The Developmental Neurotoxicity of PCBs: An Interim Report of Ongoing Neuroepodemiological and Experimental Studies

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Purpose of the studies:
Polychlorinated biphenyls (PCBs), as is true for all polyhalogenated and polybrominated compounds, represent complex mixtures of stable contaminants which, due to their biopersistence, are still present in environmental media at slowly decreasing concentrations. Their main route of intake is nutrition. A potential for developmental neurotoxicity at low levels of exposure has been demonstrated both in human infants and experimental animals [1]). Supported by research grants from the European Union our studies aim at reconsidering risk assessment of current background levels for neurodevelopmental deficit in infants and at contributing to a better understanding of the nervous system toxicity by means of neurobehavioural and neurochemical studies in rats.

1. The German Study on Neonatal PCB-exposure and Neurodevelopmental Deficit

The German perinatal study is part of a European collaborative effort combining Dutch (Rotterdam, Groningen) and Danish (Odense, Faroe Islands) study elements tied together by a common study protocol with inherent quality control aspects. The present report concentrates on the German study element and describes preliminary findings from an ongoing prospective study in a cohort of infants recruited in the city of Düsseldorf.

Methods:
Study group: A sample of 171 newborns was recruited between November 1993 and May 1995 from the obstetrical wards of three Düsseldorf hospitals (University clinic, Vincenz-Hospital, Marien-Hospital) after full informed consent of the mothers. Criteria of inclusion were: Only 1st and 2nd born children, absence of serious illness during pregnancy and delivery, absence of congenital anomalies, availability of a cordblood sample, German nationality and an Apgar-score (1 min) of at least 7. Independent variables: Basic variables were PCB-levels (sum of congeners 138, 153 and 180) in cord serum analysed by capillary gas-chromatography with electron-capture detection [2]) in Düsseldorf, and in spot milk samples collected at 2 and 4 weeks, respectively; these latter analyses were done in Kiel. Ringtests were organized between the analytical Dutch and German laboratories for quality assurance. Dependent variables: Between postnatal days 10 and 20 the early neurodevelopmental status of the infants was assessed, following the obstetrical and neurological optimality concept as developed in Groningen[3]), training of the examiners was provided by the Groningen group. At 7 months of age the Bayley Scales of Infant Development

(BSID) and the Fagan Visual Recognition Memory Test (FT) [4]) were performed for the assessment of the early psychomotor and cognitive development; to the extent possible interrater reliabilities were determined. At 18 months of age the BSID and the assessment of the neurodevelopmental status were repeated. In addition, although not reported here, spontaneous vocalizations of the infants were analyzed for mature and immature vocalizations. Statistical data analysis was done/will be done by means of multiple linear/logistic regression analysis taking the following confounding factors or covariates into account: Obstetrical optimality [3]) length of gestation, maternal age, socioeconomic status, the quality of the home environment (HOME [5]), German adaptation), birth weight, nursing habits, maternal IQ and lead in cordblood.

Preliminary Results:
PCB-concentrations in cordblood (sum of congeners 138, 153, 180), given as geometric means (geometric standard deviation =GSD), were 0.52 ng/g plasma (GSD = 1.44). Corresponding values in maternal milk were 383 ng/g fat (GSD = 1.57).

Age 10 - 20 days: Main results from multiple logistic or linear regression analyses for the associations between the neurological optimality score (NOS) and PCB-concentrations in cordblood and maternal milk (2 weeks samples), respectively, are given in table 1.

No significant associations were found between perinatal PCB-exposure and Neurological Optimality at age 10 - 20 days in the Düsseldorf cohort. More detailed analyses are needed to look into subscales of the global NOS, such as fluency or tonicity, which may well be differentially associated with early neonatal PCB-exposure.

