link between HCB and porphyria has been known for a long time, the porphyrinogenic effect of this chemical on humans has not been widely studied. The first cases of PCT induced by HCB in humans were reported in southeastern Turkey in the late 1950s (4). The outbreak was related to the inadvertent ingestion of seed wheat contaminated with the fungicide HCB. It was estimated that

5,000 subjects developed acquired PCT. The syndrome commonly consisted of weight loss, weakness, thyromegaly, hepatomegaly, gross porphyrinuria, hypertrichosis, and photosensitive dermatopathy (4). Children were affected disproportionately more than adults; it was estimated that over 3,000 children under age 16 who had ingested contaminated bread developed the disease. However, the group most severely affected by this chemical were breast-fed babies. Over 1,000 babies born to mothers with clinical symptoms of PCT or who had ingested contaminated bread during gestation or lactation or both died before the age of 12 months with weakness, convulsions, and toxic erythema known as “pembe yara.” Pembe yara was caused by HCB intake both transplacentally and via mother's milk (5). This was the first evidence that HCB is toxic to young children. Unfortunately, no dose–response data were recorded for the Turkish outbreak (6).

HCB and other organochlorine compounds are not readily metabolized or excreted. In pregnant mothers they persist in placenta tissue and are consequently transferred transplacentally from mother to

fetus (7–9). However, little information is available about the relation between prenatal exposure to HCB and porphyrin metabolism in human populations. Several studies have reported the effects of other organochlorinated compounds such as polychlorinated biphenyls, dichlorodiphenyldichloroethylene, dichlorodiphenyltrichloroethane, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (10–13) on urinary porphyrin excretion in children and neonates, but the impact of HCB on these populations has not been analyzed to date.

Previous studies made by our group, in adults of the same population, showed high atmospheric levels of HCB (mean 35 $\mu\text{g}/\text{m}^3$) in Flix (Tarragona, Catalonia, Spain), a rural village of 5,000 inhabitants located near a chlorinated solvent factory (14). We performed a cross-sectional epidemiologic study of the health effects of HCB on the population older than 14 years. We found high serum levels of HCB (mean 36.7 ng/mL), the highest ever recorded (15,16). The evaluation of the urinary porphyrin excretion showed one case of subclinical PCT and 5 subjects with coproporphyrinuria. No association between HCB serum concentrations and total urinary porphyrin excretion was found. The porphyrin profile of the highly exposed subjects was normal (17). Analysis of HCB metabolism and excretion in urine and feces revealed a strong correlation between HCB serum concentrations and pentachlorobenzene (PCBT) in urine (18) and unmetabolized HCB (19).

Although PCT has been associated most frequently with excessive alcohol consumption in middle-aged men, it has also been

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We thank C. Mazón and A. Verdú of the pediatric department of the Hospital of Móra d'Ebre for their collaboration. We are indebted to the subjects and their families for their willingness to participate, and also thank L. Alvarez for creatinine determinations.

This study was supported by grants 99/0141 and 97/1102 from Fondo de Investigación Sanitaria (FISS), Madrid, Spain, and in part by grants from Fundació La Caixa 97/009.00.

Received 12 March 2001; accepted 18 July 2001.

reported in children (20). The availability of a population with high exposure to HCB over the last four decades prompted us to investigate the possible existence of subclinical changes in urinary porphyrin excretion in neonates exposed transplacentally to this chemical, so as to broaden our understanding of its toxic effects.

Materials and Methods

Study population. We performed a cross-sectional study of all 68 full-term singleton neonates born in the Department of Obstetrics at the Móra d'Ebre Hospital between 1997 and 1999. Thirty-eight were from the exposed population (Flix); the other 30 were from neighboring villages and were selected as a nonexposed group.

Information on maternal history, sex, and fetal exposure to alcohol and tobacco consumption was obtained through a questionnaire prepared ad hoc for this study.

Using urine collection bags, we collected fresh urine samples on the third day after birth from 68 full-term neonates. Specimens were immediately frozen and stored at -20°C until assayed. We obtained 52 fetal cord blood (23 from exposed vs. 29 nonexposed) and 56 maternal serum (24 exposed vs. 32 nonexposed) samples for HCB analysis. Informed consent was obtained from parents before collection.

Urinary porphyrin measurements. All urine specimens were analyzed without previous knowledge of HCB levels in fetal cord blood and maternal serum. We determined total urinary porphyrin concentrations by spectrofluorometry (model F-2000; Hitachi Ltd., Tokyo, Japan) (21). We analyzed urinary porphyrin excretion patterns by HPLC. Briefly, 1 mL of urine sample was acidified with 50 μL of concentrated HCl, and 200 μL of this solution was injected. For HPLC determination, we used Waters equipment (Waters Corp., Milford, MA, USA): two pumps (model 515), an autosampler injector (model 717 plus), and Millennium³² software. The porphyrins were detected using a fluorescence detector (model 474), under the following conditions: excitation 405 nm and emission 618 nm, both with bandwidths of 18 nm. Porphyrin separation was achieved with an analytic column BDS-Hypersil (250 \times 4.6 mm, 5 μm particle size; Shandon HPLC, Cheshire, UK) and a gradient from 100% of solvent A (10:90 acetonitrile/ammmonium acetate 1M pH 5.16) to 95% of solvent B (10:90 acetonitrile/methanol) in 25 min. The flow rate was 1.2 mL/min (22).

Urinary creatinine determinations. We measured urinary creatinine concentration (mmol/L) using the Jaffe method (23) on a Cobas Miras (Roche Diagnostics, F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Creatinine was analyzed in the department of biochemistry at the Hospital Clínic, Barcelona.

Analysis of organochlorine compounds. All HCB sera samples were extracted with *n*-hexane and the extracts blindly assayed with gas chromatography coupled to electron capture detection (GC-ECD) in the department of environmental chemistry at the Consejo Superior de Investigación Científica in Barcelona.

Statistical analysis. Because the data distribution on porphyrins was skewed, we performed a logarithmic transformation. The variable HCB was treated as a trichotomous variable with values $\text{HCB} < 2.43$, $2.43 < \text{HCB} < 4.07$, and $\text{HCB} > 4.07$ ng/mL because the relationship between HCB and uroporphyrin isomer I (UPI), coproporphyrin isomer I (CPI), and coproporphyrin isomer III (CPIII) was not linear.

The concentration of porphyrins below the quantification limit was set at half the limit of detection. To evaluate the relationship among porphyrins (total porphyrin, UPI, CPI, CPIII) and possible confounding variables (sex, gestational age, maternal age, birth weight, alcohol, and tobacco) we used linear regression models.

We performed multiple linear regression analysis to examine the relationship between porphyrins (UP, CPI, CPIII) and HCB levels (fetal cord serum and maternal serum) adjusting for potential confounding variables

such as sex, tobacco, alcohol, and maternal age. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using Stata (StataCorp., College Station, TX, USA).

Results

The anthropometric variables, gestational age, maternal age, and alcohol and tobacco habits in mothers of the population under study are shown in Table 1. Exposed and nonexposed neonates differed in terms of maternal alcohol consumption and smoking during pregnancy. The number of males born in the exposed group was higher than in the nonexposed group.

We found detectable levels of HCB (nanograms per milliliter) in all samples of fetal cord blood and maternal serum. The medians and interquartile ranges are shown in Table 2. In fetal cord blood 59% of cases showed HCB concentrations over 1 ng/mL, a figure that rose to 91% in maternal blood. HCB levels in both sets of samples were slightly higher in the exposed group than in the nonexposed group; the difference was statistically significant ($p < 0.05$).

We analyzed total porphyrin concentrations and individual porphyrins (HPLC) in all 68 urine specimens. The median and interquartile ranges for total porphyrin and the main individual porphyrins excreted are summarized in Table 2. CPI, CPIII, and UPI were the major porphyrins excreted in

Table 1. Characteristics of the study groups.

| Variable | Total population ($n = 68$) | Exposed ($n = 38$) | Nonexposed ($n = 30$) |
|---|-------------------------------|----------------------|-------------------------|
| | Mean (SD) | Mean (SD) | Mean (SD) |
| Weight (g) | 3,281 (457) | 3,244 (503) | 3,328 (396) |
| Height (cm) | 49.5 (2.0) | 49.4 (2.1) | 49.6 (1.8) |
| Gestational age (weeks) | 39.6 (1.5) | 39.7 (1.5) | 39.5 (1.5) |
| Maternal age (years) | 30.8 (4.5) | 30.3 (4.3) | 31.5 (4.7) |
| Sex M/F | 37/31 | 27/11 | 10/20 |
| Maternal smoking during pregnancy (%) ^a | 27.9 | 39.5 | 13.3 |
| Maternal drinking during pregnancy (%) ^b | 20.6 | 26.3 | 13.3 |

Abbreviations: F, females; M, males.

^aAt least a cigarette a day during gestation. ^bModerate/high alcohol intake (as at least once a week) during gestation.

Table 2. Median (interquartile range) of HCB levels in fetal cord serum and maternal serum, and urinary porphyrin excretion in the study groups

| Variable | Total population Median (IQR) | Exposed Median (IQR) | Nonexposed Median (IQR) |
|---|-------------------------------|----------------------|-------------------------|
| ng/mL | | | |
| HCB cord blood ($n = 52$) | 1.08 (0.73–1.67) | 1.41 (0.86–2.05) | 0.92 (0.53–1.21)* |
| HCB maternal serum ($n = 56$) | 3.35 (2.11–5.47) | 3.83 (2.92–5.71) | 2.44 (1.52–3.77)* |
| $\mu\text{mol/mol}$ creatinine ($n = 68$) | | | |
| Total porphyrin | 37.3 (27.9–50.5) | 39.2 (27.4–56.6) | 36.2 (27.9–48.3) |
| UPI | 5.0 (1.9–6.6) | 5.0 (2.2–6.7) | 5.1 (1.6–6.8) |
| UPIII | 0.05 (0.05–0.5) | 0.05 (0.05–0.7) | 0.05 (0.05–0.05) |
| UPI + UPIII | 5.6 (1.9–7.3) | 5.6 (2.2–7.5) | 5.5 (1.6–6.8) |
| CPI | 13.7 (6.3–18.7) | 15.1 (6.7–21.5) | 9.8 (5.3–17.1) |
| CPIII | 10.2 (5.2–20.5) | 11.6 (5.8–22.7) | 7.4 (4.7–12.7)* |
| CPI + CPIII | 23.0 (12.6–38.7) | 27.8 (14.0–42.9) | 19.2 (12.5–30.5) |

IQR, interquartile range (25th–75th percentile).

* $p < 0.05$ compared with exposed population using Kruskal-Wallis nonparametric test for equality of populations.

both groups. Neonates from the exposed group had higher levels of CPIII ($p < 0.05$) than did those of the nonexposed group. The uroporphyrin fraction was the third most excreted porphyrin; more UPI was excreted than UPIII (Figure 1).

We detected the heptacarboxylporphyrin isomer I (hepta I) only in four cases in the exposed group (10.26%), at very low concentrations (median 0.21). We detected the heptaporphyrin isomer III (hepta III) in 14 cases (35.9%) in the exposed group and three cases (9.4%) of the nonexposed group ($p < 0.012$). All values were within the normal range (median 0.94). We found no difference in HCB concentrations between the subjects with and without hepta III. The

hexa and pentacarboxylporphyrin fractions were not detected in any group.

Table 3 shows the relationship among total porphyrin, UPI, CPI, and CPIII concentrations, HCB levels, and other characteristics of the cohort studied. We observed a decrease of urinary porphyrin excretion with HCB levels. Moreover, the neonates in the highest tertile of HCB had lower levels of CPI and CPIII ($p < 0.05$).

In neonates born to cigarette-smoking mothers, the excretion of total porphyrin and CPIII fraction were higher than in non-smokers ($p < 0.05$).

The association between CPIII and the exposed group disappeared after adjusting for smoking (Table 4), because mothers

from the exposed group smoked more during pregnancy. However, the negative association of CPI and CPIII with the highest tertile of HCB did not disappear after adjusting for smoking and alcohol, and remain statistically significant for CPI ($p < 0.05$).

Discussion

Because long-term exposure to HCB can cause its accumulation in humans, adverse effects on health are expected in exposed populations. Public attention has been drawn to HCB when high concentrations are found in the environment, as is the case in Flix (14,15). Detecting subtle alterations in urinary porphyrin excretion may be one of the most useful methods for identifying the biologic response to environmental chemicals.

In the Turkish outbreak, high HCB levels were found in maternal milk (6), but urinary porphyrin excretion was not studied in the breast-fed babies. Porphyrin excretion acquired transplacentally in response to HCB exposure has been demonstrated in experimental animals (24) but little is known about the effects in infants (25). The present study is the first to analyze the urinary porphyrin excretion in human neonates in relation to HCB levels in cord blood and maternal serum. The neonate's HCB burden depends on the mother's level of contamination. In this cohort, the mothers had been living for a long time in a population with the highest environmental (mean 35 ng/m³) (14) and serum (36.7 ng/mL) levels of this chemical ever reported (15), although in recent years HCB levels in the area around Flix have decreased (16) because of protective measures implemented in the factory. However, the HCB concentrations found in cord blood of exposed neonates and in maternal serum were still higher than in the nonexposed group (Table 2). The values in both exposed and nonexposed groups are higher than those found in neonates born in Germany between 1994 and 1995 (median 0.61 ng/mL), but lower than neonates born in Germany between 1984 and 1985 (median 2.03 ng/mL) (26). These values in Europe are much higher than those found in Canada (median 0.04 ng/mL) (27) during 1993–1995 and the United States (median 0.03 ng/g) during 1993–1998 (28).

The results of this study showed no alteration in total urinary porphyrin excretion. The quantitative analysis did not reveal any differences between groups concerning total porphyrin excretion (Table 2). The total urinary porphyrin concentrations detected here are within the ranges observed in other studies of neonates and pediatric groups (29–32). Unfortunately, in the Turkish

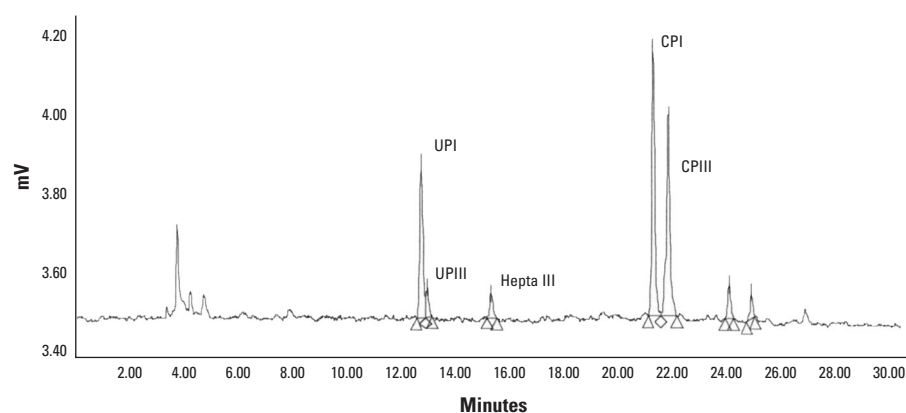


Figure 1. Porphyrin excretion pattern in a 3-day-old neonate.

Table 3. Median (IQR) of variables UPI, CPI, and CPIII, by birth weight, gestational age, maternal age, sex, smoking, alcohol, HCB maternal serum, and HCB fetal cord blood.

| Variable | No. | Total porphyrin | UPI ^a | CPI ^a | CPIII ^a |
|--------------------------|-----|-----------------|------------------|------------------|--------------------|
| Birth weight (g) | | | | | |
| < 2,500 | 3 | 41 (29–57) | 5.3 (0.1–10) | 17 (15–22) | 25 (6.4–32) |
| ≥ 2,500 | 65 | 37 (27–50) | 5.0 (2.0–6.6) | 13 (6.2–18) | 10 (5.0–19) |
| Gestational age (weeks) | | | | | |
| < 37 | 2 | 37 (33–41) | 5.1 (0.1–10) | 24 (23–25) | 15 (7.4–23) |
| ≥ 37 | 66 | 37 (27–52) | 5.0 (2.2–6.6) | 13 (6.2–17) | 10 (5.0–20) |
| Maternal age (years) | | | | | |
| ≤ 30 | 28 | 39 (33–57) | 5.5 (2.7–7.3) | 15 (7.3–23) | 11 (6.8–22) |
| > 30 | 40 | 37 (27–49) | 4.8 (1.6–6.4) | 12 (5.1–17) | 7.6 (3.5–21) |
| Sex | | | | | |
| Male | 37 | 38 (29–49) | 5.8 (2.7–7.5) | 15 (6.8–21) | 11 (3.8–20) |
| Female | 31 | 37 (25–57) | 4.0 (0.7–6.3) | 10 (5.2–17) | 9.8 (5.8–21) |
| Smoker | | | | | |
| No | 49 | 35 (26–43) | 4.9 (2.4–6.6) | 12 (5.3–17) | 7.4 (4.1–18) |
| Yes | 19 | 47 (40–60)* | 5.8 (0.1–6.9) | 15 (9.0–22) | 16 (10–23)* |
| Alcohol | | | | | |
| No | 54 | 36 (26–48) | 4.8 (1.4–6.6) | 14 (6–18) | 10 (4.7–21) |
| Yes | 14 | 42 (37–59) | 6.0 (4.1–8.0) | 15 (6.3–23) | 10 (6.4–20) |
| HCB fetal cord (ng/mL) | | | | | |
| < 0.8 | 16 | 48 (28–70) | 6.5 (2.6–8.7) | 13 (7.3–23) | 13 (5.2–23) |
| 0.8–1.48 | 20 | 39 (35–53) | 5.9 (3.8–6.6) | 15 (8.3–19) | 13 (7.4–23) |
| > 1.48 | 16 | 26 (19–39)* | 3.1 (0.9–6.2) | 6.2 (2.9–13)* | 5.1 (2.1–11)* |
| HCB mother serum (ng/mL) | | | | | |
| < 2.43 | 19 | 48 (34–68) | 6.1 (3.4–8.8) | 15 (9.7–23) | 7.4 (5.1–21) |
| 2.43–4.07 | 17 | 40 (30–45) | 5.8 (3.3–6.7) | 17 (8.3–22) | 12 (7.7–22) |
| > 4.07 | 20 | 29 (25–38)* | 4.5 (1.6–6.2) | 6.8 (5.0–14)* | 7.1 (2.1–18) |

IQR, interquartile range.

^aμmol/mol creatinine. * $p < 0.05$ using Kruskal-Wallis nonparametric test for equality of populations.

Table 4. Coefficient (SE) obtained with multivariate linear regression between log-transformed porphyrins variables and exposed group, maternal HCB and cord HCB in separate models, adjusting for mother's age, smoking, alcohol, and sex of children.

| Variable | Total porphyrin | UPI ^a | CPI ^a | CPIII ^a |
|--|-----------------|------------------|------------------|--------------------|
| Model with exposed group ^b | | | | |
| Exposed | -0.04 (0.16) | -0.21 (0.48) | 0.09 (0.22) | 0.31 (0.26) |
| Smoker | 0.32 (0.17) | -0.89 (0.51) | 0.16 (0.24) | 0.56 (0.27)* |
| Alcohol | 0.13 (0.18) | 1.02 (0.55) | 0.03 (0.25) | -0.03 (0.29) |
| Model with HCB fetal cord ^b | | | | |
| HCB fetal cord (ng/mL) | | | | |
| 0.8–1.48 | 0.05 (0.20) | 0.11 (0.61) | 0.26 (0.24) | 0.31 (0.29) |
| > 1.48 | -0.49 (0.22)* | -0.56 (0.65) | -0.69 (0.26)* | -0.58 (0.32) |
| Smoker | 0.16 (0.20) | -1.04 (0.61) | 0.32 (0.24) | 0.67 (0.29)* |
| Alcohol | 0.28 (0.24) | 1.18 (0.72) | -0.14 (0.29) | -0.46 (0.35) |
| Model with HCB maternal serum ^b | | | | |
| HCB maternal serum (ng/mL) | | | | |
| 2.43–4.07 | -0.23 (0.18) | -0.10 (0.55) | -0.09 (0.27) | 0.46 (0.35) |
| > 4.07 | -0.47 (0.19)* | -0.48 (0.56) | -0.79 (0.27)* | -0.06 (0.34) |
| Smoker | 0.08 (0.19) | -0.97 (0.57) | 0.11 (0.28) | 0.72 (0.35)* |
| Alcohol | 0.29 (0.19) | 1.29 (0.56)* | 0.19 (0.27) | 0.02 (0.35) |

^aμmol/mol creatinine. ^bCoefficient for maternal age and sex not shown. **p* < 0.05.

outbreak, urinary porphyrin concentrations were not recorded. Although levels of porphyrins were within the normal range (even subclinical normality), we observed a decrease of CPI and CPIII with the highest tertile of HCB (only statistically significant for CPI). This negative association between CP fractions and HCB levels does not agree with the results on experimental porphyria where moderate increases of CP are observed at an early stage (2). However, the small number of subjects and the known variations on the profile of porphyrin excretion during the first days of life preclude a firm conclusion.

The increase of the uroporphyrin and heptaporphyrin fractions is a specific indicator of UROD deficiency. In our cohort, no neonates showed subclinical alterations in the urinary porphyrin profile. CPI was the major porphyrin excreted, in accordance with the normal pattern observed in neonates < 7 days of age, followed by CPIII fraction (32). We found no difference in CPI excretion between both groups, but there was a statistically significant difference in CPIII excretion between exposed and nonexposed groups (Table 2). However, the increase in CPIII excretion cannot be explained by higher HCB concentrations in blood because no positive association was found between the two variables. The UP fraction, the third most excreted porphyrin, was also within normal ranges (30,31). Although the heptaporphyrin fraction is present most frequently in exposed populations, we found no evidence of a relationship between the presence of this porphyrin and HCB concentrations in blood. This lack of association between urinary porphyrin excretion and HCB levels has been observed previously in adults of the same population (17).

The information obtained through the questionnaire reflected differences in response between populations studied regarding tobacco and alcohol habits. In the exposed group the proportion of mothers who smoked and consumed alcohol was higher than in the nonexposed (Table 1), and a relationship between CP excretion and tobacco smoking was found (Table 3). Therefore, the greater CPIII excretion detected in the exposed population may be explained by the deleterious effect of tobacco smoking. Because some cytochrome P450 isoenzymes, such as P4501A2, are involved in disturbances of porphyrin metabolism a possible indirect effect of smoke through a P450 induction cannot be ruled out (33).

We conclude that although high environmental levels of HCB have been reported in Flix, no major alteration in urinary porphyrin excretion is present in neonates. The placental transfer of HCB to the fetus may not reach the threshold for subclinical alteration in porphyrin excretion pattern, considering that HCB levels in mothers are not high at the time of the study. Our findings show only a greater urinary CPIII excretion in the exposed population. However, we found no evidence for an association between HCB levels and the amount of CP. Probably, this increase of CP III is caused by a higher tobacco consumption by the mothers in this group, but further research must be done to elucidate the tobacco effect on porphyrin metabolism. The HCB levels described here could be considered a guideline for evaluating further research in other populations.

