POLYCHLORINATED BIPHENYLS BUT NOT CHLORINATED PESTICIDES ARE ASSOCIATED WITH EXTERNALIZING BEHAVIOUR IN ADOLESCENTS.

Sly PD[1]*, Chen L[1], Gangell CL[1], Scott J[1,2], Zubrick SR[3], Whitehouse AJO[3], Carpenter DO[4]

[1]Children's Health and Environment Program, Queensland Children's Medical Research Institute, The University of Queensland, Herston Rd Herston Qld, Australia; [2]Royal Brisbane and Women's Hospital, Bowen Bridge Rd, Herston, Qld, Australia; [3]Telethon Institute for Child Health Research, Roberts Rd, Subiaco, WA, Australia; [4]Institute for Health and Environment, University at Albany, Rensselaer, NY, USA.

Introduction

Mental disorders, including neurobehavioural disorders and attention deficit hyperactivity disorder, account for the largest proportion of the burden of disease for Australian children[1], yet the causes are largely unknown. While estimates vary, environmental exposures are considered to contribute to the likelihood of neurobehavioural disorders. Neurotoxicants, such as lead and polychlorinated biphenyls (PCBs), are associated with decreased IQ and neurobehavioral problems[2]; however, little systematic study has occurred on the contribution of such toxicants to behavior disturbances in children. We have taken advantage of a community-based longitudinal birth cohort study to examine the impact of PCBs and chlorinated pesticides on behavior of adolescents in Australia.

Materials and methods

The study started as a pregnancy cohort in which 2,979 women were enrolled at or before the 18th week of gestation from the antenatal booking clinics at King Edward Memorial Hospital (KEMH), the principal obstetric hospital in Perth, Western Australia. The criteria for enrolment were gestational age between 16 and 20 weeks, sufficient proficiency in English to understand the implications of participation, an expectation to deliver at the hospital, and an intention to remain in Western Australia so that follow-up through childhood would be possible. As KEMH is the only specialist obstetric care centre in Western Australia, the initial sample was at moderate obstetric risk, and the participating mothers reflect the population obtaining obstetrical care in this region. Thus this is a community-based cohort representing about 20% of all live births in Western Australia over a three year period, with exclusions only for very low birth weight or birth defects. The population was 84% Caucasian, 4% Aboriginal and 12% other (primarily Asian); reflective of the population in Western Australia.

Comprehensive data on social and demographic factors, medical and obstetric history and exposure to potential environmental and recreational toxins (alcohol, illicit drugs, medications and smoking) and allergens (pets, dust, chemicals, foods) were obtained from each parent at enrollment and, in the mother's case, updated during the 34th week of pregnancy. The initial aim of the Raine study was to test the hypothesis that intensive use of ultrasound examination and Doppler flow studies would improve pregnancy outcomes, expressed as days of neonate stay and preterm births[3]. Thus, participants were not selected on any variable that could impact on behavioural outcomes in their offspring. Subsequently, the cohort has undergone longitudinal follow-up with the general aim of determining how events during pregnancy, around birth, and in childhood influence subsequent health and development. The study continues to follow 2,868 Western Australian children, the oldest of whom is now aged 23 years. Subsequent follow-up of the children at 1, 2, 3, 6, 8, 14, 16, and 21 years took place at the Telethon Institute for Child Health Research, and assessment at 23 years will begin shortly. There is extensive survey and assessment data (socio-demographic, psychosocial and developmental) for these children and their families post-conception to 14 years of age available at present.

In this preliminary analysis we have measured serum levels of 101 PCB congeners and 3 chlorinated pesticides [hexachlorobenzene (HCB), dichlorodiphenyldichloroethylene (DDE), the major metabolite of DDT, and Mirex] in 240 children (123 male) participating in the 14 year follow-up and compared them to data obtained from the Achenbach Child Behaviour Checklist (CBCL)[4], specifically the domains assessing internalizing and externalizing behaviours. PCBs were grouped according to their structure and known activities[5]. Three kinds of internalizing behaviour are measured by the CBCL; somatic complaints, anxious/depressed behaviour and

withdrawn behaviour. Externalizing behaviour measured by the CBCL includes aggressive behaviours such as bragging, arguing, screaming, showing off, attention-seeking, teasing, being demanding, threatening and displaying a temper.

Results and discussion

Levels of PCBs and pesticides were similar to those reported in American adolescents. Group mean (sd) levels are shown for the different PCB congener groups and pesticides related to total CBCL score (Table 1), internalizing behaviour (Table 2) and externalizing behaviour (Table 3). All CBCL scores, including internalizing and externalizing behaviour scores are presented as standardised T scores. The scores have been treated as a binary variable using a recommended clinical cut point, with T scores ≥60 signifying a significant clinical problem.