Table 1: Associations between PCB (sum of 138, 153, 180) in cordblood/maternal milk and neurological optimality (60.5 - NOS) for linear regression , and logistic regression (odds ratio: NOS < 57; NOS > 57) for any doubling of exposure after adjustment for mother's age and education

	N	change	95% CL
PCB in cordblood and NOS (linear regression)	168	0.95	0.78-1.16
PCB in milk and NOS (linear regression)	128	0.90	0.74-1.09
PCB in cordblood and NOS (logistic regression)	168	1.06	0.58-1.95
PCB in milk and NOS (logistic regression	128	0.79	0.43-1.48

Age 7 months: Endpoints considered here include the Mental Development Index (MD) and the Psychomotor Development Index (PD) from the Bayley-Scales (BSID) and the "Fixation Preference Score" from the Fagan visual recognition memory test (FT). So far, only raw correlations were calculated between cordblood or milk PCB-concentrations and these cognitive endpoints. These correlation coefficients are given in table 2.

As can be seen from table 2 only few negative correlations were found between indicators of early PCB-exposure and later cognitive testing; only one of these, namely between PCB-concentrations in the 2nd breastmilk sample and the psychomotor development index (PD) of the BSID is formally significant ($p < 0.05$). Adjustment for confounding still needs to be done, however.

Table 2: Matrix of Pearson Correlation Coefficients, p-values, and no. of observations for each variable. Testing at 7 months of age

	Su Vol	BM 1	BM 2	FT II	MD 7	PD 7
Su Vol	**1** 0 169	**.080** .37 129	**.080** .47 87	**.081** .31 157	**-.014** .86 160	**.012** .88 160
BM 1	-	**1** 0 131	**.68** .0001 85	**.054** .55 125	**-.080** .37 125	**-.114** .21 125
BM 2	-	-	**1** 0 89	**.091** .41 86	**-.106** .34 85	**-.22** .05 85
FT II	-	-	-	**1** 0 158	**.115** .15 156	**.123** .13 156
MD 7	-	-	-	-	**1** 0 161	**.36** .0001 161
PD 7	-	-	-	-	-	**1** 0 161

SU Vol = Sum PCB/ng/ml (Cordblood); BM 1 = Breastmilk 1st sample (Sum PCB);
BM 2 = Breastmilk 2nd sample (Sum PCB); FT II = Fagan Test (Mean Novelty Score);
MD 7 = 7 mo BSID II (Mental Scale); PD 7 = 7 mo BSID II (Psychomotor Scale)

Conclusions:
So far, in the German perinatal study, no significant negative associations between early developmental PCB-exposure, based on the sum of congeners 138, 153 and 180, and indexes of neurodevelopment assessed at age 10 to 20 days or cognitive/motor development assessed at 7 months of age were found. This is partly at variance with published results of the Dutch breastmilk studies[6] or of earlier studies using the Fagan-test [7]):

2. Comparison of individual PCB-congeners in rats using neurobehavioural and neurochemical endpoints

Purpose of the study:
Technical PCB-mixtures have primarily been used in demonstrating the predominace of prenatal exposure for later neurobehavioral impairment [8]) It is known, however, that different PCB congeners lead to different induction of the microsomal oxidase system in the liver; coplanar congeners with chlorine substituents only in the meta- and para-possitions are ligands of the Ah-receptor and induce predominantly the gene family I (CYP1A1), whereas poly-ortho-substituted PCBs induce the gene family II [9]). Both types of PCB-congeners may also interfere differentially with nervous system functions. Orthochlorinated PCB congeners have been related to dopamine depletion both in PC-12 cells and in non-human primates [10])[11]). Since, on the other hand, metabolites of the coplanar 3,3',4,4'-TCB are known to block the binding sites of vitamin A and thyroid hormones at their plasma protein complex [12]) and since, furthermore, thyroid hormones are important regulators of neuronal development, neurobehavioral effects sof PCBs may also be mediated by this mechanism. The purpose of ongoing studies in our laboratory is to compare the effects of the ortho-chlorinated 2,2',4,4'-TCB and the coplanar 3,3',4,4'-TCB on a spectrum of

neurobehavioral functions after gestational exposure in rats and to relate observed alterations to dopaminergic and thyroid functions.