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ARTICLE 5: N Ribas-Fitó, M Sala, E Cardo, C Mazón, ME de Muga, A Verdú, E Marco, JO Grimalt, J Sunyer. **Association of hexachlorobenzene and other organochlorine compounds with anthropometric measures at birth.** *Pediatr Res* 2002; 52:163-7

L'objectiu del present treball és el d'avaluar l'associació entre l'exposició prenatal a l'hexaclorobenzè (HCB) i altres compostos organoclorats amb les mesures antropomètriques del nen al moment de néixer. Un total de 98 parelles mare-nen (83% de tots els nens nascuts entre el període 1997-1999 en una àrea específica contaminada per HCB) van ser reclutats després de donar el consentiment informat. Els nivells de compostos organoclorats es van mesurar en 72 mostres de sèrum matern al moment del part i en 70 mostres de sèrum de cordó. Dels compostos organoclorats mesurats en sèrum de cordó, la mitjana dels nivells d'HCB va ser la més elevada (mediana d'HCB = 1.13 ng/ml, p,p'DDE = 0.85 ng/ml, PCBs total = 0.27 ng/ml). Els nounats prematurs presentaven majors concentracions d'HCB [1.94 ng/ml entre els prematurs versus 1.10 ng/ml entre els no prematurs ($p < 0.10$)], p,p'DDE [2.40 versus 0.80 ($p < 0.05$)], i PCBs en sèrum de cordó [0.70 versus 0.14 ($p < 0.10$)]. Aquells infants nascuts amb una talla baixa per l'edat gestacional presentaven nivells més alts d'HCB en sèrum de cordó que aquells nounats amb una talla adequada per l'edat gestacional [1.64 ng/ml versus 1.00 ng/ml ($p < 0.05$)]. A més, els nivells d'HCB en sèrum de cordó es van associar negativament, amb una relació de dosi-resposta, amb la talla del nounat [per cada doblada de la dosi hi havia un decrement de 0.46 (e.s. = 0.22) cm] després d'ajustar per tabac, edat gestacional i altres compostos organoclorats. Les associacions entre la concentració de p,p'DDE i de PCBs amb la talla del nounat no van ser estadísticament significatives. Aquests resultats no van variar quan es va estratificar per prematuritat. Aquestes dades suggereixen que l'HCB redueix el creixement linial intrauterí del fetus.

Podeu consultar l'article a:

N Ribas-Fitó, M Sala, E Cardo, C Mazón, ME de Muga, A Verdú, E Marco, JO Grimalt, J Sunyer. "Association of hexachlorobenzene and other organochlorine compounds with anthropometric measures at birth", **Pediatric Research**, August 2002; 52 (2):163-167

ARTICLE 6: N Ribas-Fitó, M Sala, E Cardo, C Mazón, ME de Muga, A Verdú, E Marco, JO Grimalt, J Sunyer. **Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns.** *Occup Environ Med* 2003; 60:301–303 (galerades)

Objectius: Avaluar l'associació entre l'exposició prenatal als compostos organoclorats i l'estat de la glàndula tiroide en els nounats nascuts en una àrea amb alts nivells d'hexaclorobenzè (HCB).

Mètodes: Un total de 98 parelles mare-nen (83% de tots els nens nascuts entre el període 1997-1999 en una àrea específica contaminada per HCB) van ser reclutats. Els nivells de compostos organoclorats es van mesurar en 70 mostres de sèrum de cordó. Les concentracions de l'hormona estimulant de la tiroide (TSH) es van mesurar en el plasma de tots els nounats al tercer dia de vida.

Resultats: Tots els nounats presentaven concentracions de TSH dins del rang de normalitat dels valors de referència (<25 mU/l). El p,p'DDE, el β -HCH i els PCBs 138 i 118 es van associar amb un augment de les concentracions de TSH, però aquesta associació només va ser significativa pel β -HCH. Els nivells d'HCB no es van associar amb els nivells de TSH.

Conclusions: Tot i que aquesta comunitat es troba altament exposada a l'HCB, no es va trobar cap associació entre les concentracions d'aquest compost i les concentracions de TSH al néixer.

SHORT REPORT

Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns

N Ribas-Fitó, M Sala, E Cardo, C Mazón, M E de Muga, A Verdú, E Marco, J O Grimalt, J Sunyer

Occup Environ Med 2003;**60**:301–303

Aims: To assess the association between prenatal exposure to organochlorine compounds and thyroid status in newborns from an area with high levels of hexachlorobenzene (HCB).

Methods: A total of 98 mother-infant pairs (83.1% of all children born during the period 1997–99 in a specific area polluted with HCB) were recruited. Levels of organochlorine compounds were measured in 70 cord serum samples. Concentrations of thyroid stimulating hormone (TSH) were measured in plasma of all newborns three days after birth.

Results: All newborns had concentrations of TSH within the range of normal reference values (<25 mU/l). Dichlorodiphenyl dichloroethylene (p,p'DDE), beta-hexachlorocyclohexane (β -HCH), polychlorinated biphenyl (PCB) 138 and 118 were related to higher concentrations of TSH, although only significant for β -HCH. Levels of HCB were not associated with TSH.

Conclusions: Although this community is highly exposed to HCB, no association was found between this organochlorine and TSH concentrations at birth.

Exposure to some organochlorine compounds, such as polychlorinated biphenyls (PCBs), has been associated with alterations in thyroid hormone status in adult humans and animals.¹ In infants, an association of background level perinatal PCB exposure with increased thyroid stimulating hormone (TSH) concentrations at birth has been reported.²

In a rural village of 5000 inhabitants located in the vicinity of an electrochemical factory (Flix, Catalonia, Spain), unusually high atmospheric levels of hexachlorobenzene (HCB) were detected. The factory is the only one in the village. It was built in 1898 and has been producing volatile chlorinated solvents over the past four decades. HCB is released to the environment as a byproduct. Adult inhabitants studied in 1994 had the highest serum HCB levels ever found, and levels of HCB in the cord serum of newborns from this population are among the highest ever reported in the 1990s in western countries.³ The aim of the present study was to assess the association of prenatal exposure to organochlorine compounds with concentrations of TSH at birth.

SUBJECTS AND METHODS

The study area included the village of Flix and all the nearby towns of the same health area (12 000 inhabitants). A total of 110 children were born between March 1997 and December 1999 in the main hospital of the study area (93% of all children born in the study area). Children presented no congenital anomalies or diseases. Two non-Caucasian infants and two twins were excluded. A total of 98 infants born in the main

hospital of the study area were finally recruited after giving written consent. The ethical committee of the Institut Municipal d'Investigació Mèdica approved this study.

Organochlorine compounds were measured in 70 cord serum samples by gas chromatography (GC) coupled to electron capture detection and GC coupled to chemical ionisation negative-ion mass spectrometry. From the remaining newborns no information was available because of the small volume of the obtained samples. We present results for the most prevalent compounds found in sera samples: HCB, dichlorodiphenyl dichloroethylene (p,p'DDE), beta-hexachlorocyclohexane (β -HCH), and PCBs. Because the sum of PCB 118, 138, 153, and 180 represented 91% of total PCBs in cord serum, we also provide results of these four congeners. Detection limits for HCB, β -HCH, and p,p'-DDE were 0.03, 0.15, and 0.09 respectively; and for the individual PCB congeners 28, 52, 101, 118, 138, 153, and 180, were 0.17, 0.15, 0.09, 0.11, 0.15, 0.12, and 0.10 ng/ml respectively. A value of 0.01 ng/ml was given for the non-detectable levels and a value of 0.05 ng/ml for those detectable but not quantifiable. The lipid content of each serum sample was not measured because the sample volume was insufficient.

Concentrations of thyroid stimulating hormone (TSH) in plasma of 3 day old newborns are routinely measured in Spanish hospitals for the early screening of hypothyroidism. TSH was measured at the Clinical Biochemistry Institute by immunoassay (ELISA). The detection limit for TSH was 10 mU/l. Laboratory personnel were unaware of the degree of organochlorine exposure of the newborns.

Information on socioeconomic background, maternal history, parity, gender, anthropometrics, and fetal exposure to alcohol and cigarette smoking was obtained through a questionnaire prepared ad hoc for this study and from clinical records. There were no significant differences in these covariates between those mother-infant pairs with biological samples and those without.

Cord serum organochlorine concentrations were normalised by base 2 logarithmic transformation since their distribution was skewed to the right. PCB congeners were only analysed as categorical variables given the high proportion of non-quantifiable values.

TSH concentrations were dichotomised in high/low concentrations, taking the detection limit as a cut off point. Multiple logistic regression was used for analysing the associations of organochlorine compounds with concentrations of TSH. Potential confounding variables were considered for inclusion in the regression based on the literature. Selection of variables in the final regression model was data driven; only variables

Abbreviations: p,p'DDE, dichlorodiphenyl dichloroethylene; β -HCH, beta-hexachlorocyclohexane; HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; TSH, thyroid stimulating hormone

Table 1 Association of organochlorine compounds (ng/ml) in cord serum and TSH levels at birth

| | Crude association* | | Gestational age adjusted odds ratio of having TSH \geq 10 mU/l OR (95% CI) n=70 | |
|----------------|---------------------|---------------------------|---|---------------------------------|
| | TSH <10 mU/l (n=60) | TSH \geq 10 mU/l (n=10) | | |
| | Median | Geometric mean (range) | Geometric mean (range) | Doubling the dose |
| HCb | 1.14 | 1.14 (0.96–1.35) | 1.14 (0.71–1.84) | 1.15 (0.48 to 2.76) |
| p,p'DDE | 0.85 | 0.81 (0.64–1.02) | 1.20 (0.60–2.39) | 1.60 (0.87 to 2.92) |
| β -HCH | 0.54 | 0.23 (0.14–0.37) | 0.86 (0.52–1.40)† | 1.81 (1.06 to 3.11)‡ |
| Σ PCBs† | 0.27 | 0.34 (0.28–0.42) | 0.44 (0.23–0.82) | 1.38 (0.75 to 2.57) |
| | | % Quantifiable | % Quantifiable | Quantifiable v non-quantifiable |
| PCB 138 | | 25.0% | 40.0% | 2.54 (0.58 to 11.18) |
| PCB 180 | | 23.33% | 20.0% | 0.97 (0.17 to 5.47) |
| PCB 153 | | 21.67% | 20.0% | 0.90 (0.16 to 5.04) |
| | | % Detectable | % Detectable | Detectable v non-detectable |
| PCB 118 | | 45.0% | 70.0% | 2.76 (0.62 to 12.31) |

*Student's t test on base 2 log transformed variables.

†Sum of the individual congeners 28, 52, 101, 118, 138, 153, and 180.

‡p=0.03.

with associations at $p < 0.20$ with the outcome variable after inclusion of the potential confounders were selected. All statistical analyses were conducted with the SPSS and STATA packages. The criterion of statistical significance was $p < 0.05$.

RESULTS

All newborns had concentrations of TSH within the range of normal reference values (<25 mU/l). A total of 89% of the newborns had concentrations of TSH lower than 10 mU/l. Newborns with concentrations of TSH higher than 10 mU/l had a higher gestational age than those with lower concentrations of TSH ($p < 0.05$). Levels of HCB were not associated with TSH (odds ratio 1.15, 95% confidence interval 0.48 to 2.76). However, p,p'DDE, β -HCH, and PCB 138 and 118 were related to higher concentrations of TSH. Multiple regression analyses adjusting for gestational age showed that for each doubling of a dose of β -HCH there was an increase of the risk of having higher concentrations of TSH of 1.81 (table 1). This association was not modified after adjusting for the other organochlorine compounds.

DISCUSSION

These results suggest that in this cohort, conformed by a high proportion of the infants born in an HCB polluted area, there is no evidence to suggest that exposure to this chemical is associated with TSH concentrations at birth. However, those infants with higher concentrations of TSH had higher concentrations of β -HCH.

PCBs have been associated with TSH in newborns,^{2,4} although some authors state that the association with PCBs may be due to chance.⁵ Levels of PCBs in this study were lower than in other populations.⁶ However, the lack of significant association with PCB 118 and PCB 138 could be the result of a lack of statistical power. The observed association between prenatal exposure to β -HCH with concentrations of TSH in this study was unexpected and has to be considered carefully.

In this study only concentrations of TSH were measured, but no data on total thyroxine or free thyroxine were available. In a previous study on adults of the same population, an association was observed between exposure to HCB and PCBs and an increase of total thyroxine, but no association was observed with TSH or free thyroxine.⁷ A study on 1 year old Japanese infants showed decreased values of thyroxine and triiodothyronine, depending on the concentrations of PCBs in maternal milk, whereas TSH values were also unaffected.⁸

The influence of organochlorine compounds on the hypothalamic-pituitary-thyroid axis might become more pronounced later in life. In Germany, a statistically significant positive association was found between PCB 118 and TSH, and a negative relation of other congeners with triiodothyronine in

Main messages

- Organochlorine compounds may affect thyroid functioning.
- HCB levels are not associated with TSH concentrations in this highly exposed population.
- Reassessment, measuring all thyroid hormones at older ages is required.

Policy implications

- There is a need for further studies in both general and specifically exposed populations.
- Because of possible adverse effects it is important to observe the relation between brain development and thyroid function.

children 7–10 years of age.⁹ However, a study on 12 hospitalised children aged 7–14 years, who had raised concentrations of β -HCH, p,p'DDE, and PCBs, did not reveal any associations with total thyroxine or TSH.¹⁰ This population will be followed up to 4 years of life and specific thyroid hormones will be measured.

There is a need for further studies in other populations to assess the specific effects of these organochlorines on thyroid hormones at birth and at later ages. Because of possible adverse effects of these compounds on growth and development, it is also important to observe the relation between brain development and thyroid function.

ACKNOWLEDGEMENTS

We thank all the participants for their generous collaboration and all personnel of the Pediatric Department of the Hospital de Móra d'Ebre and Primary Health Care Center of Flix for their help in obtaining the data. We are also grateful to Dave Macfarlane for his help in editing the paper. The study was funded by grants from the Spanish Ministry of Health (FIS-97/1102), "Fundació La Caixa" (97/009-00), and Generalitat de Catalunya-CIRIT 1999SGR 00241.

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Accepted 20 June 2002

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ECHO

Educational level may affect mortality among working Koreans



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An epidemiological study in South Korea has shown that education is a primary influence on mortality, in contrast to studies of more developed populations.

The researchers studied deaths in the South Korean working population aged 20–64 years, as recorded on the death certificates of the Korean National Statistics Office, and obtained information on occupation and education from next of kin. They derived denominators from a 10% stratified random sample from the 1995 census.

Their data covered 287 000 deaths in nearly 17 million people. Death rates adjusted for age were greater in manual versus non-manual occupations and with lower levels of education in men (1.65 v 1.00; 5.11 v 1.00 respectively) and women (1.48 v 1.00; 3.42 v 1.00 respectively).

The relation was abolished in men (1.65 v 0.94) and more or less in women (1.48 v 1.17 when rates were adjusted for sex and education, but remained similar when educational level was adjusted for occupation.

These class differences in deaths are greater than in the west. They may reflect a huge investment in education to assure national economic survival or may be a phenomenon that has already peaked and levelled off in western populations. However, the researchers are anxious not to overstate their case. "The effects of education predominate, but the close association of the two variables, and data limitations, suggest a cautious interpretation."

▲ *Journal of Epidemiology and Community Health* 2002;**56**:798–799.

ARTICLE 7: N Ribas-Fitó, E Cardo, M Sala, ME de Muga, C Mazón, A Verdú, M Kogevinas, JO Grimalt, J Sunyer. *Breastfeeding, exposure to organochlorine compounds and neurodevelopment in infants.* Pediatrics 2003;111:e578-e583 (galerades)

Objectiu: L'exposició als compostos organoclorats (OCs) es produeix a través de l'úter i de lactància materna. Els nivells d'hexaclorobenzè (HCB) trobats en el sèrum dels nounats nascuts en una població situada al costat d'una empresa electroquímica a Espanya van ser dels més alts mai descrits. Es va estudiar l'associació entre l'exposició a compostos organoclorats i la lactància materna amb el desenvolupament neuroconductual dels nens d'un any d'edat d'aquesta població.

Mètodes: Es va reclutar el conjunt d'una cohort de nounats que incloïa 92 parelles mare-nen nascuts entre 1997-1999 en 5 poblacions veïnes (84% dels possibles reclutaments). El desenvolupament mental i psicomotor dels nens es va avaluar als 13 mesos utilitzant les escales de Bayley i de Griffiths. Les concentracions de compostos organoclorats es van mesurar en sèrum de cordó.

Resultats: Els nivells de p,p'DDE en sèrum de cordó es van associar negativament amb el desenvolupament mental i psicomotor. Per cada doblada de la dosi de p,p'DDE es va trobar un decrement resultant de 3.50 (e.s.=1.39) punts en l'escala mental i de 4.01 (e.s.=1.37) punts en l'escala psicomotora. L'exposició als PCBs només es va associar marginalment amb el desenvolupament psicomotor. L'exposició prenatal a l'HCB no va tenir cap efecte sobre el desenvolupament neurològic del nen. La lactància materna de llarga durada es va associar amb una millor resposta en les escales mental i psicomotora. Els nens que van fer lactàncies maternes de curta durada i que presentaven majors concentracions de p,p'DDE en sèrum de cordó van obtenir puntuacions més baixes en les escales mental i psicomotora. **Conclusions:** L'exposició prenatal a p,p'DDE es va associar amb un retard en el desenvolupament mental i psicomotor als 13 mesos de vida. No es va trobar cap associació amb l'exposició a HCB.

Breastfeeding, Exposure to Organochlorine Compounds, and Neurodevelopment in Infants

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ABSTRACT. *Objective.* Exposure to organochlorine compounds (OCs) occurs both in utero and through breastfeeding. Levels of hexachlorobenzene (HCB) found in the cord serum of newborns from a population located in the vicinity of an electrochemical factory in Spain were among the highest ever reported. We studied the association between exposure to OCs and breastfeeding on neurodevelopment in the 1-year-old infants of this population.

Methods. A birth cohort including 92 mother-infant pairs was recruited between 1997 and 1999 in 5 neighboring villages (84% of possible recruits). The mental and psychomotor development of each infant was assessed at 13 months using the Bayley and the Griffiths Scales of Infant Development. OCs were measured in cord serum.

Results. Dichlorodiphenyl dichloroethylene (p,p'DDE) cord serum levels were negatively associated with both mental and psychomotor development. For each doubling of a dose of p,p'DDE, we found a resultant decrease of 3.50 points (standard error: 1.39) on the mental scale and 4.01 points (standard error: 1.37) on the psychomotor scale. Exposure to polychlorinated biphenyls was only marginally associated with psychomotor development. Prenatal exposure to HCB had no effect on child neurodevelopment. Long-term breastfeeding was associated with better performance on both the mental and motor scales. Short-term breastfed infants with higher p,p'DDE levels in cord serum were associated with the lowest scores on both the mental and the psychomotor scales.

Conclusions. Prenatal exposure to p,p'DDE was associated with a delay in mental and psychomotor development at 13 months. No association was found for exposure to HCB. Long-term breastfeeding was found to be beneficial to neurodevelopment, potentially counterbalancing the impact of exposure to these chemicals through breast milk. *Pediatrics* 2003;111:e580–e585. URL: <http://www.pediatrics.org/cgi/content/full/111/5/e580>; hexachlorobenzene, dichlorodiphenyl dichloroethylene, polychlorinated biphenyls, breastfeeding, neurodevelopment.

ABBREVIATIONS. PCB, polychlorinated biphenyl; OC, organochlorine compound; HCB, hexachlorobenzene; MDI, Mental Development Index; PDI, Psychomotor Developmental Index; p,p'DDE, dichlorodiphenyl dichloroethylene; SE, standard error.

Because the brain continues to grow for at least 2 to 3 years after birth, the nervous system is vulnerable during both pre- and postnatal periods.¹ Some environmental chemicals are known to interrupt early-stage neurodevelopmental processes, affecting behavior and cognitive function.² For example, exposure to high levels of polychlorinated biphenyls (PCBs) induces significant neurologic and behavioral dysfunctions in humans and laboratory animals.³ However, existing epidemiologic studies do not allow adequate evaluation of the risk associated with neurodevelopmental effects at current levels of exposure.⁴ Evidence of the neurologic impact of other organochlorine compounds (OCs) is scarce.

Breastfeeding increases organochlorine transfer to infants,⁵ but it has also been associated with improved neurodevelopment. However, the debate regarding the potential benefits of breastfeeding on neurologic development is currently ongoing.^{6,7}

Unusually high atmospheric concentrations of hexachlorobenzene (HCB) were found in the population of a village of 5000 inhabitants in the vicinity of an electrochemical factory (Flix, Catalonia, Spain). The factory, built in 1898, has been producing chlorinated solvents for 4 decades. Production of DDT was ended in 1971, and PCB production was discontinued in 1987. In 1994, adult inhabitants of the area who were studied were found to have the highest serum HCB levels ever measured (mean of 36.7 ng/mL).⁸ In a similar study conducted in 1999, levels of HCB in the cord serum of newborns from this population were among the highest ever reported.⁹ Other OCs in this population were found to be at similar levels described in other general populations. A general population birth cohort was set up to assess the effects of prenatal and postnatal OC exposure on the neurologic development of infants and to understand the interplay between breastfeeding and in utero exposure to OCs.

METHODS

Study Population

A birth cohort was set up of 102 mother-infant pairs including 93% of all singleton children born in the main hospital of the study

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Received for publication Jun 13, 2002; accepted Dec 20, 2002.

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area during the period March 1997 to December 1999. The study area included the village of Flix and all other towns of the same administrative health area (12 000 inhabitants). None of the children had major congenital anomalies or other diseases. Ten mother-infant pairs were lost to the 1-year follow-up. This study was approved by the ethics committee of the Institut Municipal d'Investigació Mèdica, and all mothers gave informed written consent.

Neurodevelopmental Tests

The children's mental and psychomotor development was assessed at 13 months (± 6 weeks) using the Bayley Scales of Infant Development¹⁰ and the Griffiths Mental Development Scales.¹¹ The Bayley scales yield 2 indices, the Mental Development Index (MDI) and the Psychomotor Developmental Index (PDI). The Griffiths is divided into 5 subscales (Locomotor, Personal-Social, Hearing and Language, Eye-Hand Coordination, and Performance). The Pearson correlations between the MDI and those mental areas from the Griffiths were 0.76 for Personal-Social, 0.69 for Hearing and Language, 0.73 for Performance, and 0.74 for Eye-Hand Coordination. Correlations between the PDI were 0.84 for Locomotor and 0.60 for Eye-Hand Coordination. All testing was done at the Primary Health Care Centre of Flix in the presence of the mother by the same 2 field workers (E.C. and M.E.d.M.). The field workers were unaware of the child's organochlorine background exposure or place of residence, whether the parents worked in the electrochemical factory, and whether the mother preferred to breastfeed her child.