Table 1: Association of PCB congeners and pesticides to total CBCL scores

Toxicant	**CBCL total score**		
	<60 (n=209)	≥60 (n=29)	p
Total PCB ppt/ww	736.2 (589.1)	1165.9 (1615.6)	0.012
Dioxin-like TEQ ($x10^{-5}$) ppt/ww	9.92 (25.9)	1.46 (25.4)	0.36
Di-ortho PCB ppt/ww	388.8 (329.1)	503.6 (743.4)	0.15
Tri+tetra-ortho PCB ppt/ww	129.4 (224.5)	249.4 (319.4)	0.012
Estrogenic PCB ppt/ww	169.3 (231.7)	189.3 (380.7)	0.69
Mono-ortho PCB ppt/ww	98.5 (141.2)	156.9 284.5)	0.075
HCB	30.4 (25.9)	31.6 (17.7)	0.81
DDE	532.5 (518.7)	455.1 (353.8)	0.44
Mirex	9.93 (17.5)	16.7 (34.2)	0.093

Table 2: Association of PCB congeners and pesticides to CBCL internalizing scores

Toxicant	**Internalizing behaviour**		
	<60 (n=219)	≥60 (n=19)	p
Total PCB ppt/ww	779.9 (885.8)	888.4 (682.8)	0.60
Dioxin-like TEQ ($x10^{-5}$) ppt/ww	10.1 (25.9)	15.0 (25.4)	0.43
Di-ortho PCB ppt/ww	403.3 (410.6)	396.6 (297.0)	0.94
Tri+tetra-ortho PCB ppt/ww	134.8 (229.6)	251.3 (331.8)	0.042
Estrogenic PCB ppt/ww	176.5(263.1)	116.7(66.3)	0.33
Mono-ortho PCB ppt/ww	105.1 (169.6)	110.8 (112.3)	0.89
HCB	30.5 (25.5)	30.6 (19.0)	0.99
DDE	531.2 (513.3)	429.8(334.2)	0.40
Mirex	10.3 (17.8)	15.8 (39.2)	0.26

Table 3: Association of PCB congeners and pesticides to CBCL externalizing scores

Toxicant	**Externalizing behaviour**		
	<60 (n=198)	≥60 (n=40)	p
Total PCB ppt/ww	729.9 (547.4)	1079.1 (1728.7)	0.02
Dioxin-like TEQ ($x10^{-5}$) ppt/ww	10.2 (27.0)	11.9 (19.7)	0.70
Di-ortho PCB ppt/ww	377.2 (293.8)	529.6 (726.2)	0.028
Tri+tetra-ortho PCB ppt/ww	137.6 (241.7)	176.0 (234.7)	0.36
Estrogenic PCB ppt/ww	157.7 (214.3)	241.2 (390.7)	0.057
Mono-ortho PCB ppt/ww	93.8 (107.1)	164.2 (323.2)	0.014
HCB	30.2 (26.2)	32.0 (18.3)	0.69
DDE	521.8 (450.5)	529.4 (709.6)	0.93
Mirex	10.9 (20.8)	10.3 (17.6)	0.86

These data demonstrate that the mean serum levels of total PCBs and of several congener groups were significantly higher in those adolescents who are in the clinical range with emotional and behavioural problems as measured by the CBCL. In particular, those adolescents with elevated scores on the externalizing behaviour domain more commonly had higher levels of PCBs. The associations appear with groups of PCBs having *ortho*-substituted chlorines, and not with dioxin TEQs. No associations were seen between levels of the 3 chlorinated pesticides and emotional or behavioural problems as measured by the CBCL.

This is a pilot study that is underpowered to fully examine associations between toxicant levels and behaviour in these adolescents. In addition, while behavioural problems are more common in adolescent boys, we have not stratified our results by sex due to the small numbers with abnormal scores. We have serum samples from approximately 1000 more children from this cohort that have not yet been analysed. Future analyses with larger numbers will provide more detailed information on the impact of these chlorinated organic chemicals on neurobehavioural development during childhood. However the preliminary data are consistent with the hypothesis that exposure to PCBs containing *ortho* chlorines increases risk of anti-social behaviours.

Acknowledgements:
We thank Gretchen Welfinger-Smith who performed all PCB and pesticide analyses. The authors would like to acknowledge the National Health and Medical Research Council (NHMRC) and the Telethon Institute for Child Health Research (TICHR) for their long term contribution to funding the study over the last 20 years. Core Management of the Raine study is funded by the University of Western Australia (UWA), Curtin University, the UWA Faculty of Medicine, Dentistry and Health Sciences, the Raine Medical Research Foundation, TICHR and the Women's and Infants Research Foundation. The authors are extremely grateful to all of the families who took part in this study and the whole Raine Study team, which includes the Cohort Manager, Data Manager and data collection team.

References:
[1]Begg S, Vos T, Barker B, Stevenson C, Stanley L, Lopez AD (2007). The Burden of Disease in Australia 2003. Canberra: Australian Institute of Health and Welfare; 2007. Report No: PHE82.
[2]May M (2000). Disturbing behavior. Environ Health Perspect 108: A262-A267.
[3]Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI (1993). Effects of frequent ultrasound during pregnancy: a randomised controlled trial. Lancet 342: 887-891.
[4]Achenbach, Thomas M., and Leslie A. Rescorla. (2001). *Manual for the ASEBA School-Age Forms & Profiles: Child Behavior Checklist for Ages 6-18, Teacher's Report Form, Youth Self-Report, An Integrated System of Multi-informant Assessment*. Burlington: University of Vermont, Research Center for Children, Youth, and Families.
[5] Goncharov A, Haase RF, Santiago-Rivera A, Morse G, Akwesasne Task Force on the Environment, McCaffrey RJ, Rej R and Carpenter DO (2008) High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a Native American population. Environ Res 105: 226-239.