Methods:
45 time-pregnant Wistar rats were exposed by daily s.c. injections between gestational days (GD) 7 and 18 with either 2,2'4,4'-TCB or 3,3',4,4'-TCB (1 mg/kg b.w. in olive oil). In a 2nd experiment for the assessment of dopaminergic and thyroid functions 38 time pregnant Wistar rats were exposed between GD 7 and 18 to 3,3',4,4'-TCB (s.c. injections of 1 mg/kg b.w. in olive oil), or were given 0.05 mg PTU/l in drinking water (6-n-Propyl-2-thiouracil). Neurobehavioral tests conducted at different ages in the offspring of treated or control animals included the open field (activity), the radial arm maze (spatial learning; hippocampal functions), active avoidance learning, passive avoidance learning (amygdala), catalepsy following an i.p. injection of haloperidol (0.3. mg/kg b.w.), and delayed spatial alternation (prefrontal cortex). PCB concentrations in different tissues were measured by GC-MS following a column clean-up liquid chromatographic method based on a Florisil column. Dopamine and DOPAC levels as well as serotonin were measured in different brain regions, as well.

Results:
Tissue concentrations of 2,2',4,4'-TCB in treated dams at GD19 were 0.56 (brain) and 6.87 µg/g (adipose tissue), and of 3,3',4,4'-TCB 0.1 (brain) and 2.47 µg/g (adipose tissue). AT PND 21 TCB-concentrations in brain tissue remained largely unchanged, whereas they were reduced in adipose tissue to 1.76 µg/g (2,2',4,4'-TCB) and 1.82 µg/g (3,3',4,4'-TCB). Behavioral impairment due to gestational TCB-exposure was seen for haloperidol-induced catalepsy (figure 1) and passive avoidance performance) (figure 2) [13].

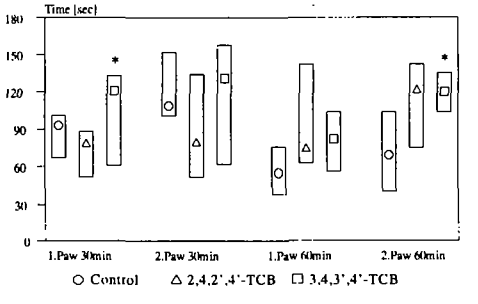

Figure 1: Catalepsy test. Time to remove the both paws from the bar is given (median and 25/75 interquartile range). *) $p < 0.05$; $n = 10$.

At PND 180 the degree of catalepsy in the offspring at PND 180 was increased by gestational exposure to 3,3',4,4'-TCB relative to controls which may be interpreted as being suggestive of dopamine-depletion in nigro-striatal structures due to the coplanar but not to the orthochlorinated congener. Also, at PND 220 in the passive avoidance task, TCB-induced impairment was more pronounced for the coplanar relative to the orthochlorinated TCB (figure 2): The latency to step down from the platform, possibly indicative of retention deficit in the exposed animals, was significantly shorter at 4 and 24 hours following the initial shock experience only in the animals having experien- ced estational exposure to 3,3',4,4'-TCB. In a 2nd experiment concentrations of neurotransmitters

were measured in the offspring following gestational exposure to either 3,3',4,4'-TCB or PTU. The most significant finding was an increase of both dopamine and of DOPAC in prefronatal cortex in the TCB-exposed animals relative to controls and PTU-treated rats at PND40.

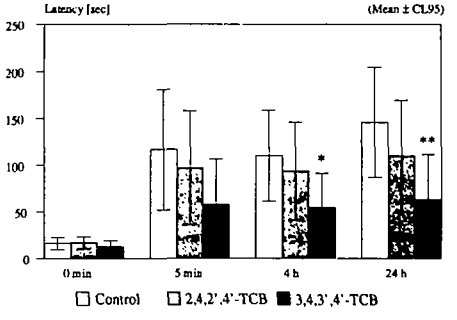

Figure 2: Performance in the passive avoidance task (step down). Latencies to descend from the platform are given (means ± CL 95). *) $p < 0.05$, $n = 10$.

Conclusions:
These observations from still ongoing experimental studies suggest that gestational exposure to 3,3',4,4'-TCB, a coplanar cogener, produces longlasting neurobehavioral impairment in terms of increased catalepsy following a haloperidol challenge, whereas the orthochlorinated congener 2,2',4,4'-TCB is less effective in this respect. It remains to be shown which neurotransmitters besides dopamine are involved in this longlasting neurotoxicity, and if and to what extent disruption of thyroid functions may also be involved in PCB-induced developmental neurotoxicity.

3. References

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