Organochlorine Exposure

OCs in cord serum were measured by gas chromatography with electron capture detection and gas chromatography coupled to chemical ionization negative-ion mass spectrometry. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL was used for the analyses. Serum samples were stored at -40°C until analysis. All analysis was conducted in the Department of Environmental Chemistry. We present results for the most prevalent compounds found in sera samples: HCB, dichlorodiphenyl dichloroethylene (p,p'DDE), and PCBs are presented as the summation of the individual congeners 28, 52, 101, 118, 138, 153, and 180. Detection limits for HCB and p,p'DDE were 0.03 and 0.09, respectively, and ~ 0.10 for the individual PCB congeners. A value of 0.01 ng/mL was given for nondetectable levels, and a value of 0.05 ng/mL was given for those that were detectable but not quantifiable. The

between-assay coefficient of variation for the assays was 6.4% for HCB, 8.6% for p,p'DDE, and between 6% and 11% for the individual PCB congeners.

Other Variables

Information on socioeconomic background, maternal diseases and obstetric history, parity, gender, fetal exposure to alcohol (at least 2 drinks a week during the entire pregnancy) and cigarette smoking (at least 1 cigarette a day during the last trimester), type and duration of breastfeeding, and maternal intelligence (Raven Progressive Matrices) was obtained through questionnaires administered in person after delivery and at 13 months. Duration of breastfeeding was categorized as formula-fed, short-term breastfed (2–16 weeks), and long-term breastfed (>16 weeks). Sixteen weeks was the median of the duration.

Statistics and Data Analysis

Neurodevelopmental scores followed a normal distribution, whereas cord serum organochlorine levels were skewed to the right and were normalized by base 2 logarithmic transformation. The dependent variable (mental or psychomotor development) was examined in relation to level of organochlorines and the study variables using linear regression models. Both continuous and categorized levels of HCB, p,p'DDE, and total PCBs in cord serum were used in the regression analysis. Study variables were treated as potential confounding factors and were selected on the basis of previous studies.¹² Adjustment of the association between OCs and neurodevelopmental scales for the confounding variables was conducted using multivariate linear regression models after inclusion of variables with $P < .20$. Diagnosis of statistical assumptions of the models was conducted through visual inspection of the standard plots of the residuals. All statistical analysis was conducted with the STATA 6.0 statistical software package. Criteria of statistical significance was $P < .05$.

RESULTS

Table 1 provides a snapshot of study participants according to type and duration of breastfeeding and participation. Nonparticipants had lower education levels and a higher unemployment rate. Mothers whose children were breastfed were younger, had

TABLE 1. Characteristics of the Study Population According to Type and Duration of Breastfeeding, and Participation ($n = 102$)

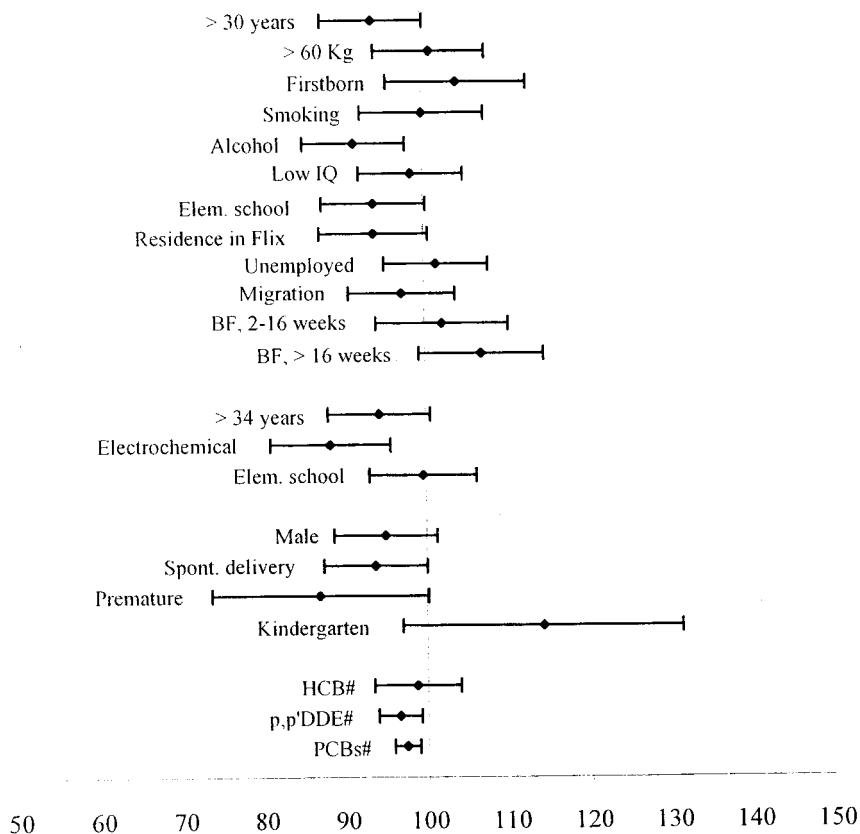
| | Participants | | | Nonparticipants ($n = 10$) |
|------------------------|-----------------------------|---|--|---------------------------------|
| | Formula-Fed ($n = 27$) | Breastfed 2–16 Weeks ($n = 30$) | Breastfed >16 Weeks ($n = 35$) | |
| Maternal variables (%) | | | | |
| Age >30 y | 67 | 43* | 54 | 60 |
| Weight >60 kg† | 72 | 40* | 35* | 67 |
| Parity: firstborn | 41 | 60 | 51 | 30 |
| Smoking in pregnancy | 41 | 43 | 14* | 31 |
| Alcohol in pregnancy | 11 | 20 | 14 | 20 |
| Low IQ‡ | 48 | 18* | 18* | - |
| Elementary school | 58 | 30 | 34 | 86* |
| Residence in Flix | 44 | 67 | 54 | 40 |
| Unemployment | 37 | 40 | 37 | 70* |
| Migration <10 y | 42 | 38 | 26 | 25 |
| Paternal variables (%) | | | | |
| Age >34 y | 52 | 40 | 40 | 40 |
| Electrochemical worker | 19 | 20 | 23 | 40 |
| Elementary school | 50 | 40 | 40 | 71 |
| Child variables (%) | | | | |
| Female gender | 63 | 40 | 60 | 70 |
| Spontaneous delivery | 59 | 63 | 57 | 30 |
| Gestational age <37 wk | 4 | 10 | 6 | 20 |
| Kindergarten <12 mo | 4 | 3 | 3 | 0 |

* $P < .05$ in comparison with formula-fed.

† Maternal weight measured at first trimester of pregnancy.

‡ Low IQ was defined as a scoring <45 points in the Raven Progressive Matrices.

Fig 1. Changes and 95% confidence interval (CI) on the MDI per each study variable and OCs in cord serum. This figure shows how individual components reduce or increase performance on the MDI. Considering the mean of the MDI as 100, each line represents the estimated change (and 95% CI) in the MDI scoring that would accompany each specific variable (ie, a child whose mother was older than 30 years would score -6.00 points [SE: 3.18] less in the MDI than a child whose mother was younger than 30). BF, breastfeeding; #change for a doubling of the concentration in nanograms per milliliter of each OC in cord serum.



higher IQ scores, and were thinner; those with a longer duration of breastfeeding also smoked less.

Figure 1 shows the relationship between MDI, OCs in cord serum, and other study variables. Mothers with low education levels, fathers who worked at the electrochemical plant, and levels of p,p'DDE and PCBs in cord serum were negatively associated to MDI ($P < .05$). Maternal age above 30 (mean in this population), maternal unemployment and migration, paternal age above 34 (mean in this population), and all of the children's variables were also associated with decreased MDI scores, although results were only marginally significant ($P < .1$). Breastfeeding and kindergarten attendance were also associated with better performance, although again, results were only marginally significant ($P < .1$). Figure 2 shows the impact of the same potential stressors on the PDI. The impact of breastfeeding on the PDI was lower than for the MDI, whereas the effects of kindergarten attendance and maternal smoking and drinking were stronger in comparison with MDI. A negative association was found between the PDI scores and exposure to p,p'DDE and PCBs in cord serum. HCB was not associated with either the MDI or the PDI.

The effects of p,p'DDE on both mental and psychomotor development and of PCBs on psychomotor development persisted after adjusting for potential confounding variables such as parents' education levels (for each doubling of a dose of p,p'DDE, there was a decrease of 3.50 points [standard error (SE): 1.39] on the MDI and 4.01 points [SE: 1.37] on the PDI). In addition, a negative dose-response relation-

ship between levels of p,p'DDE in cord serum (measured in tertiles) and MDI and PDI scores was observed. Statistically significant decreased scores were observed for the highest level of exposure, with a coefficient of -12.25 (SE: 4.90; $P = .02$) for the MDI and -12.11 (SE: 4.58; $P = .01$) for the PDI. After including all 3 organochlorines (HCB, p,p'DDE, and PCBs) in the same model, only the negative effect of p,p'DDE persisted (Table 2). Cord serum levels of p,p'DDE had the strongest negative association with the Locomotor, Personal-Social, and Performance areas on the Griffiths Scales. Duration of breastfeeding was positively associated with both the mental and the psychomotor scales (Table 2).

Table 3 depicts performance on the MDI and PDI according to type and duration of breastfeeding and levels of p,p'DDE in cord serum (categorized as above or below the median of 0.85 ng/mL). Infants with lower levels of p,p'DDE exhibited better performance than those with higher levels of exposure (110.88 vs 106.86; $P = NS$). The longer the breastfeeding period, the better the child's performance was (P trend = .04). When we considered infants with the lowest exposure and a longer duration of breastfeeding, only infants who were highly exposed and breastfed short term performed worse in a statistically significant way (Table 3). They also performed worse in the PDI.

DISCUSSION

p,p'DDE cord serum levels were negatively associated with both mental and psychomotor development. Exposure to PCBs was only marginally asso-

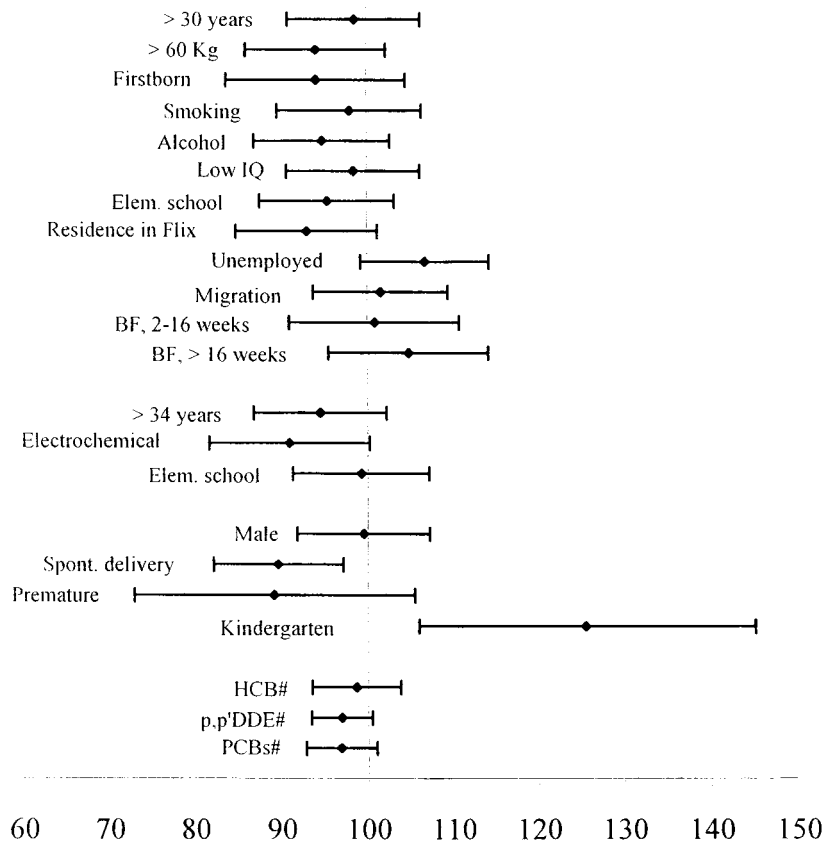


Fig 2. Changes and 95% CI on the PDI per each study variable and OCs in cord serum. This figure shows how individual components reduce or increase performance on the PDI. Considering the mean of the PDI as 100, each line represents the estimated change (and 95% CI) in the PDI scoring that would accompany each specific variable (ie, a child whose mother was older than 30 years would score -1.36 points [SE: 3.92] less in the PDI than a child whose mother was younger than 30). #Change for a doubling of the concentration in nanograms per milliliter of each OC in cord serum.

TABLE 2. Regression Coefficients (and SEs) of Bayley and Griffith Scales on Breastfeeding and OCs in Cord Serum*

| | Bayley Scales | | Griffiths Scales | | | | |
|-----------------------|---------------|---------------|------------------|---------------|----------------------|-----------------------|---------------|
| | MDI | PDI | Locomotor | Social | Hearing and Language | Eye-Hand Coordination | Performance |
| Constant | 100.98 | 82.64 | 87.23 | 84.15 | 113.04 | 102.69 | 98.20 |
| Breastfeeding, <16 wk | 1.69 (4.82) | -1.76 (5.04) | 3.96 (5.75) | 9.83 (5.70) | — | 0.25 (5.41) | 1.49 (5.22) |
| Breastfeeding, >16 wk | 10.71 (4.48)‡ | 8.97 (4.53)‡ | 12.43 (5.23)‡ | 12.73 (5.18)‡ | — | 9.79 (5.09)§ | 10.67 (4.74)‡ |
| p,p'DDE (ng/ml)† | -3.44 (1.39)‡ | -3.83 (1.46)‡ | -4.02 (1.64)‡ | -4.66 (1.74)‡ | -1.00 (1.34) | -2.88 (1.62)§ | -2.78 (1.39)‡ |
| HCB (ng/ml)† | — | — | — | — | — | — | — |
| ΣPCBs (ng/ml)† | — | -2.84 (1.72)§ | -3.45 (2.01)§ | — | — | — | — |

* OCs were analyzed in the same model. Adjusted by maternal age, tobacco and alcohol exposure during pregnancy, maternal education, migration, paternal occupation, gender, kindergarten, and breastfeeding.

† Each unit change represents a doubling of the concentration in ng/ml of each OC in cord serum.

‡ $P < .05$.

§ $P < .10$.

ciated with psychomotor development. Prenatal exposure to HCB had no effect on child neurodevelopment. Duration of breastfeeding was positively associated with performance on the mental and psychomotor scales, but infants who were breastfed short-term and had higher p,p'DDE levels in cord serum had the lowest scores on the mental and psychomotor scales.

The impact of organochlorine chemicals on neurodevelopment has been studied in a number of cohorts, most of them focusing on PCBs. Existing research suggests that PCBs hinder neurodevelopment among children exposed early in life.⁴ Information about the neurodevelopmental effects of other specific OCs is scarce. In a study conducted in North Carolina, no relationship was observed between prenatal and postnatal exposure to p,p'DDE and mental

or motor development at 12 months.¹³ In a study conducted in Oswego, New York, cord blood levels of DDE failed to predict infant intelligence at 12 months of age.¹⁴ However, in the Oswego study, levels of p,p'DDE were lower than those encountered in our population. PCB levels, alternatively, were higher.¹⁵ We are not aware of any study evaluating the neurotoxic effects of HCB in children.

The present study was conducted in an area where organochlorines are the main pollutant. The population living in this area has the highest levels of HCB ever reported.¹⁶ In addition, the population has been found to have high levels of p,p'DDE as a result of the intensive use of organochlorine chemicals in agriculture in the past. In contrast to the study conducted in 1994, we observed that levels of HCB among women in the present cohort were 61% lower

TABLE 3. Bayley MDI and PDI According to Type and Duration of Feeding and Levels of p,p'DDE in Cord Serum

| | Type of Breastfeeding | | |
|---|----------------------------|-----------------------------|--------------------|
| | Breastfeeding >16 Weeks | Breastfeeding 2–16 Weeks | Formula Feeding |
| MDI | | | |
| p,p'DDE in cord serum ≤ 0.85 ng/mL | 110.94 (6.60) | 103.24 (9.25) | 101.47 (11.11) |
| p,p'DDE in cord serum > 0.85 ng/mL | 106.79 (9.58) | 96.58 (10.66)* | 101.37 (9.58) |
| PDI | | | |
| p,p'DDE in cord serum ≤ 0.85 ng/mL | 97.26 (5.61) | 85.83 (6.61) | 91.81 (5.95) |
| p,p'DDE in cord serum > 0.85 ng/mL | 90.57 (4.74) | 81.16 (7.25)* | 86.85 (6.81) |

* $P < .05$. The reference category were those infants who were long breastfed and whose levels in cord serum were low. Adjusted by maternal age, tobacco and alcohol exposure during pregnancy, maternal education, paternal occupation, gender, and kindergarten.

than those observed in women of the same age in the previous study (10.6 vs 4.1 ng/mL). Levels of p,p'DDE also were shown to have decreased in the present cohort, although this diminution was not statistically significant (2.6 vs 2.0 ng/mL). PCB levels were found to have increased in relation to those measured in 1994 (1.4 vs 1.9 ng/mL).¹⁷ It is possible that a neurotoxic co-pollutant such as methyl-mercury could be present in this population,¹⁸ but a recent study among children aged 6 to 16 years from Flix indicated that mercury concentrations in hair were lower than those found in other populations.¹⁹

The effect of these chemicals on brain growth (both pre- and postnatally) is an important issue of concern. Most of the available studies suggest that the prenatal nervous system is more vulnerable to the harmful effects of organochlorine chemicals than the early postnatal nervous system. However, some recent studies support the hypothesis that an additional effect of postnatal exposure through breastfeeding is likely.²⁰ We have observed in the infants of this population that those who breastfed increased their concentrations of organochlorine chemicals during the first weeks of life (N. Ribas-Fitó, submitted for publication). Long-term breastfeeding, however, seems to be beneficial to the infant.

This uncertainty in identifying the susceptible periods for each area of development complicates our understanding of the interrelation between breastfeeding as an exposure pathway and the benefits of breastfeeding itself. A recent study reported that formula-fed infants had significantly lower cognition abilities than breastfed infants and, moreover, that the effects of prenatal PCB exposure were more pronounced in formula-fed than in breastfed infants.²¹ However, this study concluded that the differences in vulnerability of the 2 groups were more likely to be related to parental and home characteristics than to the beneficial effects of breastfeeding per se. Several studies have also attempted to understand the role of breastfeeding on IQ, and although some authors conclude that the observed advantage of breastfeeding on IQ is related only to genetic and socioenvironmental factors, a recent meta-analysis showed that after adjustment for appropriate key co-factors, breastfeeding was associated with significantly higher scores for cognitive development than formula feeding.⁶ Longer duration of breastfeeding has also been positively associated with intelligence

in adulthood.²² We also observed the benefits of long-term breastfeeding on mental indices, along with the indirect benefit of balancing the impact of exposure to p,p'DDE after adjustment for some socioeconomic variables.

Because multiple variables play important roles in the development of the human brain, it is difficult to elucidate the interactions and relations between all variables. The magnitude of the effect of prenatal exposure to organochlorine chemicals seems to be of the same degree as other preventable variables. This is an important concern because environmental exposures are unintentional and the degree of the exposure to these mixtures of chemicals is unknown. We did not assess the home environment with a standardized tool such as the HOME Inventory²³ because of cross-cultural differences. We did include some specific factors, but correlation with maternal education was high. Besides, the correlations between environmental measures and measures of cognitive development have not been shown to be particularly strong until children approach 2 years of age.²⁴

As has been previously reported,²⁵ the scores obtained from the Griffiths Scale were consistently higher than those obtained from the Bayley Scale, although the correlation between the 2 tests was high ($r = 0.881$, $P < .01$). Knowledge of the specific cognitive and motor skills that might be affected after exposure to each individual organochlorine is scarce. It is also not known whether each organochlorine might act on a different site or whether the time window in which humans incorporate them is the same per each chemical. In fact, it is not even clear which of the affected areas might remain affected in later childhood, although possible persistence into school age has been described.²¹ The possible mechanisms of resilience after exposure to these chemicals have not yet been established. Neurotoxic exposures that affect subtle brain functioning manifest themselves only when this functioning is needed, and might never be detected if cognitive or behavioral functioning is within normal limits.²⁶ The effects of environmental pollutants on health are most often subtle, because they usually occur at concentrations that are not expected to result in acute toxic symptoms, but these probable small effects at the individual level might have a large impact at the population level.²⁷

Despite the relatively small size of the cohort, this study reports significant results. This might be explained by the strength of the associations. Although the participation rates in the study were high, there was a significant reduction in participation of children from less-educated and unemployed mothers. This difference could bias our results by underestimating the organochlorine effect. A follow-up is under way, with 1 aim being to evaluate whether the neurodevelopmental effects observed in early life persist later in life.

In conclusion, in this population, prenatal exposure to p,p'DDE was associated with a delay in the mental and psychomotor development at the age of 1 year. No association was found for exposure to HCB. Long-term breastfeeding was found to be beneficial for the neurodevelopment of the child, helping to counterbalance the potential impact of the exposure to these chemicals through breast milk. In clinical terms, practicing pediatricians should be aware of the organochlorine exposure to infants in utero and through breastfeeding. However, they should continue to encourage long-term breastfeeding to balance the potential impact of organochlorine exposure through breast milk.

ACKNOWLEDGMENTS

This study was funded by grants from the Spanish Ministry of Health (FIS-97/1102), "Fundació La Caixa" (97/009-00 and 00/077-00), and the Generalitat de Catalunya-CIRIT 1999SGR 00241.

We thank all of the study participants for generous collaboration and all of the personnel in the pediatric department of the Hospital de Móra d'Ebre and the Primary Health Care Center of Flix for help in obtaining data, in particular Rosa M. Sabaté.

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DISCUSSIÓ

1. SOBRE L'EXPOSICIÓ A COMPOSTOS ORGANOCOLORATS

La producció de la majoria de compostos organoclorats està actualment prohibida a molts països degut a la seva toxicitat en models animals i en humans. De totes maneres, la seva alliberació al medi ambient persisteix en l'actualitat degut a la seva fabricació (malgrat que alguns països industrialitzats ja n'han prohibit el seu ús), i degut a la seva emissió com a subproductes en processos industrials o d'incineració (Carpenter et al., 1998).

Tots els nounats estudiats en aquesta cohort han presentat concentracions detectables de compostos organoclorats al moment de néixer. Els nivells més elevats al moment de néixer es corresponen a l'HCB, seguit del p,p'DDE i de la suma de PCBs. Els nivells d'HCB van ser més elevats en les mares i els nounats de Flix que en els de les poblacions veïnes. Això es correspon amb les troballes de Grimalt on les concentracions d'HCB a l'atmosfera propera a Flix són més elevades que uns quilòmetres més enllà mentre que la distribució dels altres compostos organoclorats és més homogènia entre els diferents pobles de la zona (Grimalt, comunicació oral).

Els nivells d'HCB en el sèrum matern al moment del part són dels més elevats descrits en poblacions occidentals a la dècada dels 90. La mitjana geomètrica en aquesta població és de 3.19 ng/ml mentre que a Noruega l'any 1988 es descriuen nivells de 2 ng/ml (Skaare et al., 1988) i a la resta de poblacions occidentals els valors reportats eren majoritàriament inferiors a 1 ng/ml (Needham et al., 1990; Krauthacker, 1991; Jarrell et al., 1993). Per contra, els nivells de PCBs, p,p'DDE i β -HCH s'han trobat dins del rang observat a altres poblacions (Sauer et al., 1994; Greizerstein et al., 1999; Patandin et al., 1999b). Sobre els nivells d'HCB en sèrum de cordó la informació és molt més escassa, però els pocs estudis existents posen en evidència que el grau de contaminació dels nounats d'aquesta població (mediana=1.13 ng/ml) és dels més elevats si es compara amb el d'altres poblacions occidentals (Lackmann et al., 1996;

Rhainds et al., 1999; Covaci et al., 2002). Els nivells de p,p'DDE en la nostra població (mediana=0.85 ng/ml) també són més elevats que els observats recentment a altres poblacions europees (Covaci et al., 2002). Per contra, els nivells de PCBs en el sèrum de cordó dels nounats de la nostra població són inferiors als observats a altres poblacions (Huisman et al., 1995b; Lackmann et al., 1996; Rhainds et al., 1999; Covaci et al., 2002). Aquests fets reflecteixen els usos agrícoles i industrials de les darreres dècades. La transferència transplacentària dels compostos organoclorats de la mare al nen s'ha estudiat en nombrosos estudis (Skaare et al., 1988; Lackmann et al., 1999) però la informació referent a l'HCB és més limitada. En aquest estudi s'ha trobat una associació positiva entre els nivells d'HCB, de p,p'DDE i de β -HCH de la mare al moment del part amb els del nen.

Les concentracions dels compostos organoclorats també es van trobar presents a totes les mostres de calostro i de llet materna de les mares d'aquest estudi. Les concentracions d'aquests compostos en calostro es van correlacionar amb les concentracions al sèrum del nen a les 8 setmanes de vida. Aquells infants que van fer lactància materna van presentar unes concentracions més elevades a les 8 setmanes que aquells que van fer lactància artificial. Els nivells d'HCB i de p,p'DDE en el calostro de les mares d'aquesta població, de la mateixa manera que les concentracions al moment de néixer, són de les més elevades de les descrites a les poblacions occidentals (Hernandez et al., 1993; Schade et al., 1998; Noren et al., 2000). Els nivells de PCBs també es van trobar dins del rang de l'observat a altres estudis (DeKoning et al., 2000; Solomon et al., 2002b). A part de descriure el grau de contaminació de les llets de les mares, aquest treball va pretendre conèixer quin podria ser el període crític d'exposició a compostos organoclorats a través de la lactància. S'ha observat que les concentracions d'OCs en sèrum del nen a les 8 setmanes es correlacionen millor amb les concentracions en calostro que en la llet materna obtinguda a les 3 setmanes després del part. De la mateixa manera, s'ha observat que les concentracions en la llet de la mare a les tres setmanes són més baixes que les trobades en calostro. Aquesta tendència decreixent, que podria explicar una major transferència dels OCs durant les primeres lactàncies que posteriorment, també s'ha observat recentment a Mèxic (Waliszewski et al., 2002).

En aquest estudi s'han observat diferències en la transferència dels diferents OCs a través de la llet. Per l'HCB, el major contaminant de la zona, s'observa com la lactància materna és només en part responsable de l'increment en les seves concentracions en el nen durant les primeres 8 setmanes de vida. Aquest fet podria estar explicat perquè en aquesta zona, i concretament a Flix, la via d'incorporació de l'HCB majoritària és a través de la seva inhalació. Per contra, les concentracions de p,p'DDE són les que augmenten més després de l'alletament matern. Els orígens de les concentracions tan elevades de p,p'DDE a Espanya es desconeixen, però la presència de valors elevats també s'ha descrit en els aliments (Kalantzi et al., 2001).

Tot i que el grau de contaminació per HCB en aquesta població continua essent molt elevat, s'ha observat que els nivells en sang venosa de les dones de 18 a 40 d'aquest estudi han estat un 61% més baixos que els de les dones estudiades l'any 1994 (veure annex 1). Les millores realitzades en els processos d'incineració per part de l'empresa electroquímica podrien explicar aquesta disminució.

Existeixen una sèrie de dificultats metodològiques en la mesura de l'exposició a aquests compostos que limiten la comparabilitat dels diferents treballs i en dificulten la interpretació de les troballes. Per una banda no queda molt clar quin és el substrat biològic més adequat per identificar millor l'exposició prenatal. Alguns autors han utilitzat el calostre com a indicador de l'exposició prenatal (Rogan et al., 1991) i altres apunten que el sèrum de la mare al final de l'embaràs seria la millor mesura de l'exposició prenatal (Koopman-Esseboom et al., 1999). Pel que fa a l'exposició postnatal tampoc queda clar quin seria el moment en què el pas d'aquests compostos a través de l'alletament matern seria més important. També es desconeix si les diferències en la transferència dels diferents compostos tenen a veure amb les seves diferents lipofilitats. Una altre de les limitacions pel que fa a la mesura de l'exposició és el tema de l'ajust per lípids. Degut a l'elevada lipofilitat d'aquests compostos alguns autors proposen la necessitat d'ajustar les concentracions dels OCs pel contingut lipídic de la mostra (Needham et al., 2002). En el present treball es van ajustar les concentracions d'OCs de les mostres de calostre i de llet pel seu contingut en triglicèrids. L'ajust de les mostres de sèrum no va ser

possible degut al baix volum obtingut de l'extracció de les mostres sanguínies. De totes maneres, en un estudi recent s'ha descrit que la correlació entre les concentracions de p,p'DDE i de PCBs en sèrum ajustades per lípids i les no ajustades per lípids és de 0.9 (Karmaus et al., 2002)

2. SOBRE ELS EFECTES SOBRE LA SALUT INFANTIL

Degut a la transferència d'aquests compostos de la mare al nen i al fet que les concentracions d'HCB i de p,p'DDE en el conjunt d'aquesta població siguin relativament elevades, cal descartar qualsevol associació entre els OCs i la salut infantil.

Aquest estudi és el primer que analitza el patró d'excreció de porfirines urinàries en nounats en relació als nivells d'HCB en sèrum de cordó. No es va observar cap alteració en el patró d'excreció de porfirines urinàries en el conjunt dels nounats. Les concentracions de porfirines urinàries es van trobar dins dels rangs observats a altres poblacions de nounats (Bloom et al., 1991; Pfluger et al., 1994, Ozalla et al., 1999). De totes maneres, es va observar un decrement de CPI i de CPIII amb el tertíl superior d'HCB. Aquesta associació negativa entre les fraccions de coproporfirines i els nivells d'HCB no es troba en concordança amb els resultats obtinguts en models experimentals on s'observa un augment moderat de la coproporfirina (Elder, 1975). Als nounats de Flix es va observar una major excreció de CPIII que als nounats de les poblacions veïnes. De totes maneres, el fet que les mares de Flix fossin més fumadores que les de les poblacions veïnes podria explicar aquestes diferències en el patró d'excreció de la CPIII, ja que el tabac és un inductor de la P450 i que alguns isoenzims de la P450, com el P4501A2, estan involucrats en el metabolisme de les porfirines (Sinclair et al., 2000). De totes maneres, aquestes troballes s'han de prendre amb molta precaució ja que durant els 10 primers dies de vida els nivells d'uroporfirines són molt variables. Una anàlisi posterior de l'associació entre les uroporfirines individuals amb les concentracions d'HCB en el sèrum de la població adulta de Flix també va mostrar que l'HCB no s'associava amb un augment de les uroporfirines

individuals i que, en tot cas, l'associació era amb una disminució de les coproporfirines (veure annex 2).

Per altra banda, en els nounats d'aquesta cohort s'ha pogut establir que l'HCB redueix el creixement linial durant la gestació, ja que les concentracions d'HCB en sèrum de cordó s'han associat a una disminució de la talla dels nens. Pocs estudis han avaluat els efectes dels compostos organoclorats sobre la talla al moment de néixer i la majoria s'han limitat a estudiar els efectes dels PCBs dins dels diferents OCs (Rogan et al., 1988; Patandin et al., 1998). De les diferents mesures antropomètriques, la més estudiada ha estat el pes al moment de néixer. En nombrosos estudis s'ha trobat una associació dels PCBs amb nounats de baix pes (Fein et al., 1984; Rogan et al., 1988; Patandin et al., 1998; Rylander et al., 1998) tot i que a North Carolina (Rogan et al., 1986) i a Finlàndia (Vartiainen et al., 1998) no s'ha trobat aquest efecte. També s'han reportat associacions entre l'HCB (Schade et al., 1998) i el p,p'DDE (Longnecker et al., 2001) amb un pes més baix al moment de néixer. Els nounats prematurs van presentar concentracions més elevades de tots els compostos organoclorats, particularment de p,p'DDE. Recentment s'ha descrit aquesta mateixa associació entre el p,p'DDE i la prematuritat (Longnecker et al., 2001; Torres-Arreola et al., 2003).

Aprofitant la informació existent sobre els nivells de TSH al tercer dia de vida (a Catalunya es mesura de forma rutinària per fer el cribratge de l'hipotiroïdisme) es va estudiar l'associació entre l'exposició prenatal als OCs i l'estat de l'hormona tiroide en els nounats de la cohort. No es va trobar cap evidència que l'exposició a HCB s'associés amb les concentracions de TSH al moment de néixer. En aquest estudi només es van mesurar els nivells de TSH i no es va obtenir informació de les tiroxines totals i lliures. En l'estudi anterior realitzat en la població adulta de Flix es va observar que l'HCB i els PCBs augmentaven els nivells de tiroxina total però no els de tiroxina lliure o de TSH (Sala et al., 2001). En un estudi amb infants japonesos d'un any d'edat també es va observar com els nivells de PCBs s'associaven amb els nivells de tiroxines i de triiodotironina, però no amb els de TSH (Nagayama et al., 1998). Una altra limitació és el fet que l'impacte dels compostos organoclorats sobre les hormones tiroïdees es podria fer palès

més endavant, per això, als 4 anys d'edat dels nens d'aquesta població es farà un estudi més complet de la seva funció tiroïdea.

L'impacte dels compostos organoclorats sobre el desenvolupament s'ha estudiat en algunes cohorts, la majoria limitades a l'estudi dels PCBs. La revisió realitzada en el marc d'aquesta tesi suggereix que els PCBs tenen un efecte sobre el desenvolupament neuroconductual dels nens exposats en les primeres etapes de la vida. La informació referent a altres compostos organoclorats és molt més limitada. En aquest estudi s'ha observat que el p,p'DDE s'associa negativament amb el desenvolupament de les àrees mental i psicomotora del nen al primer any de vida, contràriament al descrit fins ara (Gladen et al., 1988; Darvill et al., 2000). L'exposició als PCBs només s'associa marginalment amb el desenvolupament psicomotor dels nens i l'exposició prenatal a l'HCB no ha tingut cap efecte sobre el desenvolupament neurològic del nen. La durada de la lactància materna s'ha associat amb una millora en la resposta de les àrees mentals i motores dels infants, però els nens que van fer lactàncies curtes i que al moment de néixer tenien concentracions més elevades de p,p'DDE van presentar les pitjors puntuacions.

La majoria d'estudis han descrit el període prenatal com el període més vulnerable pel desenvolupament del sistema nerviós, de totes maneres, alguns estudis suggereixen un efecte addicional de l'exposició a través de l'aliment matern (Walkowiak et al., 2001). Un estudi recent a Holanda ha observat que els nens en edat escolar que van fer lactància artificial van presentar puntuacions més baixes en les habilitats cognitives que els nens que van fer lactància materna i, afegeix, que els efectes de l'exposició prenatal als PCBs van ser més pronunciats en els nens alimentats amb lactància artificial que amb lactància materna (Vreugdenhil et al., 2002; Vreugdenhil et al., 2002). De totes maneres, aquest mateix estudi va concloure que aquestes diferències entre els dos grups es podrien atribuir més a diferències socio-ambientals en el context familiar que a la mateixa lactància materna. El debat sobre el rol de la lactància materna per se sobre el desenvolupament neurològic dels infants continua obert. Els beneficis de la lactància materna són evidents, però alguns autors ho atribueixen a diferències socio-econòmiques. Tot i això, una meta anàlisi va concloure que després d'ajustar per les

covariables apropiades, hi havia un benefici residual associat únicament a les característiques de la llet materna (Anderson et al., 1999).

Un altre punt important és el coneixement de quines són les àrees específiques afectades per l'exposició a aquests compostos i quines són les àrees que es mantindran afectades en edats posteriors, tot i que s'han descrit efectes en edat escolar (Vreugdenhil et al., 2002).

També és important tenir en compte que, tot i que les troballes no tinguin una transcendència clínica important i, per la seva magnitud, tampoc una transcendència individual (en el cas del desenvolupament neurològic, una pèrdua de 7 punts en el coeficient intel·lectual (mitja desviació estàndard) és poc significativa a escala individual), aquests canvis poden tenir un impacte important en Salut Pública. Si tenim en compte que l'exposició a aquests compostos tòxics persistents és universal, un canvi mitjà de petites dimensions com el descrit de 7 punts pot tenir un impacte poblacional considerable. En el cas del plom, es va poder constatar que una disminució mitjana de 7 punts en el coeficient intel·lectual dels nens implicava passar del 4% de nens amb coeficients intel·lectuals inferiors a 80 al 12% de nens, fent augmentant per tant el nombre de nens que requeririen un suport psicopedagògic (Tong et al., 1998; Burns et al., 1999) (figura 13).

Figura 13. Impacte poblacional de l'exposició al plom (Tong et al., 1998).

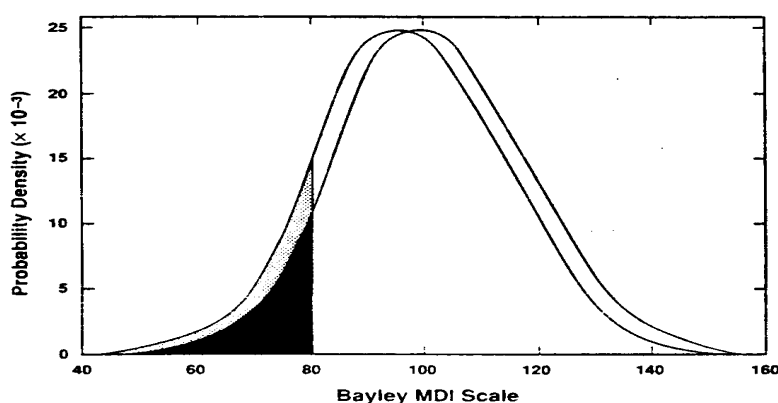


FIG. 1. Normal Probability Densities with Means of 96 and 100 and Standard Deviations of 16.

En aquest estudi, a nivells no molt superiors als que es troben arreu hem identificat efectes notables de

l'exposició prenatal als OCs (una disminució del creixement longitudinal al néixer per una banda, i una disminució del desenvolupament mental i motor a l'any de vida per l'altra). Aquests impactes s'han observat majoritàriament pel p,p'DDE i, en menys importància, per l'HCB malgrat ser el contaminant majoritari de la zona. Fins ara, la recerca epidemiològica en aquesta àrea de recerca s'ha centrat en l'estudi de la toxicitat dels PCBs, minimitzant l'interès per l'estudi d'altres OCs. A Catalunya i a la resta de l'Estat espanyol, els PCBs no són els contaminants majoritaris, sinó que trobem concentracions més elevades de p,p'DDE i, sobretot, d'HCB. De totes maneres, el fet que els humans estiguem exposats a una barreja d'aquests compostos en dificulta el poder establir l'efecte individualitzat de cadascun d'ells. Tampoc podem descartar que les diferències observades en els diferents estudis entre l'associació als diferents OCs amb cadascun dels efectes vinguin determinades pel fet que sigui el contaminant més prevalent de cada zona el que s'associï més fortament a cadascun dels efectes estudiats, o fins i tot a les interaccions entre ells.

En aquest estudi hem trobat que l'HCB només s'associa a una talla baixa dels nens al néixer i que a l'any d'edat l'efecte més fort sobre el desenvolupament neuroconductual és pel p,p'DDE. Aquestes desavinences entre els diferents contaminants podrien venir explicades per diferents motius. Per una banda, el fet que el creixement linial del fetus es produeixi majoritàriament al segon trimestre de l'embaràs podria indicar que la transferència de l'HCB de la mare al fetus es produeix majoritàriament durant aquest període, mentre que el fet que el p,p'DDE afecti la prematuritat del nounat podria indicar una transferència durant el tercer trimestre. De totes maneres, no es coneix en quin moment aquests compostos es transfereixen de la mare al nen, ni si variables com el canvi de pes durant la gestació o canvis en la dieta influeixen i de quina manera aquesta transferència. Un altre explicació podria venir donada per diferències en l'especificitat de la seva toxicitat, l'HCB afectant únicament el creixement linial dels infants i el p,p'DDE la prematuritat i el desenvolupament neuroconductual. Es coneix per diferents estudis que els nens prematurs presenten més problemes en el desenvolupament cognitiu i en les funcions motores i que aquests dèficits es fan palesos fins a l'adolescència (Allin et al., 2001). Alguns autors expliquen aquesta relació pel fet que en els nens prematurs, la transferència de la tiroxina materna de la mare al nen queda interrompuda donant lloc a una

hipotiroxinèmia (Morreale de Escobar, 2001). Les hormones tiroïdees són necessàries pel desenvolupament del cervell durant el període perinatal i l'hipotiroïdisme s'ha associat amb una alteració de les funcions cognitives i motores (Morreale de Escobar, 2001) i atencionals (Rovet et al., 2001). En aquest estudi els nens prematurs han presentat valors més elevats de p,p'DDE i ha estat aquest compost el que més s'ha associat amb efectes en el desenvolupament neurològic dels infants (prematurs i nascuts a terme). Pel que fa a les hormones tiroïdees tots els nounats presentaven valors dins del rang de la normalitat, però sí que va cridar l'atenció el fet que els nounats amb nivells de TSH més elevats presentaven concentracions més elevades de p,p'DDE (encara que no estadísticament significatives) mentre que els nivells d'HCB eren iguals en ambdós grups.

Per altra banda, si tenim en compte que el p,p'DDE és el compost majoritari en la llet materna, que entre els nens que fan alletament matern el p,p'DDE és el compost que augmenta més durant les primeres 8 setmanes de vida i que la correlació entre les concentracions de p,p'DDE en sèrum de cordó i les del calostre i la llet materna és molt elevada, no podem descartar que part dels efectes observats sobre el desenvolupament neuroconductual en els nens al primer any de vida vinguin explicats per un efecte afegit de l'exposició postnatal. De totes maneres, degut al rol protector de la lactància materna, és difícil determinar l'efecte de l'exposició postnatal d'aquests compostos sobre la salut infantil.

Desconeixem doncs si el diferent paper dels OCs s'explica per diferències en la seva toxicitat o en la seva transferència. O bé si, l'efecte és inespecífic entre els diferents OCs, o fins i tot entre els diferents COPs, ja que els estudis epidemiològics observen associacions amb el contaminat més prevalent de la zona (PCBs a la zona dels grans llacs d'EUA (Jacobson et al., 1996) o mercuri a les Illes Faroe (Grandjean et al., 1998)).

3. SOBRE LES LIMITACIONS DEL TREBALL

Tot i la grandària petita de la cohort, aquest estudi ha trobat resultats significatius. La força de les associacions i l'ús de variables d'efecte en escala contínua ho podrien explicar. Tot i que la participació és elevada, les mares que van decidir no participar a l'estudi tenien un nivell educatiu més baix, en un major percentatge estaven en atur i, encara que no estadísticament significatiu, en un major percentatge eren fills de treballadors de l'empresa.

Totes les mostres es van analitzar al CSIC i els qui van realitzar les anàlisis desconeixien de quin nen procedia cada mostra. Pel que fa a la mesura de l'exposició, la mesura de les uroporfirines i dels nivells de TSH es va realitzar desconeixent a quin nen pertanyia cada mostra, la mesura de l'antropometria es va realitzar amb els mateixos aparells a l'Hospital de Móra d'Ebre i la mesura del desenvolupament mental i psicomotor a partir de dos tests estandarditzats es va realitzar per dues neuropediatres que desconeixien la residència, el grau d'exposició als compostos organoclorats i el tipus de lactància realitzada. Per tant, no és probable que existeixi un biaix diagnòstic diferencial.

Malgrat les troballes estadísticament significatives del present treball, l'epidemiologia ambiental afronta el repte d'estudiar efectes en general poc freqüents o efectes més funcionals que patològics a nivells d'exposició molt més baixos que els derivats d'exposicions accidentals o ocupacionals. Sovint, la manca de poder estadístic limita l'elaboració de conclusions clares. Per això és cada vegada més necessari unir esforços per poder realitzar estudis multicèntrics amb un major poder estadístic i representativitat de la mostra, per tal de poder mesurar les interaccions entre la lactància i les concentracions postnatsals d'OCs sobre el desenvolupament neuroconductual i tenir en compte la interacció entre els diferents compostos i la interacció amb altres neurotòxics ambientals com el plom o el metilmercuri (Bemis et al., 1999; Grandjean et al., 1998).

4. SOBRE LES NOVES LÍNIES DE RECERCA

El projecte *Infancia y Medio Ambiente* (INMA) pretén seguir de manera prospectiva el desenvolupament d'uns 5000 nens des del moment de la gestació fins l'adolescència a diferents àrees geogràfiques d'Espanya. Algunes de les cohorts seran de nova formació i altres seran cohorts existents. Entre elles hi haurà la cohort del present treball que, en aquests moments, està fent un seguiment dels nens als 4 anys de vida per tal d'avaluar la persistència i irreversibilitat dels efectes observats. Els objectius d'aquest projecte són múltiples però bàsicament pretén unir el coneixement i la metodologia de treball dels diferents grups de l'Estat espanyol que estudien els efectes del medi ambient sobre la salut infantil, descriure el grau de contaminació individual i la càrrega d'exposició durant la gestació i la primera infància, avaluar el paper dels contaminants ambientals i els factors dietètics sobre el creixement fetal i el desenvolupament neuro-endocrino-immunitari, i ampliar el nombre de cohorts existents per tal de contrastar les hipòtesis generades a les cohorts existents (p.e. en el cas del present treball, contrastar si la lactància materna antagonitza l'efecte de l'exposició postnatal als compostos organoclorats sobre el desenvolupament neuroconductual).

Un dels altres objectius d'aquest projecte és el de crear un pla de formació dels diferents grups de treball en aspectes de comunicació científica, amb especial èmfasi en el coneixement dels sistemes de funcionament dels mitjans de comunicació i en les diferents formes de relació entre els investigadors i periodistes i, dissenyar i executar un pla de comunicació amb l'objectiu de difondre públicament els coneixements que es vagin generant i promoure canvis en les conductes en la societat en general o en determinats grups.

5. SOBRE LES IMPLICACIONS EN SALUT PÚBLICA

Pel que fa a la salut pública de la zona de Flix i de les poblacions veïnes les troballes d'aquest estudi tenen molta importància. Per una banda, els beneficis de la lactància de més de 16 setmanes (moment que coincideix amb la fi de la baixa laboral per maternitat) sobre el desenvolupament neuroconductual dels nens, a pesar del risc d'incorporar més tòxics ambientals, s'hauria de tenir en compte per part dels pediatres de la zona. Per altra, les

troballes sobre els nivells de contaminació en aquesta població ens indiquen que pel que fa a la majoria d'OCs, els nivells observats a aquesta zona es corresponen amb el d'altres poblacions estudiades ja que la incorporació majoritària vindria donada per la dieta. Per contra, tot i que els nivells d'HCB hagin disminuït, aquest estudi posa en evidència que els nens de Flix continuen incorporant nivells elevats d'HCB. Per tant, seria recomanable prendre més mesures per tal de disminuir la contaminació de l'aire i dels aliments per OCs.

A nivell més global, aquest estudi evidencia el fet que s'hagi de recomanar la lactància materna de llarga durada i el fet que la incorporació de compostos tòxics per la salut de forma continuada des de les primeres etapes de la vida és una realitat i que caldria millorar la producció, el control i la distribució d'aquests compostos.

A nivell científic, les troballes d'aquest estudi han ajudat a conèixer una mica millor la toxicitat d'aquests compostos i han obert noves hipòtesis que caldria corroborar i aprofundir en estudis posteriors.

6. SOBRE LES IMPLICACIONS POLÍTICO-SANITÀRIES

A Catalunya i a la resta de l'Estat espanyol la informació sobre el grau d'exposició a compostos orgànics persistents és molt limitada i encara ho és més el coneixement de les concentracions d'aquests compostos en humans. Si bé és cert que la situació de Flix és molt particular i concreta i que és difícil fer inferències de les troballes a la població general, també és cert que Flix respon a la contaminació específica d'un d'aquests compostos, l'hexaclorobenzè. Es desconeix l'origen dels nivells tan elevats de p,p'DDE trobats a la zona, però tot i la poca informació existent es pot aventurar que les concentracions de p,p'DDE a Catalunya es podrien trobar en el rang mig-alt dels valors observats a altres països europeus. Els PCBs sembla ser que estarien situats en el rang mig de l'observat a altres zones. Les concentracions d'HCB, a part del cas particular de Flix, també es trobarien en el rang alt comparat amb altres països de la Unió Europea (veure annex 3).

Si el desconeixement sobre el grau de contaminació dels aliments i de les persones a Catalunya és important, el desconeixement sobre l'impacte d'aquests processos ambientals sobre la salut humana és desmesurada. Aquest dèficit d'indicadors poblacionals és només una de les conseqüències de les múltiples deficiències que presenten les polítiques ambientals a l'Estat espanyol (Ballester, 2000; Daponte et al., 2000).

Conjuntament amb més de cent països, Espanya es prepara per implementar el Conveni d'Estocolm. Acol·lit pel Programa Ambiental de les Nacions Unides, el Conveni d'Estocolm o el Tractat sobre els Compostos Orgànics Persistents pretén acabar amb l'ús d'alguns COPs i reduir-ne l'ús d'uns altres (Karlagnis et al., 2001; www.chem.unep.ch/sc; www.ipen.org; www.worldwildlife.org/toxics). Això obligarà als països signants la dinamització dels programes de salut pública, de medi ambient i de seguretat alimentària, enfortint les estratègies polítiques i científiques per tal de disminuir el grau de contaminació dels ciutadans i millorar-ne la seva qualitat de vida (veure annex 3).

De totes maneres, establir aquest lligam entre factors ambientals i perill per la salut sovint inclou un cert grau d'incertesa científica. Moltes vegades es recomana utilitzar el principi de precaució quan es tracta de noves substàncies on la informació sobre els seus possibles efectes adversos és desconeguda i molt limitada. Tal i com es va dir a la Tercera Conferència Ministerial sobre Medi Ambient i Salut de la Organització Mundial de la Salut l'any 1999, el principi de precaució i la prevenció de les exposicions haurien de formar part de les polítiques de medi ambient i salut centrant-se en les vulnerabilitats particulars dels infants.

Actualment existeix una necessitat urgent per avaluar i reduir l'exposició dels infants a riscos ambientals, des del moment de la concepció fins a l'adolescència, tenint en compte la seva susceptibilitat particular i els seus patrons d'activitat. Els nens i els infants no es poden considerar com a petits adults, per això, la Quarta Conferència Ministerial sobre Medi Ambient i Salut de la OMS (Budapest 2004) es centrarà en la salut dels infants i de les futures generacions en un context més ampli de desenvolupament sostenible.

La salut com un dret humà, la igualtat i la solidaritat, el dret a saber, el desenvolupament sostenible i el principi de precaució són només alguns dels principis bàsics que haurien de regir el suport polític per desenvolupar ambients sans pels nostres infants.

CONCLUSIONS I IMPLICACIONS

CONCLUSIONS

Sobre l'estat de la qüestió

1. La recerca actual suggereix que els PCBs tenen un efecte sobre el desenvolupament neurològic dels infants exposats en les etapes primerenques de la vida.
2. L'heterogeneïtat existent entre el disseny dels diferents estudis no permet obtenir una mesura quantitativa conjunta de l'associació entre l'exposició a PCBs i els efectes sobre el desenvolupament neurològic dels infants.
3. L'exposició universal als compostos organoclorats fa necessària la posada en marxa de nous estudis de recerca.

Sobre la transferència dels compostos organoclorats.

4. Els nivells d'HCB i de p,p'DDE en el sèrum de cordó i en el calostre de les mares d'aquesta població són dels més alts mai descrits en poblacions occidentals.
5. L'HCB, de la mateixa manera que altres compostos organoclorats, es transfereix a través de la placenta implicant que les mares més exposades tinguin fills amb majors concentracions.
6. La lactància materna implica un canvi d'aquests contaminants al cos i incrementa el grau de contaminació dels lactants durant les primeres setmanes de vida.

Sobre els efectes sobre la salut del nen durant el primer any de vida.

7. Tot i que el grau d'exposició a HCB en aquesta població és molt elevada no s'han trobat associacions amb el patró d'excreció de porfirines, les concentracions de TSH al néixer i el desenvolupament neuroconductual a l'any de vida.
8. Les concentracions d'HCB al moment de néixer s'han associat amb una disminució de la talla del nounat al naixement.
9. L'exposició prenatal a p,p'DDE s'ha associat amb un retard en el desenvolupament mental i psicomotor als 13 mesos de vida.

10. La lactància materna de llarga durada (de més de 16 setmanes) s'ha associat amb una millor resposta en les escales mental i psicomotora.

IMPLICACIONS

Els resultats d'aquest estudi tenen molta importància en salut pública i en la clínica habitual. Els pediatres haurien de ser conscients de l'exposició dels infants als compostos organoclorats a través de la placenta i de la lactància. Tot i que la lactància materna suposi una via d'entrada d'aquests compostos al nen, els beneficis de la lactància materna, sobretot si és de llarga durada, són innegables. Per tant, s'hauria de promoure la lactància materna de llarga durada per tal de contrarestar el possible impacte de l'exposició a compostos organoclorats a través de la lactància materna.

ANNEXES

ANNEX 1: N Ribas-Fitó, J Sunyer, M Sala, JO Grimalt. **Cambios en las concentraciones de compuestos organoclorados en las mujeres de la población de Flix, Tarragona.** Gac Sanit 2003 (en premsa)

Cambios en las concentraciones de compuestos organoclorados en las mujeres de la población de Flix, Tarragona

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Recuento de palabras

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RESUMEN

La población de Flix (comarca de la Ribera del Ebro, Tarragona) se encuentra altamente expuesta a hexaclorobenceno (HCB) debido a la proximidad a una empresa electroquímica. Aunque los niveles de contaminación por HCB en esta población continúan siendo elevados, se ha observado que las concentraciones en sangre venosa de las mujeres de 18 a 40 años en 1997-99 fueron un 61% más bajas que en 1994 (4.1 ng/ml vs. 10.6 ng/ml). Las concentraciones de diclorodifenil dicloroetano (p,p'DDE) y beta-hexaclorociclohexano (β -HCH) también mostraron esta tendencia a la baja, aunque su disminución no fue estadísticamente significativa. Por el contrario, los niveles de bifenilos policlorados (PCBs) en 1997-99 aumentaron con relación a 1994 aunque la diferencia no fue estadísticamente significativa.

Palabras clave:

Compuestos organoclorados, tendencias temporales, Flix, Hexaclorobenceno, PCBs, p,p'DDE, β -HCH

ABSTRACT

The population of Flix (region of the Ribera del Ebro, Tarragona, Spain) is highly exposed to hexachlorobenzene (HCB) due to the vicinity of an electrochemical factory. Although the degree of HCB contamination in this population is still high, it has been observed that concentrations of HCB in sera among women aged 18-40 years in the period 1997-99 were a 61% lower than those observed in 1994 (4.1 ng/ml vs. 10.6 ng/ml). Concentrations of dichlorodiphenyl dichloroethane (p,p'DDE) and beta-hexachlorocyclohexane (β -HCH) also shown these decrease although its diminution was not statistically significant. On the contrary, although the difference was not statistically significant, concentrations of polychlorinated biphenyls (PCBs) in 1997-99 increased in relation to 1994.

Key Words:

Organochlorine compounds, Temporal trends, Flix, Hexachlorobenzene, PCBs, p,p'DDE, β -HCH

INTRODUCCIÓN

Los compuestos organoclorados como el hexaclorobenceno (HCB) y los bifenilos policlorados (PCBs) son compuestos ubicuos en la naturaleza que se incorporan en el organismo humano principalmente a través de la dieta (1,2). En la actualidad, la mayoría de estos compuestos están prohibidos, pero siguen presentes en todos los seres humanos debido a su uso en países del tercer mundo, su lenta biodegradación y su formación actual como sub-productos dentro de la síntesis de disolventes clorados y de otros compuestos organoclorados.

Se desconoce cuál es la evolución temporal de la dosis interna de estos compuestos en humanos. Mientras que algunos autores sugieren una reducción (3), otros estudios han puesto de manifiesto una situación estacionaria para algunos compuestos, y en ocasiones incluso un cierto aumento (4). Ciertamente, la escasa información dificulta la derivación de conclusiones. En nuestro entorno sólo existe un estudio longitudinal que evalúe los cambios en tiempo de la carga interna de compuestos organoclorados (4). En 1994 un estudio en la población de Flix (Ribera del Ebro, Tarragona) puso en evidencia que sus habitantes presentaban unas concentraciones en suero muy elevadas de HCB debido a la proximidad a una empresa electroquímica (5). Asimismo se observó que los niños nacidos en dicha población entre 1997-99 presentaban ya en el momento de nacer concentraciones muy elevadas de HCB y que todos ellos presentaban concentraciones en sangre detectables de PCBs y de DDE (6). El objetivo del presente estudio fue el de comparar las concentraciones de compuestos organoclorados entre las mujeres de 18 a 40 años no nulíparas del estudio de 1994 y el del período 1997-99 para observar la tendencia temporal de las concentraciones de dichos compuestos en la población de Flix.

MÉTODOS

En 1994 se llevó a cabo un estudio transversal con 1800 habitantes de Flix mayores de 14 años (43% del total de la población). Se obtuvo información sobre estilo de vida, historia ocupacional y médica a partir de un cuestionario y muestras de sangre de 608 individuos (5). En 1997 se inició un estudio longitudinal en la misma población y en poblaciones vecinas, con el objetivo de evaluar la transferencia de los compuestos organoclorados a través de la placenta y de la lactancia materna y de sus efectos sobre la salud del niño durante el primer año de vida (6). El reclutamiento de las parejas madre-niño se produjo en el momento del parto en el Hospital de Mora de Ebro entre 1997 y 1999. De las mujeres que dieron muestra de sangre en el momento del parto 41 eran residentes de Flix.

Los dos estudios fueron aprobados por el Comité Ético del Institut Municipal d'Investigació Mèdica. Del estudio de 1994 seleccionamos a las 86 mujeres no nulíparas y de edades comprendidas entre los 18 y los 40 años (rango de edad de las mujeres del estudio de 1997-99). Una de las mujeres participó en las dos fases del estudio y se excluyó del análisis. Las concentraciones de organoclorados en suero se midieron por cromatografía de gases (CG) acoplada a detección de captura de electrones y por CG acoplada a un espectrómetro de masas de ionización negativa (6). Todas las muestras fueron analizadas en el Departamento de Química Ambiental del CID-CSIC. A partir de cuestionarios se obtuvo información sobre la edad de las mujeres, el número de hijos, el índice de masa corporal, el tiempo de residencia en Flix, la ocupación, y el consumo de alcohol y/o tabaco.

Las posibles correlaciones entre las concentraciones en suero de organoclorados y las distintas covariables se analizaron con modelos de regresión multivariada previa transformación logarítmica de las variables respuesta.

RESULTADOS

Las características de las mujeres de Flix y sus concentraciones de organoclorados en 1994 y 1997-99 aparecen resumidas en la tabla 1. Las mujeres del primer estudio eran mayores y con mayor número de hijos que las del segundo período ($p < 0.05$). Las concentraciones de HCB en las mujeres de Flix en 1997-99 fueron un 61% más bajas que en 1994 (tabla 2). Las concentraciones de p,p'DDE y β -HCH también mostraron esta tendencia a la baja, aunque su disminución no fue estadísticamente significativa. Por el contrario, las concentraciones de PCBs en suero en 1997-99 aumentaron con relación a 1994. El grado de exposición de las mujeres a los distintos compuestos organoclorados se asoció positivamente con la edad de la mujer, su índice de masa corporal y de su paridad. A su vez, las concentraciones de HCB y de PCBs también se asociaron positivamente con el tiempo de residencia en la población de Flix. Los modelos de regresión multivariada tras ajustar por estas variables también mostraron que las concentraciones de HCB en suero en las mujeres de Flix disminuyeron de 1994 a 1997-99 (tabla 2), así como el análisis estratificado (i.e. tanto en primíparas como en multíparas).

DISCUSIÓN

En España es muy escasa la información sobre el grado de contaminación por compuestos organoclorados en poblaciones generales y sólo existe un estudio sobre las tendencias temporales de dicha contaminación (7). Aunque el tamaño muestral es reducido y la exposición a HCB de esta población se puede considerar singular por la proximidad de la industria electroquímica, el presente estudio constituye una aportación novedosa.

A pesar de que el grado de contaminación por HCB en esta población continúa siendo elevado, en el presente estudio se ha observado que éste ha sido el compuesto organoclorado que más ha disminuido en los últimos años. Esta disminución podría ser debida a las mejoras realizadas en los procesos de incineración en la empresa electroquímica.

El único estudio que ha realizado mediciones repetidas a lo largo del tiempo en población general española ha puesto en evidencia que las concentraciones de PCBs aumentaron un 12% entre 1995 y 1997 (4). El presente estudio, basado en dos cortes transversales en la misma población, sugiere que la contaminación por la mayoría de compuestos organoclorados en Flix ha disminuido de un período a otro, pero, al igual que en el estudio de Mataró, las concentraciones de PCBs en suero han subido a lo largo del tiempo.

Sin embargo, al provenir los datos de este trabajo de cortes transversales de estudios con otros diseños, el trabajo presenta algunas limitaciones. El número de mujeres incluido en este estudio es reducido, especialmente para el segundo período. El primer grupo de mujeres procede de una muestra poblacional mientras que en el segundo grupo se trata de madres recientes. En las madres recientes los procesos del parto y de la lactancia constituyen una vía de eliminación de estos compuestos. Otra limitación importante podría ser el hecho de que no se ajustaron las concentraciones de organoclorados por lípidos ya que no se disponía de dicha información en ninguno de los grupos de estudio.

Dado el posible impacto que los procesos ambientales tienen sobre la salud humana, es necesario esclarecer mediante más estudios cuales son las concentraciones de estos compuestos en nuestra población. El Convenio de Estocolmo (8, 9), pendiente de implementación por muchos países, es una buena oportunidad para mejorar la información existente sobre las concentraciones de compuestos organoclorados y otros contaminantes orgánicos persistentes.

Tabla 1: Características y concentraciones de compuestos organoclorados (ng/ml) en sangre venosa de las mujeres de Flix en los períodos 1994 y 1997-99.

| | 1994 (n=85) | | | | | 1997-1999 (n=40) | | | | | p |
|----------------------------|-------------|------|------|------|------|------------------|------|------|------|------|--------|
| | p5 | p25 | P50 | p75 | p95 | p5 | p25 | p50 | p75 | p95 | |
| Edad (años) | 24 | 31 | 33 | 36 | 39 | 18.2 | 27.6 | 30.2 | 33.8 | 36.3 | 0.002 |
| Tiempo en Flix (años) | 5 | 12 | 31 | 35 | 38 | 2 | 24.5 | 29 | 31.5 | 35 | 0.55 |
| IMC (kg/m ²) | 18.7 | 20.9 | 22.5 | 24.6 | 28.5 | 18.6 | 21.4 | 23 | 25.2 | 30.3 | 0.53 |
| HCB (ng/ml) | 4.4 | 7.0 | 10.6 | 14.4 | 24.1 | 0.6 | 2.7 | 4.1 | 6.3 | 11.6 | <0.001 |
| p,p'DDE (ng/ml) | 0.05 | 1.4 | 2.6 | 4.6 | 15.9 | 0.5 | 1.1 | 2.0 | 3.5 | 6.2 | 0.26 |
| ΣPCBs (ng/ml) | 0.1 | 0.8 | 1.4 | 2.2 | 4.8 | 0.2 | 1.2 | 1.9 | 2.3 | 5.1 | 0.15 |
| β-HCH (ng/ml) | 0.01 | 1.3 | 2.7 | 3.8 | 4.2 | 0.05 | 0.5 | 1.2 | 2.0 | 5.2 | 0.46 |
| | | | % | | | | | % | | | |
| Consumo tabaco | | | 40 | | | | | 38 | | | 0.77 |
| Consumo alcohol | | | 40 | | | | | 40 | | | 0.90 |
| Trabajadora electroquímica | | | 3 | | | | | 3 | | | 0.96 |
| Primípara | | | 33 | | | | | 60 | | | 0.005 |

IMC: Índice de masa corporal; ΣPCBs: suma de los congéneres de PCBs 28, 52, 101, 118, 138, 153, 180

Tabla 2: Efecto (coeficiente y error estándar) del periodo de estudio en las concentraciones de compuestos organoclorados.

| | Coeficiente | Error estándar | p | % cambio |
|---------|-------------|----------------|--------|----------|
| HCB | -1.04 | 0.14 | <0.001 | -61% |
| p,p'DDE | -0.37 | 0.24 | 0.12 | -23% |
| ΣPCBs | 0.22 | 0.24 | 0.36 | +35% |
| β-HCH | 0.23 | 0.42 | 0.56 | -55% |

Cada coeficiente deriva de un modelo multivariante distinto. Cada unidad representa el cambio de pertenecer al período 1997-99 respecto al período 1994. Las unidades se basan en unidades logarítmicas. Ajustado por edad, paridad, índice de masa corporal, tiempo de residencia en Flix y ocupación en la empresa electroquímica.

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ANNEX 2: J Sunyer, C Herrero, D Ozalla, M Sala, **N Ribas-Fitó**, JO Grimalt, X Basagaña.
Serum organochlorines and urinary porphyrin pattern in a population highly exposed to hexachlorobenzene. Environ Health Glob Access Sci Source 2002;1:1-8

Research

Serum organochlorines and urinary porphyrin pattern in a population highly exposed to hexachlorobenzene

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Published: 19 July 2002

Received: 17 June 2002

Environmental Health: A Global Access Science Source 2002, 1:1

Accepted: 19 July 2002

This article is available from: <http://www.ehjournal.net/content/1/1/1>

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Keywords: coproporphyrin I, coproporphyrin III, hexachlorobenzene, organochlorinated compounds, porphyria, uroporphyrin

Abstract

Background: Porphyria cutanea tarda (PCT) is caused by hexachlorobenzene (HCB) in several species of laboratory mammals, but the human evidence is contradictory. In a study among adults of a population highly exposed to HCB (Flix, Catalonia, Spain), the prevalence of PCT was not increased. We aimed at analysing the association of individual urinary porphyrins with the serum concentrations of HCB and other organochlorine compounds in this highly exposed population.

Methods: A cross-sectional study on total porphyrins was carried out in 1994 on 604 inhabitants of the general population of Flix, older than 14 years. Of them, 241 subjects (comprising a random sample and the subgroup with the highest exposure) were included for the present study. The porphyrin profile was determined by high-pressure liquid chromatography. Serum concentrations of HCB, as well as common organochlorine compounds, were determined by gas chromatography coupled to electron capture detection.

Results: Coproporphyrin I (CPI) and coproporphyrin III (CPIII) were the major porphyrins excreted, while uroporphyrins I and III were only detected in 2% and 36% of the subjects respectively, and heptaporphyrins I and III in 1% and 6%, respectively. CPI and CPIII decreased with increasing HCB concentrations ($p < 0.05$). This negative association was not explained by age, alcohol, smoking, or other organochlorine compounds. No association was found between uroporphyrin I and III excretion, nor heptaporphyrin excretion, and HCB. CPIII increased with smoking ($p < 0.05$).

Conclusion: HCB exposure in this highly exposed population did not increase urinary concentrations of individual porphyrins.

The porphyrias are disorders of the haem biosynthesis in which specific patterns of overproduction of haem precursors are associated with particular clinical features. Porphyria cutanea tarda (PCT) is one of the major potential toxic manifestations of hexachlorobenzene (HCB) in several species of laboratory mammals [1]. However, the porphyrinogenic effect of this chemical on humans has not been widely studied. The first cases of PCT induced by HCB in humans were reported in south-eastern Turkey in the late 1950s due to food poisoning in undernourished children [2–4]. Levels of HCB were not measured. In addition, there have been some case reports of workers exposed to HCB developing PCT [5], but there was no association between exposure to HCB and PCT in small studies in workers [6–8].

High atmospheric levels of HCB (mean 35 $\mu\text{g}/\text{m}^3$) were detected in Flix (Catalonia, Spain), a village of 5,000 inhabitants located in the vicinity of an electrochemical factory, the only industry in town [9]. Internal dose concentrations (i.e., serum) found were the highest ever reported in human general populations [10]. In a survey of 604 inhabitants only one subject had abnormally high levels of total porphyrins, compatible with PCT [11], resulting in a prevalence of PCT not higher than expected [12]. The association between individual urinary porphyrins and the internal dose of HCB was studied only in the 15 subjects with extremely high levels of HCB. Although concentrations of total porphyrins were within the range of clinical normality in this adult population [11], variations within the normality could be informative of sub-clinical harmful effects (i.e., functional effects). In addition, functional effects may be applied to risk assessment and prevention for its potential impact on susceptible populations [13].

Our objective was to determine the association between individual urinary porphyrin levels and serum concentrations of HCB and other organochlorine compounds in the general population of Flix.

Material & Methods

Study population

An epidemiological cross-sectional study was carried out on the 4,178 inhabitants of Flix older than 14 years in 1994 and details of participation and sociodemographic characteristics are described elsewhere [14]. Total porphyrins were analysed in 604 of these participants [11], a subsample of 241 of them being included in the present study. This subsample was composed of a 100 subjects previously selected for the studies of HCB biokinetics [15] consisting of those 8 subjects with the four highest and four lowest HCB levels, complemented by a random sample of 92, supplemented by a further 141 subjects who had worked in the electrochemical factory. Workers were

included because they had the highest levels of HCB in Flix [10]. In total, 177 out of the 241 subjects had worked in the electrochemical factory at some time, of whom 55 were currently employed. The individual with PCT was excluded from this analysis. All subjects were informed of the purpose of the study and signed a written consent form approved by the ethical committee at IMIM.

Urinary porphyrin measurements

Urine samples (24 h) were collected in 2 L plastic flasks. Immediately after collection, sodium bicarbonate was added to obtain a solution of 5 g/L, and 5 ml aliquots were transferred to polypropylene tubes and stored at -20°C until analysis. All urine specimens were analysed without previous knowledge of HCB levels in serum. Urinary porphyrin excretion patterns were analysed by high performance liquid chromatography (HPLC). Briefly, 1 mL of urine sample was acidified with 50 μL of HCl and 200 μL of this solution was injected. The HPLC determination was performed with Waters equipment (Waters Corp. Mildford, MA, USA) (2 pumps mod. 515, an autosampler injector (mod. 717 plus) and Millennium software). The porphyrins were detected using a fluorescence detector (mod. 474), under the following conditions: excitation 405 nm, emission 618 nm, both with band widths of 18 nm. Porphyrin separation was achieved with an analytical column BDS-Hypersil (250 \times 4.6 mm, 5 μm particle size) (Shandon HPLC, Cheshire, U.K) and a gradient from 100% of solvent A (10:90 acetonitrile/ammmonium acetate 1 M pH 5.16) to 95% of solvent B (10:90 acetonitrile/methanol) in 25 min. The flow rate was 1.2 mL/min [16].

Analysis of organochlorine compounds

Organochlorine compounds in serum were analysed by gas chromatography (GC) coupled to electron capture detection and GC coupled to chemical ionisation negative-ion mass spectrometry. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL was used for the analyses. All the analyses were carried out in the Department of Environmental Chemistry (CID-CSIC). Details of the methodology have been reported elsewhere [10,15]. We present results for the most prevalent compounds found in sera samples: HCB, dichlorodiphenyl dichloroethene (p,p'-DDE), and polychlorinated biphenyls (PCBs) which we present as the summation of the individual congeners 28, 52, 101, 118, 138, 153, and 180. Because the PCB congeners 138, 153 and 180 represented around 90% of total PCBs, we also provide results of these individual congeners. Detection limits for HCB and p,p'-DDE were 0.2 ng/mL, and for the individual congeners of PCBs were the following: 0.17, 0.15, 0.09, 0.11, 0.15, 0.12, and 0.10. The concentration of organochlorine compounds below the detection limit were set at half the limit of detection.

Statistical analysis

Individual porphyrins were treated both as a dichotomous variable (detectable/non-detectable) and as continuous variable after assigning half the detectable level to represent the non-detectable values. Total porphyrins were analysed only as a continuous variable since all observations were detectable, while uroporphyrins were analysed only as a dichotomous variable since most of the observations were not-detectable. The crude association between porphyrins and the study variables (organochlorine compounds as well as confounding variables such as age, sex, alcohol, smoking and duration of the residence) was assessed with the non-parametric Wilcoxon test for linear trend, given the non-normal distribution of porphyrins. To control the association between porphyrins and organochlorine compounds for the confounding variables both linear regression models and logistic regression models were fitted. Since the data distribution of individual porphyrins was skewed, a logarithmic transformation was carried out. Organochlorine compounds were treated both as trichotomous variables and as continuous variables, given a certain linearity in the association with porphyrins. Organochlorine compounds were also logarithmically transformed (in base 10) to achieve a normal distribution. Multiple linear regression analysis was performed to examine the relationship between continuous values of porphyrins (total porphyrins, CP I, CPIII) and organochlorine levels adjusting for potential confounding variables such as creatinine in serum [14]. Association was measured with the regression coefficient. The antilogarithm of the coefficient yields the relative change in porphyrin levels for each 10-fold increase in organochlorine

compound levels. Multiple logistic regression models were fitted to estimate the adjusted association between categorical values of uroporphyrins I and III and organochlorine compounds. The measure of association was the odds ratio. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using Stata (StataCorp. TX, USA).

Table 1: Characteristics of the 241 subjects

| Variable | n (%) |
|------------------------------|----------|
| Male | 180 (75) |
| Age | |
| < 45 years | 89 (37) |
| 45–64 years | 84 (35) |
| > 64 years | 68 (28) |
| Alcohol | |
| No | 73 (30) |
| Occasional | 61 (25) |
| Habitual (> 3 drinks a week) | 107 (45) |
| Smoking | |
| Never | 73 (30) |
| Occasional | 11 (5) |
| Ex | 82 (34) |
| Current | 75 (31) |
| <20 cigs./day | 33 (13) |
| ≥ 20 cigs./day | 42 (18) |
| Years living in Flix | |
| 1–9 | 4 (2) |
| 10–19 | 19 (8) |
| >20 | 218 (90) |

Table 2: Distribution of urinary uroporphyrins (µmol/L) and serum concentrations of organochlorinated compounds (ng/mL) (n = 241 subjects)

| | % detectable | Percentiles among detectables | | | | |
|------------------|--------------|-------------------------------|------|------|------|------|
| | | Min | p25 | p50 | p75 | Max |
| Total porphyrins | 100% | 13.0 | 63.8 | 98.5 | 157 | 497 |
| Uro I | 36% | 2.0 | 6.0 | 10.0 | 16.0 | 37.0 |
| Uro III | 2% | 1.0 | 1.0 | 1.0 | 8.0 | 8.0 |
| Hepta I | 1% | 2.0 | 2.0 | 2.5 | 3.0 | 3.0 |
| Hepta III | 6% | 2.0 | 2.8 | 4.0 | 6.8 | 13.0 |
| Copro I | 76% | 1.0 | 4.0 | 7.0 | 14.0 | 134 |
| Copro III | 86% | 2.0 | 10.0 | 21.5 | 37.0 | 154 |
| HCB | 100% | 2.30 | 13.2 | 21.7 | 37.9 | 1616 |
| DDE | 97% | 0.1 | 2.59 | 6.15 | 14.4 | 67.4 |
| Total PCBs† | 94% | 0.2 | 1.89 | 3.78 | 7.02 | 143 |
| CB-138 | 87% | 0.1 | 0.58 | 1.03 | 1.85 | 35.0 |
| CB-153 | 85% | 0.1 | 0.67 | 1.18 | 2.32 | 40.9 |
| CB-180 | 89% | 0.1 | 0.58 | 1.43 | 2.66 | 63.5 |

Uro: uroporphyrins; Hepta: heptaporphyrins; Copro: coproporphyrins. † PCBs = Σ (28, 52, 101, 118, 138, 153, 180) congeners

Results

Table 1 presents the socio-demographic and behavioural characteristics of the subjects included in this study. Not surprisingly, most of them are males given that we enriched the sample with the electrochemical workers in order to have the individuals with the highest HCB levels in the study. It is noticeable that most of them had lived in the town for more than 20 years.

Distributions of total porphyrin concentrations and individual urinary porphyrins as well as organochlorine compound levels in serum are presented in table 2. Coproporphyrin isomers I-III (CPI; CPIII) were the major porphyrins excreted. The uroporphyrin I (UPI) fraction was the third most excreted porphyrin. The heptacarboxylporphyrin isomer I (hepta I) was only detected in 1% of subjects and the heptaporphyrin isomer III (hepta III) in 6%. The hexa- and pentacarboxylporphyrin fractions were not detected. Among the organochlorine compounds, all subjects had detectable concentrations of HCB, and most of them of *p,p'*-DDE. The highest concentrations were found for HCB. The most prevalent PCB congener was CB-180.

Total porphyrins decreased with age, years of residence in Flix, and organochlorine concentrations (except CB-153) and increased with male gender and tobacco smoking, in a statistically significant way, while no association was observed with alcohol consumption (table 3). A similar pattern was observed with CPI and CPIII, except that the association with sex disappeared. However, for CPI the only statistically significant differences found were for age, years of residence, HCB and *p,p'*-DDE, but not for smoking or PCBs. For CPIII, the associations with smoking and PCBs, in addition to age, years of residence, HCB and *p,p'*-DDE, were significant. The negative association of CPI and CPIII with HCB occurred both in subjects working in the electrochemical factory (with the highest HCB levels, median of HCB = 79.2 ng/mL) and in non-workers (median HCB = 14.2 ng/mL). Workers had lower average levels of CPIII than non-workers (20.6 and 33.3 $\mu\text{mol/L}$, respectively) and of CPI (6.8 and 11.2 $\mu\text{mol/L}$, respectively) (all $p < 0.05$). UPI and UPIII did not differ for any of the study variables (table 3), and nor did heptaporphyrin.

The negative association of HCB with total porphyrins and CPIII remained after adjusting for the confounding variables (table 4). The association between HCB and CPI was also negative but with a p value of 0.06. When HCB was treated as a trichotomous variable in the multivariate models, results were very similar to those in table 3 (where they were unadjusted), with a p value < 0.05 for CPIII. In contrast, the associations of *p,p'*-DDE and PCBs disappeared in the multivariate model. HCB remained as-

sociated with total porphyrins and CPIII after adjusting for *p,p'*-DDE and PCBs. In the multivariate models, the positive associations of smoking with total porphyrins (coefficient (se) = 0.18 (0.08)), and mainly with CPIII (coefficient (se) = 0.30 (0.13)) remained, and so did the negative association of age with total porphyrins, CPI and CPIII. The adjusted association with UPI and UPIII remained non-significant.

Discussion

In experimental porphyria in laboratory animals, intake of HCB was followed initially by a moderate increase of coproporphyrins, and later by an increase of highly carboxylated porphyrins, such as uroporphyrins and heptaporphyrins [17]. In our human population, we observed that levels of individual urinary porphyrins (uroporphyrins, heptaporphyrins, coproporphyrins) did not increase with serum HCB concentrations. Even though our results contrast with the findings to be expected from experiments in animals [17], in general, they coincide with older studies in human populations (table 5) since no increase of uroporphyrins was observed in any of these studies. In addition, in contrast with animals, coproporphyrins (mainly CPIII) were lower in subjects with higher levels of HCB which agrees with an old study in workers from a chlorinated solvents plant [8]. Furthermore, neonates of Flix also showed a decrease of coproporphyrins in relation to HCB [18], although levels of urinary porphyrins during the first 10 days of life are highly variable [19], and results difficult to interpret. The negative association with coproporphyrins was not found in a cross-sectional study in the general population in Louisiana, USA [20], where plasma concentrations of HCB were positively correlated with levels of urinary coproporphyrin, though in a weak and crude way (correlation coefficient = 0.15). However, serum concentrations of HCB among these subjects were very low in contrast with spray workers from the same location among whom there was no association between HCB and individual coproporphyrins [7]. Finally, in a study comparing 9 smelter workers exposed to HCB and to octachlorostyrene with 18 non-exposed workers, CPIII was higher among the workers [21]. Only this latter study, in addition to the studies conducted in Flix, measured the porphyrin profile using a reliable method such as the high-pressure liquid chromatography. However, the authors did not report the levels of HCB, and comparisons were not adjusted for other variables related with porphyrins such as age and smoking.

Epidemiological studies on porphyrins with other environmental agents are rare. The possible role of other halogenated compounds, such as dioxins (TCDD), in the disturbance of the porphyrin metabolism was proposed due to some case reports of PCT among workers [22]. However, this effect was mostly attributed to a concomi-

Table 3: Association between urinary porphyrine concentrations and the study variables (age, sex, alcohol, smoking, years of residence, and organochlorinated compounds in serum).

| | TP | Copro I | | Copro III | | Uro I + UroIII |
|-----------------------|------------------|-------------------|--------------------|-------------------|--------------------|-------------------|
| | Median (25%-75%) | Detectables n (%) | Median (25%-75%) † | Detectables n (%) | Median (25%-75%) † | Detectables n (%) |
| Age | | | | | | |
| <45 | 117 (73-185) | 70 (79) | 7 (3-15) | 85 (96) | 29 (11-46) | 31 (35) |
| 45-64 | 89 (62-132) | 62 (74) | 4 (0.5-8) | 67 (80) | 14 (4.5-27.5) | 24 (29) |
| >64 | 83 (56-126)* | 51 (75) | 4 (1.3-9)* | 56 (82)* | 12.5 (3-30)* | 27 (40) |
| Sex | | | | | | |
| Female | 85 (62-112) | 42 (69) | 5 (0.5-11) | 60 (98) | 20 (10-31) | 17 (28) |
| Male | 107 (65-165)* | 141 (78) | 5 (2-11) | 148 (82)* | 19 (5-37) | 65 (36) |
| Alcohol | | | | | | |
| No | 99 (62-144) | 52 (71) | 5 (0.5-11) | 67 (92) | 18 (6-35) | 23 (32) |
| Ocasional | 86 (64-143) | 45 (74) | 5 (0.5-10) | 52 (85) | 19 (7-35) | 21 (34) |
| Habitual | 103 (66-162) | 86 (80) | 5 (3-12) | 89 (83) | 20 (5-35) | 38 (36) |
| Smoking | | | | | | |
| Never | 86 (62-124) | 52 (71) | 5 (0.5-10) | 64 (88) | 19 (6-29) | 19 (26) |
| Ocasional + Ex | 92 (60-146) | 65 (70) | 4 (0.5-10) | 76 (82) | 12 (4-35) | 33 (35) |
| < 20 cigs./day | 103 (83-159) | 30 (91) | 6 (4-9) | 28 (85) | 20 (7-39) | 12 (36) |
| ≥ 20 cigs./day | 127 (81-588)* | 36 (86)* | 6.5 (3.5-15) | 40 (95) | 32 (12-46)* | 18 (43) |
| Years living in Flix | | | | | | |
| ≤ 20 years | 165 (86, 240) | 21 (91) | 7 (3, 19) | 22 (95) | 27 (17,51) | 10 (43) |
| > 20 years | 94 (62, 143)* | 162 (74) | 5 (0.5, 10)* | 186 (85) | 19 (6, 34)* | 72 (33) |
| HCB ng/ml (tertiles) | | | | | | |
| <16.1 | 129 (81-176) | 60 (77) | 7 (2-15) | 73 (94) | 26 (8-43) | 29 (37) |
| 16.1-29.5 | 82 (55-126) | 62 (75) | 4 (0.5-10) | 70 (84) | 18 (5-31) | 28 (34) |
| >29.5 | 92 (64-130)* | 61 (76) | 5 (2-8.5)* | 65 (81)* | 14 (5-31.5)* | 25 (31) |
| DDE ng/ml (tertiles) | | | | | | |
| < 3.22 | 124 (74-179) | 62 (78) | 7 (2-15) | 74 (94) | 26 (7-49) | 31 (39) |
| 3.22 - 9.88 | 105 (65-161) | 60 (75) | 4 (0.5-10) | 64 (80) | 20 (4.5-35) | 22 (28) |
| > 9.88 | 82 (57-117)* | 61 (74) | 5 (2-8.5)* | 70 (85) | 12 (5-26)* | 29 (35) |
| PCBs ng/ml (tertiles) | | | | | | |
| < 2.08 | 108 (74, 166) | 63 (80) | 6 (2-13) | 72 (91) | 22 (7, 35) | 21 (27) |
| 2.08-4.89 | 99 (61, 172) | 60 (77) | 4 (2-12) | 69 (88) | 20 (6, 38) | 30 (38) |
| > 4.89 | 87 (59, 130)* | 60 (73) | 4.5 (0.5-9) | 67 (80)* | 13.5 (4, 30)* | 31 (37) |
| CB-138 (tertiles) | | | | | | |
| < 0.55 | 108 (73-116) | 63 (79) | 5.5 (2.5-13) | 73 (91) | 20 (7.5-36.5) | 19 (24) |
| 0.55 - 1.29 | 99 (61-146) | 63 (80) | 5 (2-12) | 69 (87) | 21 (6-38) | 36 (46) |
| > 1.29 | 89 (60-130)* | 57 (70) | 4 (0.5-9) | 66 (80)* | 13 (4-29)* | 27 (33) |
| CB-153 (tertiles) | | | | | | |
| < 0.61 | 109 (73-161) | 65 (81) | 6 (3-13.5) | 74 (93) | 21 (8.5-35) | 21 (26) |
| 0.61 - 1.36 | 98 (61-173) | 58 (73) | 4 (0.5-11) | 66 (84) | 20 (5-39) | 30 (38) |
| > 1.36 | 87 (61-130) | 60 (73) | 4 (0.5-9)* | 68 (83) | 13 (5-32) | 31 (38) |
| CB-180 (tertiles) | | | | | | |
| < 0.61 | 110 (73-168) | 64 (80) | 5 (2-11.5) | 72 (90) | 21 (7-38.5) | 22 (28) |
| 0.61 - 1.80 | 94 (61-143) | 59 (75) | 5 (0.5-12) | 73 (92) | 20 (6-37) | 26 (33) |
| > 1.80 | 90 (60-141)* | 60 (73) | 4.5 (0.5-10) | 63 (77)* | 14 (3-29)* | 34 (41) |

TP: total porphyrins; CPI, CPIII: coproporphyrins I, III; UPI, UPIII: uroporphyrins I, III. * p < 0.05 using the extension of the non-parametric Wilcoxon test for linear trend † non-detectable values were set at the median value between 0 and the detectable level

Table 4: Adjusted association between urinary porphyrin levels and serum concentrations of organochlorinated compounds (n = 241).

| Organochlorinated compound | TP | Copro I | Copro III |
|----------------------------|-----------------|----------------|-----------------|
| Coefficient (se)† | | | |
| Single pollutant model | | | |
| HCB | -0.265 (0.078)* | -0.321 (0.171) | -0.395 (0.187)* |
| DDE | -0.024 (0.049) | 0.048 (0.115) | -0.051 (0.123) |
| PCBs | -0.005 (0.048) | -0.039 (0.111) | -0.019 (0.120) |
| Multipollutant model†† | | | |
| HCB | -0.281 (0.079)* | -0.315 (0.174) | -0.420 (0.190)* |
| DDE | -0.056 (0.053) | 0.039 (0.127) | -0.092 (0.137) |
| PCBs | 0.005 (0.052) | -0.059 (0.121) | -0.011 (0.131) |

TP: Total porphyrins. CPI and CPIII: coproporphyrins, I and III, respectively. † The coefficient gives the relative change in porphyrin levels for a 10-fold concentration increase in the organochlorinated compound, adjusted for age, smoking, alcohol, years of residence in Flix, and creatinine in serum. †† Adjusted for age, smoking, alcohol and years of residence in Flix, as well as for the other organochlorinated compounds in the table. * p < 0.05

Table 5: Studies on urinary porphyrins in human populations exposed to moderate levels of hexachlorobenzene (HCB).

| Author year/country (ref) | N | Period | Serum-HCB (range in ng/mL) | Porphyrins UP | CP | Comments |
|---------------------------|-----|-------------|----------------------------|---------------|----|----------------------------|
| WORKERS | | | | | | |
| Morley 1973/Australia [6] | 54 | 1950's-60's | NA | NS | NS | only 1 worker with high UP |
| Burns 1974/USA [7] | 20 | 1973-74 | <1-310 | NS | NS | - |
| Currier 1980/USA [8] | 50 | 1974-7 | 3-1121 | NS | ↓ | ↓ CP if HCB > 200 |
| Selden 1999/Sweden [21] | 27 | 1980's | NA(9E/18NE) | NS | ↑ | ↑ CPIII in E vs NE |
| GENERAL POPULATION | | | | | | |
| NEONATES | | | | | | |
| Ozalla 2002/Spain [18] | 68 | 1997-99 | 0.4-21 | NS | ↓ | ↓ CPI and CPIII |
| ADULTS | | | | | | |
| Burns 1975/USA [20] | 120 | 1972 | 0-23 | NS | ↑ | - |
| This study/Spain | 242 | 1994 | 2-1616 | NS | ↓ | ↓ CPI and CPIII |

NA: not available. NS: no statistically significant difference. UP: uroporphyrins. CP, CPI and CPIII: coproporphyrins total, I and III. E/NE: number of subjects exposed/non-exposed

tant exposure to HCB [22]. Recent epidemiological studies in human populations did not find any association of TCDD exposure with regard to urinary uroporphyrin or coproporphyrin levels [23,24]. A negative association between arsenic concentration in urine and CPIII was found in 36 individuals from the general population in an area of Mexico with high levels of arsenic in drinking water compared with 31 non-exposed subjects [25]. In contrast with studies on HCB, a concomitant increase in uroporphyrins occurred. Also, in a cross-sectional study carried out in a convention of dentists, the 38 dentists with levels of

total mercury higher than 20 µg/l had higher levels (twice) of urinary coproporphyrins than the 23 dentists with no detectable levels of mercury [26]. Mechanisms of action for these compounds, however, could be of a different nature than those related with HCB.

In accordance with our previous observation of an association of passive smoking with CPIII in neonates [18], active smoking is associated with an increase of porphyrins in the present study. In patients with intermittent porphyria, smoking was associated with induction of repeated

acute attacks of porphyria [27]. This finding agrees with a possible indirect effect of smoke through a cytochrome P450 induction, since some P450 isoenzymes such as P4501A2s, which is affected by smoking, are involved in disturbances of porphyrin metabolism [28]. Recently it has been suggested that HCB should be classified as a dioxin-like compound that binds the Aryl hydrocarbon (Ah) receptor inducing hepatic cytochrome p4502B activity [29]. Why smoking is associated with an increase of CPIII while HCB was associated with a decrease of CPI and CPIII, without modifying uroporphyrin levels, is an intriguing problem to solve.

Overall, the epidemiological studies in human populations have to be considered with caution given their size, the methodology used to measure the porphyrin profile, the control of the effect of variables such as age and smoking and the difficulties in disentangling the individual pollutant effects from the pollution mixtures. A conclusion from such review is the lack of a common procedure in environmental studies of porphyria. In addition, porphyrin increases have been related with the multiple chemical sensitivity syndrome (MCS) [13]. However, there is currently no convincing evidence that MCS syndrome affects the haem synthesis [13]. Our findings confirm that it is premature and speculative to relate porphyrin increases with current environmental exposures.

The present results do not seem affected by a selection bias given that similar findings were observed both in the random and the enriched sample. Cross-sectional bias also seems improbable since exposure to HCB could not be determined by knowledge of levels of uroporphyrins and viceversa. A confounding effect due to an unmeasured variable seems also unlikely. Positive serology against virus C was only found in 4 of the subjects, and renal dysfunction was detected in a very small proportion [14]. A final explanation could be the presence of an error due to misclassification of uroporphyrins or HCB levels, non-differential, that led to the dilution of the association between HCB and uroporphyrins. Nevertheless, an error of this type should also have affected the association with smoking, while a positive association with smoking was found. In addition, in the present study porphyrin patterns and HCB levels were determined with the best current technology. These facts suggest that the effect of this misclassification error if any must be small and unlikely to explain the complete lack of porphyrin increase according to the categories of HCB.

The present study is the largest ever conducted, used a reliable method of measuring porphyrin patterns and incorporated analysis of cofactors. In addition, the population under study is specifically exposed to high levels of HCB

(serum concentrations were around 20 times higher than in unexposed Europeans of the same age [10], and levels of other pollutants such as PCB, DDE or dioxins were within the range of most populations [9]).

Conclusion

The present results suggest that current levels of HCB are not associated with an increase of the individual uroporphyrins, and if anything are associated with a decrease of the coproporphyrins, which challenges the concept of a porphyrinogenic effect of environmental pollutants at current levels of exposure.

Abbreviations

PCT Porphyria Cutanea Tarda

HCB Hexachlorobenzene

CPI Coproporphyrin I

CPIII Coproporphyrin III

p,p'-DDE Dichlorodiphenyl Dichloroethene

PCB's Polychlorinated Biphenyls

UPI Uroporphyrin I

Hepta I Heptacarboxylporphyrin isomer I

Hepta III Heptaporphyrin isomer III

MCS Multiple Chemical Sensitivity

UPIII Uroporphyrin III

Authors' contribution

JS was the principal investigator and drafted the manuscript, CH and DO carried out the porphyrin analysis and actively participated in the drafting. MS and NRF participated in the design of the study, the contact with participants and the freeze chain. JO did the organochlorine analyses and XB the statistical analysis.

Competing Interests

None

Acknowledgement

We thank Carlos Mazón MD and Antoni Verdú MD of the pediatric department of the Hospital of Móra d'Ebre for their collaboration. We are indebted to the participants for their altruism in participating, and also thank Luisa Alvarez MD for creatinine determinations.

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Pre-publication history

The pre-publication history for this article can be downloaded from here:

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ANNEX 3: M Porta, M Kogevinas, E Zumeta, J Sunyer, N Ribas-Fitó, Grupo de Trabajo sobre Compuestos Tóxicos Persistentes y Salud del IMIM. **Concentraciones de compuestos tóxicos persistentes en la población española: el rompecabezas sin piezas y la protección de la salud pública.** Gac Sanit 2002;16:257-66

Concentraciones de compuestos tóxicos persistentes en la población española: el rompecabezas sin piezas y la protección de la salud pública

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Recibido: 18 de diciembre de 2001.

Aceptado: 23 de febrero de 2002.

(Concentrations of persistent toxic compounds in the Spanish population: a puzzle without pieces and the protection of public health)

Resumen

La contaminación por compuestos tóxicos persistentes (CTP) de la población general es un hecho relevante desde una perspectiva de salud pública. Es, asimismo, importante para el sistema sanitario asistencial y para las políticas ambientales, alimentarias, industriales y económicas.

Aunque en España los conocimientos sobre la contaminación de los alimentos por CTP presentan grandes vacíos temporales y geográficos, aún es menor la información sobre sus concentraciones en las personas: no existe ningún estudio representativo de una población general sana efectuado en una zona geográfica amplia. Los estudios disponibles indican que un 80-100% de la población tiene concentraciones detectables de DDE, policlorobifenilos, hexaclorobenceno o lindano. En España el número de estudios sobre los efectos que los CTP tienen en las personas es todavía más exiguo. Los estudios internacionales sugieren que dosis de algunos CTP por debajo de las que normalmente se consideran «seguras» pueden causar efectos biológicos y clínicos relevantes. Los mecanismos de acción no comprenden sólo la disrupción endocrina. La valoración de la significación clínica y social del espectro de efectos más sutiles y con períodos de latencia mayores de los CTP presenta interesantes retos y oportunidades.

España y otros países europeos sufren un déficit de indicadores poblacionales sobre el impacto que los procesos ambientales tienen en la salud humana. Los distintos ámbitos de la Administración deben monitorizar los valores biológicos de los CTPs y valorar sus posibles riesgos para la salud.

Junto con más de cien otros países, próximamente España intentará implementar el Tratado sobre los Contaminantes Orgánicos Persistentes (Convenio de Estocolmo). Ello constituye un nuevo motivo para desarrollar programas más eficientes de vigilancia y control de los residuos de los CTP en alimentos, humanos y medio ambiente. Como parte de la aplicación del Convenio, es necesario iniciar un informe pe-

Summary

The contamination by persistent toxic compounds (PTCs) of the general population is a fact of relevance from a public health perspective. It is also relevant to health care professionals, as well as for environmental, food, industrial and economic policies.

Though in Spain information on food contamination by PTCs shows large time and geographic gaps, the scarcity of data is even more severe on the concentrations that PTCs have in people: a representative study of a general healthy population living in a wide geographic area has never been conducted in the country. However, the available studies indicate that around 80-100% of the population has detectable concentrations of DDE, PCBs, hexachlorobenzene or lindane. Studies on the effects that PTCs have upon humans are extremely infrequent in Spain. Yet, the international literature suggests that some PTCs may induce significant biological and clinical effects at doses below those traditionally deemed "safe". The mechanism of action of PTCs are not restricted to endocrine disruption. Assessing the clinical and social relevance of the more subtle and long-term effects of PTCs presents interesting challenges and opportunities.

Spain and other European countries lack population indicators on the impact that environmental processes have on human health. Several government levels have a role to fulfill in the monitoring of biological levels of PTCs among persons in order to assess the risks of adverse health effects.

Along with over a hundred other countries. Spain will soon try to implement the Stockholm treaty on persistent organic pollutants (POPs). This constitutes a new opportunity to develop more efficient policies to control PTC residues in food, humans and the environment. As part of the treaty implementation it is necessary to launch a Report on factors that influence body concentrations of PTCs in the Spain general population.

Key words: Persistent toxic compounds. Persistent organic

riódico sobre los factores que condicionan las concentraciones internas de CTP en la población general española.

Palabras clave: Compuestos tóxicos persistentes. Agentes químicos ambientales. Compuestos organoclorados. DDT. DDE. Policlorobifenilos. Dioxinas. Hexaclorobenceno. Lindano. Convenio de Estocolmo.

pollutants (POPs). Organochlorine compounds. Environment/chemistry. DDT. DDE. Polychlorinated biphenyls. Dioxins. Hexachlorobenzene. Lindane. Stockholm treaty.

Introducción

Entre las complejas y apasionantes cuestiones que nos plantean los compuestos tóxicos persistentes (CTP), especialmente a quienes trabajamos desde y para la salud pública, el hecho que nos parece de mayor relevancia es la contaminación por CTP de vastos sectores de la población general; cuanto menos, como punto de partida y de llegada. En efecto, la mayoría de los habitantes de la Tierra almacenamos en nuestro organismo cantidades apreciables de CTP. Entre ellos, el plaguicida DDT, el DDE (principal producto en el que se degrada el DDT), ciertos policlorobifenilos (más conocidos por PCB, sus siglas en inglés), las dioxinas, el hexaclorobenceno, los hexaclorociclohexanos y otros residuos de compuestos organoclorados¹⁻⁶. Aunque la importancia de la impregnación corporal por CTP de las personas puede parecer obvia, este hecho es a menudo soslayado desde otras perspectivas (p. ej., la estrictamente económica o industrial). Por ende, la conciencia social de este hecho y de sus implicaciones es todavía débil en bastantes países europeos, incluido el nuestro.

Para referirse a ciertos CTP algunas organizaciones y científicos utilizan la expresión *compuestos* (o a veces *contaminantes*) *orgánicos persistentes* (COP; en inglés, POP), que a nuestro juicio tiene menos ventajas. En este trabajo utilizaremos la expresión CTP, que incluye a todos los COP.

En España, el DDT se utilizó ampliamente como plaguicida desde mediados de los años cincuenta hasta la mitad de la década de los setenta, y menos posteriormente. Aunque la orden que prohibió su uso entró en vigor en 1977^{7,8}, no está documentado cuándo terminó realmente su utilización, si es que ha terminado completamente. Esta duda obedece a varias razones. En primer lugar, el DDT se sigue usando para fabricar productos, como el herbicida dicofol, que en consecuencia contienen DDT. En segundo lugar, existen indicios de que cantidades menores de DDT podrían estar entrando ilegalmente en España procedentes de otros países. En tercer lugar, periódicamente se tiene noticia de usos ocasionales en explotaciones agrícolas y ganaderas. En cuarto lugar, el Instituto de Toxicología (antes Instituto Nacional de Toxicología), dependiente del Ministerio de Justicia, recibe periódicamente notificaciones y consultas relacionadas con personas que

sufren episodios de intoxicación aguda por DDT (ponencia oral presentada por el director del Instituto de Toxicología, Rafael Cabrera, en el Congreso sobre Implementación del Convenio sobre COP, Madrid, 27 de noviembre de 2001).

En la bibliografía académica no aparece una revisión completa sobre la historia de la fabricación y uso de DDT en España, realizada por ejemplo desde las perspectivas de la historia económica o de la salud pública; disponer de dicho análisis ayudaría a explicar las concentraciones que actualmente hallamos en los alimentos y en las personas (concentraciones cuya evolución espaciotemporal tampoco ha sido objeto de revisión sistemática). En la tabla 1 se sintetizan las principales incógnitas acerca del DDT en España. El razonamiento subyacente a esas preguntas sería aplicable de forma similar a otros CTP. Sí se sabe que la empresa Erquimia (situada en Flix, Tarragona) fue la principal productora española de DDT durante años. También produjo DDT la empresa Montecinca (en Monzón, Huesca), que actualmente fabrica dicofol; asimismo, produce dicofol alguna otra empresa española.

Los PCB se han utilizado como aislantes en equipos eléctricos, como lubricantes, en plásticos, tintas y otras múltiples aplicaciones industriales y domésti-

Tabla 1. Algunas incógnitas acerca del DDT en España

- A partir de 1955, fecha en la que parece que empieza su uso en España ¿cómo se difunde geográficamente y temporalmente su uso en la agricultura española? ¿Cuáles fueron los principales determinantes políticos y económicos de su utilización durante la dictadura franquista?
- ¿Cuál es la aplicación espacio-temporal real de la legislación que prohíbe su uso al principio de la transición democrática y posteriormente, hasta la actualidad?
- ¿Cuánto DDT se produjo en España? ¿Cuánto y de dónde se importaba? ¿Qué fiabilidad tienen las estadísticas oficiales al respecto?
- ¿Cuándo se detecta por primera vez DDT en una población humana española? ¿Cómo evolucionan esos valores?
- ¿Cuándo se detecta por primera vez en alimentos? ¿Cómo evolucionan esos valores?
- ¿Qué efectos agudos y crónicos ha tenido el DDT y está teniendo el DDE sobre la salud de las personas, la agricultura, la fauna y el ambiente?
- ¿De dónde procede el DDT que actualmente se detecta en los alimentos, las personas y el ambiente? ¿Se trata exclusivamente del resultado de exposiciones históricas o existe todavía exposición *de novo*? Y en este último caso, ¿se produce sólo por piensos, grasas y alimentos importados?

Tabla 2. Un ejemplo de los retos pendientes: la Directiva sobre eliminación de los policlorobifenilos y policloroterfenilos

- El Ministerio de Medio Ambiente (MIMAM) calcula que en España existen unas 210.000 toneladas de tales compuestos: 70.000 toneladas de PCB y aparatos que los contienen junto con otras 140.000 procedentes de aceites dieléctricos y aparatos potencialmente contaminados^a
- En su artículo 4, la Directiva 96/59/CE sobre eliminación de los policlorobifenilos y de los policloroterfenilos (PCB/PCT) (que se transpone al derecho interno mediante el RD 1378/99) establece que los «estados miembro garantizarán que se realicen inventarios de los aparatos que contengan un volumen de PCB superior a 5 dm³ y enviarán un resumen de dichos inventarios a la Comisión, a más tardar 3 años después de la adopción de la Directiva» (es decir, antes del 16 de septiembre de 1999). Los inventarios deben incluir: nombre y dirección del poseedor, ubicación y descripción del aparato, cantidad de PCB contenidos, fechas y tipo de gestión prevista y fecha de la declaración. Por tanto, una simple estimación o declaración de cantidades no es suficiente^b
- El artículo 11 de la Directiva establece que en el mismo plazo de 3 años se elaborarán: un plan para la descontaminación y la eliminación de los aparatos que figuran en el inventario y un proyecto de recogida y posterior eliminación de los aparatos que no estén en el inventario. El Gobierno publicó en el BOE su Plan Nacional el 18 de abril de 2001. Está inicialmente dimensionado para unas 232.000 toneladas, pero se "prevé su revisión con el fin de actualizarlo a medida que las comunidades autónomas vayan realizando los inventarios"^a. A falta de un inventario exhaustivo, el Plan Nacional tampoco puede ajustarse a los requerimientos de la Directiva
- El Plan Nacional se aprobó con año y medio de retraso, sin haber sido sometido a la preceptiva información pública, tal y como establece el artículo 5.2 de la Ley de Residuos, sin dar oportunidad para hacer alegaciones a las organizaciones sociales. Aunque formalmente el anuncio de información pública apareció en el BOE de 25 de octubre de 2000, no se llegó a someter ningún documento a la misma, pues no existía^b
- Una vez que se conozcan las dimensiones reales estaremos ante el reto de gestionarlo correctamente (regulado en el artículo 8 de la Directiva). Es de esperar que tantas toneladas de PCBs (en el ámbito estatal y europeo) no acaben incinerándose, pues pueden constituir una fuente importante de emisión de dioxinas y furanos; además, sería incoherente con la puesta en marcha del Convenio de Estocolmo
- El gobierno tiene pendiente implementar sus propuestas para que se cumpla el objetivo de descontaminar y eliminar los PCB «lo antes posible» (artículo 3 de la Directiva), sin esperar a la fecha límite, diciembre de 2010

^aFuente: MIMAM. Información de Medio Ambiente 2001;95.

^bBlount E, comunicación personal (enero de 2002).

cas^{3-6,9,10}. En España existe un escaso conocimiento sobre la cantidad de PCB almacenados y las condiciones de las instalaciones que los contienen. Reflejo de ello es que en la actualidad el gobierno atiende en el Tribunal de Justicia de Luxemburgo una denuncia presentada por la Comisión Europea por incumplir la Directiva sobre eliminación de los PCB y policloroterfenilos (tabla 2). Este problema ejemplifica algunos de los problemas que deben resolver nuestras sociedades. Ilustra, asimismo, el esfuerzo técnico y político que exigirá la aplicación del Convenio de Estocolmo, al que nos referimos más adelante.

En Europa occidental las principales fuentes de contaminación por dioxinas son las incineradoras de residuos y las industrias de reciclaje de metal¹¹.

Contaminación de los alimentos

Los CTP tienen una gran inercia química (persistencia en el medio, efectos a largo plazo, bioacumulación); se han dispersado y contaminan amplias zonas del planeta, y son muy difíciles de excretar por el cuerpo humano, en el que tienen una larga vida media, y se acumulan en los tejidos grasos¹⁻⁶. Llegan hasta nuestro organismo a través de una exposición ambiental «de fondo», continua, a dosis muy bajas. Fundamentalmente, a través de la dieta; sobre todo a partir de las partes más grasas de los alimentos, incluyendo las grasas recicladas para fabricar productos (pastelería, piensos) que humanos y animales comemos^{6,9,10}.

Diversos estudios han observado que en España muchas muestras de carne, pescado, huevos, leche, mantequilla, queso o cereales contienen residuos de DDE, PCB, hexaclorobenceno e isómeros del lindano, como el β -hexaclorociclohexano^{3,10,12,13}; su revisión rebasa el ámbito del presente debate. En cuanto a las dioxinas, se calcula que un 95-98% entran en el cuerpo humano a través de los alimentos (más del 80%, por los de origen animal)¹¹. No obstante, en España la magnitud poblacional del problema es relativamente mal conocida, básicamente por dos razones: a) los estudios científicos se han hecho sin continuidad temporal ni exhaustividad sociodemográfica, y b) los estudios o actuaciones puntuales efectuadas por la Administración parecen ser escasos o incompletos, son poco difundidos o adolecen de importantes limitaciones metodológicas. Aunque otros países europeos sufran una situación similar, es necesario alcanzar un conocimiento más sistemático del estado de la contaminación por CTP de los alimentos, difundir ese conocimiento a la ciudadanía y, probablemente, aplicar de forma más metódica la legislación vigente.

Contaminación de las personas

La información sobre las concentraciones que los CTP tienen en la población española es más escasa que en el caso de los alimentos: no existe ningún estudio de una población general sana efectuado en una zona geográfica amplia y bien definida. La posible excepción sería el estudio desarrollado en el pueblo de Flix^{14,15}. Pero en realidad no es tal, pues Flix tiene menos de 5.000 habitantes, está expuesto a una fuente puntual (industrial) y los resultados, obviamente, no son

extrapolables a la mayoría de las poblaciones españolas.

Con sus limitaciones, los estudios realizados en España sugieren que la mayoría de las personas tenemos concentraciones apreciables de CTP. Es habitual encontrar que un 80-100% de la población tiene concentraciones detectables de DDE, PCB (particularmente, de los congéneres 118, 138, 153, 170, 180 o 187), hexaclorobenceno y hexaclorociclohexanos^{1,3,7,8,14-26}. Una de las incógnitas por resolver es a qué factores obedece la presencia de DDT en la sangre de los recién nacidos^{1,3,23,26,27}, pues la prohibición de este compuesto y su degradación a DDE podrían llevar a esperar que ya no se detectase en ellos.

En la tabla 3 se presentan las principales características y resultados de los estudios españoles más destacables en cuanto a las concentraciones de DDT y DDE en humanos^{7,8,14,17-26}. Como puede observarse, prácticamente siempre los períodos y las zonas geográficas cubiertas por esos estudios son limitados, las poblaciones son a menudo «de conveniencia» o tienen otras peculiaridades, el número de individuos incluidos es bajo, y apenas se incluyen subgrupos que merecen especial atención (embarazadas, niños, ancianos). Por todo ello, las concentraciones observadas son sólo orientativas. Con estas salvedades, se puede aventurar que las concentraciones de DDT y DDE podrían encontrarse en el rango medio-alto de los valores observados en otros

países europeos^{8,10,16}. En cuanto a los valores tisulares de otros compuestos, como los PCB, los valores observados por algunos estudios parecen situarnos en el rango medio, mientras que las concentraciones de hexaclorobenceno observadas sugieren que los españoles podemos tener valores superiores al promedio de la Unión Europea.

En España sólo se ha publicado un estudio que haya medido las concentraciones de CTP en una población humana de forma longitudinal; es decir, que haya efectuado mediciones de CTP con la misma metodología en la misma población y en más de un momento. Se trata de un trabajo efectuado en Mataró (en el norte de Barcelona), a raíz de la apertura de una planta incineradora^{28,29}. En la figura 1 se presentan las concentraciones sanguíneas de dioxinas en los habitantes de la zona, según la distancia entre el lugar de residencia y la incineradora, en cada uno de los tres cortes temporales. Por una parte, se observa que quienes residen cerca de la incineradora no presentan concentraciones mayores que los que residen más lejos de aquélla; los valores son incluso algo inferiores a los de una población control no expuesta a las emisiones de la planta, el pueblo de Arenys de Mar (fig. 1; última barra a la derecha; año 1999). Pero el hallazgo más relevante, en el contexto que nos ocupa, es que durante los 4 años las concentraciones de dioxinas y furanos aumentaron: aproximada-

Tabla 3. Principales características y resultados de diversos estudios españoles sobre valores de DDT y DDE en humanos

| Autor (año publicación) | Cita | Año ^a | Lugar | Población | Muestra |
|------------------------------|------|------------------|-------------------------------|---|---|
| Pérez de Ciriza et al (1988) | 17 | Años ochenta | Navarra | Agricultores | Suero |
| Pérez de Ciriza et al (1988) | 18 | Años ochenta | Navarra | Madres que habían alumbrado 4-5 días antes | Leche materna |
| Camps et al (1989) | 7 | 1985-1987 | Lleida | Autopsias | Tejido adiposo abdominal |
| Martí Lloret et al (1988) | 19 | 1987 | Alicante | Autopsias y pacientes intervenidos quirúrgicamente | Tejido adiposo abdominal |
| Gómez-Catalán et al (1993) | 20 | 1985-1987 | Lleida | Autopsias | Tejido adiposo abdominal |
| Ídem | 20 | 1986-1988 | Olot-Garrotxa | Pacientes intervenidos quirúrgicamente | Tejido adiposo abdominal |
| Ídem | 20 | 1987-1988 | Barcelona | Autopsias | Tejido adiposo abdominal |
| Ídem | 20 | 1987-1988 | Tarragona | Pacientes intervenidos quirúrgicamente | Tejido adiposo abdominal |
| Barrotx et al (1995) | 22 | 1989-1994 | Lleida | Autopsias, excluyendo muertos por intoxicación | Tejido adiposo abdominal |
| Hernández et al (1993) | 8 | 1991 | Madrid | Madres de área urbana de Madrid | Leche materna |
| Gómez-Catalán et al (1995) | 21 | 1991 | Navarra | Pacientes intervenidos quirúrgicamente | Tejido adiposo abdominal |
| Martínez et al (1993) | 23 | 1992 | Huelva | Madres y sus hijos recién nacidos | Sangre materna y de cordón umbilical y leche materna |
| Porta et al (1999, 2000) | 24,3 | 1992-1995 | Barcelona, Mallorca, Elche | Pacientes con cáncer de páncreas | Suero |
| Ídem | 24 | 1995 | Barcelona | Controles hospitalarios | Suero |
| Sala et al (1999) | 14 | 1994 | Flix | Muestra aleatoria de la población general | Suero |
| Van't Veer et al (1997) | 25 | 1996 | Málaga | Mujeres posmenopáusicas con cáncer de mama | Grasa subcutánea |
| Ídem | 25 | 1996 | Málaga | Controles del estudio de casos y controles | Grasa subcutánea |
| Sala et al (2001) | 26 | 1997-1999 | Flix y cercanías | Madres y sus hijos recién nacidos | Sangre materna y de cordón umbilical |

Tabla 3, continuación.

| Autor (año publicación) | Cita | Método analítico | Número de varones | Número de mujeres | Edad Media (DT) | Concentración DDT Media (DT) | Unidades ^c | Concentración DDE Media (DT) |
|------------------------------|------|------------------|-------------------|-------------------|-----------------|------------------------------|-----------------------|------------------------------|
| Pérez de Ciriza et al (1988) | 17 | CG-DCE | 147 | 0 | 49,6 (10,9) | 5,46 (2,50) | ng/ml | 15,17 (12,20) |
| Pérez de Ciriza et al (1988) | 18 | CG-EM | NA | 45 | 26,7 (5,5) | 0,28 (0,36) | µg/g | 1,47 (0,96) |
| Camps et al (1989) | 7 | CG-DCE | 71 | 16 | ND | 1,50 (0,89) | µg/g | 6,27 (5,67) |
| Martí Lloret et al (1988) | 19 | CG | 5 | 8 | 45 | 6,20 (9,18) | µg/g | |
| Gómez-Catalán et al (1993) | 20 | CG-DCE | 41 | 12 | 51 (22) | 1,40 (0,85) | µg/g | 6,84 (6,13) |
| Ídem | 20 | CG-DCE | 23 | 27 | 54 (22) | 0,69 (0,35) | µg/g | 3,73 (2,37) |
| Ídem | 20 | CG-DCE | 41 | 27 | 57 (22) | 1,35 (0,80) | µg/g | 6,98 (6,85) |
| Ídem | 20 | CG-DCE | 35 | 50 | 55 (18) | 1,20 (0,84) | µg/g | 6,03 (5,32) |
| Barrotx et al (1995) | 22 | CG-DCE | 51 | 26 | ND ND | 0,56 (0,56) | µg/g | 5,11 (4,44) |
| Hernández et al (1993) | 8 | CG-DCE | 0 | 51 | 29 ND | 0,01 ND | µg/g | 0,60 ND |
| Gómez-Catalán et al (1995) | 21 | CG-DCE | ND | ND | ND ND | 0,40 (0,43) | µg/g | 3,93 (4) |
| Martínez et al (1993) | 23 | CG-EM | NA | 50 | ND ND | 1,62 ^b ND | µg/g | ND ND |
| Porta et al (2001) | 24,3 | CG-DCE | 28 | 23 | 66 (12) | 0,21 (0,25) | µg/g | 2,73 (2,79) |
| Ídem | 24 | CG-DCE | 12 | 14 | 73 (10) | 0,08 (0,11) | µg/g | 1,40 (1,06) |
| Sala et al (1999) | 14 | CG-DCE | 249 | 359 | 50,2 (47,9) | 0,94 ND | ng/ml | 9,61 ND |
| Van't Veer et al (1997) | 25 | CG-DCE | NA | 56 | 62 ND | ND ND | µg/g | 2,56 ND |
| Ídem | 25 | CG-DCE | NA | 64 | 62 ND | ND ND | µg/g | 3,13 ND |
| Sala et al (2001) | 26 | CG-DCE | NA | 72 | 30,5 (4,7) | 0,27 (0,52) | ng/ml | 3,20 (3,46) |

^aEstimación del año de recogida de las muestras.

^bSuma de análogos del DDT.

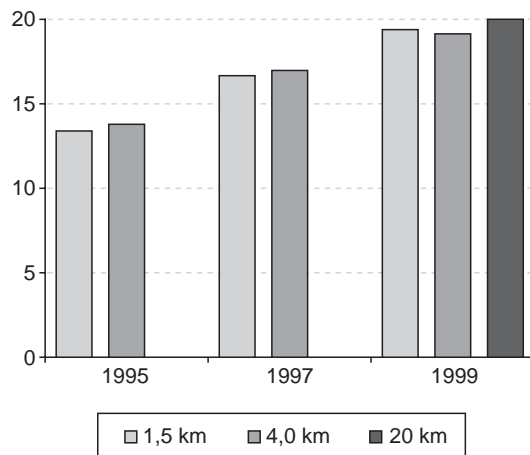
^cng/ml equivale a partes por billón (ppb) (billón: en la acepción anglosajona, es decir, mil millones). µg/g equivale a partes por millón (ppm).

CG: cromatografía de gases. CG-DCE: cromatografía de gases con detección por captura de electrones. CG-EM: cromatografía de gases con espectrometría de masas; DT: desviación típica. ND: no disponible; NA: no aplicable.

mente un 45% en la población de Mataró que vive lejos de la planta y un 40% en el grupo que reside cerca de ella. La explicación más probable es la contami-

nación alimentaria²⁸. Además, el valor de dioxinas en las poblaciones de Mataró y Arenys es aproximadamente un 25% superior al observado en otros países desarrollados²⁸.

Figura 1. Concentraciones sanguíneas de dioxinas (media en I-TEQ) según distancia entre la residencia de las personas y la incineradora. Mataró (Barcelona) 1995, 1997 y 1999²⁹. Figura reproducida con permiso de *Epidemiology*. I-TEQ: *international toxic equivalent* (equivalente tóxico internacional). Incluye a las policloro-dibenzo-para-dioxinas (PCDD) y a los policloro-dibenzo-furanos (PCDF); no incluye a los PCB parecidos a las dioxinas (*dioxin-like PCB*).



Informe sobre la Exposición Humana a Agentes Químicos Ambientales

Desde las perspectivas de la salud pública y la ecología –y también desde otras– es preocupante que en España no exista un estudio representativo de alguna zona geográfica amplia que haya analizado los factores que condicionan los valores de CTP en el organismo de las personas. De hecho, el déficit de indicadores poblacionales sobre el impacto que los procesos ambientales tienen en la salud humana es sólo una consecuencia más de las múltiples deficiencias que en España presentan las políticas ambientales³⁰⁻³². Es cierto que éste no es el único país europeo con dichas carencias; por ejemplo, la Comisión Europea considera que los valores actuales de contaminación por dioxinas, furanos y PCB de los alimentos y piensos son «inaceptables». Por ésta y otras razones bien conocidas, la UE promueve cambios sustanciales en las políticas públicas; entre ellos, normativas para que todos los ámbitos de las Administraciones desarrollen sistemas más

eficaces para monitorizar y reducir los valores biológicos de los CTP y para evaluar los riesgos de efectos adversos para la salud^{9,33-36}.

Es lógico que, al no disponer todavía de sistemas de información ambiental desarrollados, desconozcamos cuáles son las concentraciones corporales de CTP en los ciudadanos, según comunidades autónomas, grupos de edad y género, hábitos alimentarios, ocupación, educación o clase social. Un componente importante de dichos sistemas consistiría en un Informe sobre la Exposición Humana a Agentes Químicos Ambientales en la población general española. ¿Cuáles serían sus principales usos en salud pública? En la tabla 4 se sintetizan³⁶.

Es preciso desarrollar este proyecto de Informe con una visión salubrista y ambiental integradora, que permita estimar las vías de exposición y que periódicamente incorpore datos sobre otros agentes químicos ambientales que también comportan riesgos: compuestos organofosforados y organobromados, ftalatos, metales (plomo, mercurio, cromo, cadmio), disolventes como el benceno o humo ambiental del tabaco. Esta información podría entonces relacionarse con otros indicadores de salud ya disponibles (p. ej., los obtenidos mediante las encuestas de salud y otros). En algunos

países existen referentes de estudios de esta índole, bien como parte de encuestas de salud, bien estudios *ad hoc* sobre contaminantes específicos^{33,36-40}.

El Informe sería una de las actuaciones en las que plasmar la ratificación del Convenio de Estocolmo, como veremos a continuación. Pero antes es necesario resumir algunas ideas acerca de los efectos de los CTP en las personas, pues ellos son una razón fundamental para abordar estas cuestiones.

Efectos sutiles y a largo plazo

Tras constatar la escasez de estudios sobre las concentraciones de CTP en los alimentos y la aún mayor carencia de estudios en las personas, el tercer peldaño que bajamos es el de los efectos que los CTP tienen en las personas: la escasez de estudios es aquí perfectamente descriptible^{1,3,15,24,25,41-43} (la de estudios lamentablemente, no la de efectos). Nos referimos a trabajos que hayan medido la impregnación corporal por CTP y la hayan relacionado con variables fisiológicas, clínicas y sociales en algún grupo humano del Estado español.

Sin embargo, las investigaciones efectuadas en otros países indican que dosis de algunos contaminantes por debajo de las que actualmente se suelen considerar «seguras» (partes por billón) pueden causar efectos biológicos y clínicos relevantes^{1,4,44,45}, (como se puede observar en la tabla 2, muchos CTP se encuentran en el organismo humano a concentraciones de partes por billón, mientras que otros, como las dioxinas, es más frecuente que se hallen a concentraciones de partes por trillón; por otra parte, su toxicidad es superior¹¹). Algunos de tales efectos se producen porque algunos CTP son disruptores endocrinos^{1-3,6,46}. Pero los mecanismos mediante los cuales actúan son más amplios^{2,4,24,40}, p. ej., algunos compuestos pueden alterar los procesos de maduración inmunológica y neuro-conductual durante el desarrollo fetal y la infancia mediante mecanismos de otra índole^{26,41-47}. Asimismo, algunos PCB pueden modular la expresión de oncogenes, como los genes de la familia *ras*. Algunas mezclas de PCB pueden aumentar los valores de ARNm de los genes *raf* y *erb* en células hepáticas humanas; es decir, pueden actuar como «promotores tumorales» mediante la activación de vías de proliferación celular^{48,49}. Asimismo, durante la oxidación de los PCB menos clorados se producen radicales libres y daño oxidativo al ADN^{50,51}.

Hansen⁴ señala que los PCB son tóxicos de amplio espectro y que suelen formar parte de mezclas. Por ende, la valoración de sus riesgos encuentra abundantes dificultades. Hasta ahora, las investigaciones se han centrado en los PCB planares, es decir, en los congéneres más agonistas del receptor intracelular Ah, que ac-

Tabla 4. Informe sobre la Exposición Humana a Agentes Químicos Ambientales. Propósito y usos^a

Propósito general

Proporcionar a los agentes sociales, a las autoridades (sanitarias, laborales, ambientales, económicas) y a los expertos información válida sobre las dosis internas de agentes químicos ambientales (AQA) –como los CTP– en una muestra representativa de la población general, con el propósito de ayudar a prevenir trastornos de salud provocados por la exposición a tales agentes

Usos concretos del Informe

1. Conocer las concentraciones corporales de determinados AQA (CTP y otros)
2. Analizar específicamente dichas concentraciones en subgrupos de la población general, como las niñas y niños, las personas de las clases sociales inferiores, los ancianos, las mujeres en edad fértil o determinados colectivos de trabajadores
3. Establecer las actuales vías de entrada en el organismo de dichos agentes, identificando en particular los productos alimentarios responsables
4. Valorar la efectividad de los programas (seguridad alimentaria, plaguicidas, instalaciones industriales) para reducir la exposición de los ciudadanos a AQA concretos
5. Establecer valores de referencia, que indiquen si una persona tiene una concentración inaceptablemente alta de un AQA en su organismo
6. Hacer un seguimiento a lo largo del tiempo de la exposición de la población a AQA
7. Establecer prioridades de investigación sobre los efectos que los AQA tienen en la salud humana y sobre la gestión de estos riesgos

^aModificado de: National Center for Environmental Health, National Report on Human Exposure to Environmental Chemicals³⁶.

túan de forma similar a las dioxinas pero no son los más ubicuos; ello ha retrasado el análisis de los efectos de los PCB no planares, más prevalentes en el ambiente y en los organismos vivos, lo que crea la impresión de que éstos conllevan pocos riesgos⁴.

La investigación sobre éstos y otros mecanismos biológicos «indirectos» (p. ej., epigenéticos) es muy dinámica; justifica el desarrollo de nuevos criterios de clasificación de los CTP, más acordes con su multiplicidad de efectos sobre la salud humana⁴; en la UE están cambiando sustancialmente las valoraciones de riesgos (p. ej., neurotóxicos, conductuales o carcinogénicos), y en algunos países favorece la evolución de disciplinas como la química ambiental, la toxicología genética, la ecotoxicología o la epidemiología molecular (existen incluso experiencias de integración de estas disciplinas en la práctica de la salud pública).

Un problema de salud pública multidimensional y «glocal»

En los próximos años seguiremos asistiendo a la emergencia de fascinantes hallazgos (moleculares, fisiológicos, clínicos, epidemiológicos o ambientales) sobre los efectos de los CTP. Cabe esperar que se genere, asimismo, nuevo conocimiento y nueva praxis sobre las dimensiones culturales, éticas, socioeconómicas y políticas, pues también están en constante mutación los conocimientos y las actitudes en relación con las causas estructurales de la contaminación por CTP. Estos cambios tienen impactos relevantes en cuestiones tan diversas como los procesos de construcción social de los riesgos alimentarios, ambientales o laborales; la normativa de la UE sobre contaminación de piensos y alimentos; el control de los usos ilícitos de los CTP⁵²; el tratamiento de los residuos y la prevención de los vertidos ilegales³⁰⁻³²; o la búsqueda de organizaciones capaces de gobernar problemáticas que son genuinamente globales y locales («glocales»⁵³) a la vez.

Valorar mejor la significación social de los conocimientos sobre el espectro de efectos más sutiles y con períodos de latencia más largos de los CTP es uno de los grandes retos actuales^{3,11,54,55}. Lo es también —naturalmente y de forma singular— para los especialistas en salud pública. Existen diversas razones para ello: a) el vasto número de personas expuestas; b) la carga comunitaria que pueden representar los efectos de los CTP, lo que incluye impactos importantes sobre el sistema de salud; c) las competencias que las autoridades de salud pública municipales, autonómicas, estatales y comunitarias tienen en áreas estratégicas para prevenir la exposición a los CTP, monitorizar valores e intervenir; d) la complejidad quimicobiológica de las ex-

posiciones, sus mecanismos de acción y sus efectos funcionales, en cuya valoración debemos participar, y e) el arraigo estructural que las fuentes y las vías de exposición tienen en nuestro sistema económico y ecológico (que al cabo son uno solo), y en sus subsistemas agrícola, industrial o alimentario⁵⁶.

El Convenio de Estocolmo: ¿se puede gobernar globalmente la salud pública?

Los CTP son más que un símbolo de una cierta «globalización»⁵⁶: son contaminantes estrictamente planetarios. Y no sólo en la dimensión biofísica: globales o planetarios son los procesos causales distales que mejor explican sus efectos en todas las partes de la Tierra. ¿Se puede hacer algo «glocalmente» útil?

Aunque existen múltiples experiencias —como las que durante décadas ha desarrollado la Organización Mundial de la Salud (OMS)—, la gobernabilidad democrática global de la salud pública es difícil de poner en práctica. Algo similar ocurre con las políticas ambientales, y ahí están propuestas como el Código Internacional de Conducta para la Distribución y Uso de Pesticidas (1985), las Directrices de Londres para el Intercambio de Información en el Comercio Internacional de Productos Químicos (1987) o el capítulo 19 de la «Agenda 21» (1992)⁵⁷.

Nos encontramos, no obstante, ante una nueva oportunidad histórica: la resultante del proceso de negociación, acuerdo y ratificación del Tratado de Estocolmo, o Convenio de los COP^{6,57-61}. Auspiciado por el Programa Ambiental de las Naciones Unidas, este acuerdo internacional persigue acabar con el uso de varios COP y reducir el de otros, en total, 12 (tabla 5). La redacción final del tratado se fraguó durante la Convención para el control de los COP celebrada en Johannesburgo en diciembre de 2000^{52,60,61}. El acuerdo fue posteriormente suscrito por un centenar de países —entre ellos, España— durante la reunión celebrada al efecto en Estocolmo en mayo de 2001. Los países firmantes deberán ratificarlo en los próximos meses, aunque se cree que transcurrirán 3 o 4 años antes de que 50 países lo hayan hecho, tras lo cual el tratado entrará en vigor. (En el momento de revisar este artículo lo habían ratificado 6 países.) Entonces se celebrará otra Conferencia de las Partes, que deberá concretar numerosas cuestiones de enorme importancia práctica.

En noviembre de 2001, a iniciativa del Instituto Sindical de Trabajo, Ambiente y Salud (ISTAS), la posible aplicación en España del Convenio concitó —por vez primera en España— a un nutrido grupo de ambientalistas, sindicalistas, salubristas, representantes empresariales y autoridades políticas⁵⁸. Es significativo que

Tabla 5. Principales características del Convenio de Estocolmo sobre Contaminantes Orgánicos Persistentes (COP)⁵⁷⁻⁵⁹

El Convenio contiene tres anexos con los listados de los 12 COP.

Anexo A: establece la eliminación de la producción y el uso de aldrin, bifenilos policlorados (PCB), clordano, dieldrina, endrina, heptacloro, hexaclorobenceno, mirex y toxafeno. Anexo B: establece la restricción de la producción y el uso del DDT (con las excepciones de uso contra vectores de enfermedad y para la fabricación de dicofol). Anexo C: establece la reducción de la emisión de subproductos no intencionados como las dioxinas y los furanos

Cada parte elaborará un plan para el cumplimiento del Convenio y lo transmitirá a la Conferencia de las Partes dos años después de la entrada en vigor del Convenio, y actualizará este plan periódicamente (artículo 7)

Las partes identificarán las existencias, productos, artículos en uso y residuos que contengan o estén contaminados por COP y gestionarán esas existencias de forma que se destruyan totalmente o se transformen hasta que pierdan las características de un COP (artículo 6)

Cada país adoptará medidas para prevenir la producción y utilización de nuevos productos químicos que posean las características de los COP (artículo 3)

Se establece un procedimiento para la inclusión en el Convenio, en el futuro, de otros COP (artículo F y anexos D, E y F). Cualquier país podrá presentar una propuesta de inclusión de un producto químico que cumpla los requisitos de persistencia, bioacumulación, potencial de transporte a larga distancia en el ambiente y efectos adversos especificados en el Convenio (artículo 8)

Se alude a la definición del Principio de Precaución de la Declaración de Río de Janeiro. Se enuncia en el preámbulo y en el objetivo general (artículo 1), y se menciona en el cuerpo principal del texto

Cada país designará un centro nacional de coordinación para el intercambio de información acerca de la reducción o eliminación de la producción y alternativas a la utilización de los COP (artículo 9)

Se promoverán campañas de sensibilización, se facilitará la participación pública y se promoverá la capacitación de los trabajadores y el personal científico, técnico y directivo. Cada país podrá difundir información acerca de las cantidades anuales de COP que se liberan o eliminan (artículo 10)

Las partes desarrollarán programas de investigación y vigilancia de los COP y sus alternativas (artículo 11)

Las partes prestarán asistencia técnica y recursos a los países en desarrollo para la aplicación del Convenio (artículos 12 y 13). Hasta que se establezca el conjunto de mecanismos financieros, el cumplimiento del Convenio se apoyará en el Fondo Global para el Ambiente (GEF) (artículo 14)

Dos de las debilidades del Convenio son: que no se establecieron límites cuantitativos para la reducción de los subproductos no intencionados; y que, pese a las intenciones de la UE de ampliar el alcance del Convenio, finalmente éste sólo incluyó las 12 sustancias propuestas desde un principio⁵⁷

contaminantes. Todo ello favorecerá la consolidación de las estrategias más eficientes que ya funcionan, y la puesta en marcha de nuevas políticas; para vigilar y controlar, por ejemplo, la contaminación industrial por PCB de los alimentos, el agua, el aire y los suelos; sobre las condiciones de trabajo, para proteger a los trabajadores expuestos; para controlar el uso de plaguicidas en la industria agrícola, o sistemas eficaces de inspección de los residuos organoclorados en los alimentos^{9,30-34,58}. Al mismo tiempo, será esencial apoyar con mayor decisión la investigación que más ayude a comprender y controlar las consecuencias económicas, laborales, culturales, ambientales y sanitarias que tienen los residuos de CTP y otros agentes químicos ambientales. Además de proporcionar beneficios sociales directos, estas estrategias servirán —a quienes las apliquen— para demostrar a la ciudadanía que es posible actuar a un mismo tiempo global y localmente, a corto y largo plazo. Hay que tener cuenta que, por ejemplo, el Convenio de Estocolmo contempla el progresivo abandono de los PCB para 2020-2030, mientras que la Directiva sobre PCB trabaja con el horizonte de 2010 (tabla 2). Puesto que a menudo el tiempo de vida media de estos compuestos es de décadas, si se cumplen las normas legales y no cambian otros determinantes, en los próximos 10-30 años la impregnación corporal por muchos CTP sólo habrá descendido a la mitad, a un tercio o quizá a una cuarta parte de las concentraciones actuales. Obviamente, los principales beneficios de lo que ahora hagamos sólo podrán ser percibidos por nuestros descendientes. Esta escala temporal plantea incógnitas socioculturales y técnicas inéditas.

En cualquier población, la información sobre las concentraciones de los CTP es un puzzle técnicamente complejo y cambiante en el tiempo. Pero las piezas del rompecabezas disponibles en España son exiguas. Si nuestras prioridades reales son la salud pública y el equilibrio ambiental, esta situación debe cambiar. El Convenio de Estocolmo es una nueva oportunidad social, política y científica para lograrlo.

Agradecimientos

Los autores agradecen sinceramente las aportaciones de Estefanía Blount, Joan Grimalt, Esther Marco, Mary Woolf, Kirsten Moysich, David J. MacFarlane, Àlex Amorós, Olga Juan y Puri Barbas. Las ideas y propuestas contenidas en este artículo fueron discutidas en diversos encuentros: IV Conferencia sobre Disruptores Endocrinos y IX Jornada Técnica de la Sociedad Española de Sanidad Ambiental (Ministerio de Sanidad y Consumo, Madrid, 3 abril 2001); Inauguración de los Cursos de verano de la Universidad de Alicante (Cocentaina, 9 julio 2001); I Reunión Nacional sobre Dioxinas, Furanos y Compuestos Orgánicos Persistentes Relacionados

dicha organización sindical articulase y auspiciase el debate, pues *a priori* cabría esperar que lo hubiesen hecho otras organizaciones profesionales, científicas o gubernamentales.

En consonancia con las previsiones ya apuntadas, creemos que en los próximos años en España seguirá aumentando la concienciación social sobre los riesgos que suponen los CTP. También es previsible que los gobiernos (europeo, central, autonómicos y municipales) dinamicen sus programas de salud pública, medio ambiente o seguridad alimentaria y apliquen con mayor vigor la existente y la emergente legislación sobre

(CSIC, Madrid, 18 octubre 2001); y Congreso sobre Implementación del Convenio sobre COPs (Instituto de Salud, Trabajo y Ambiente-CC.OO., Madrid, 27 noviembre 2001). Nin-

guna idea madura fuera del contexto social. Sin estos foros, las ideas aquí plasmadas tampoco habrían alcanzado su forma actual.

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