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ScienceDirect
NeuroToxicology 28 (2007) 471–477
NeuroToxicology

In utero exposure to DDT and performance on the Brazelton neonatal behavioral assessment scale

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Received 3 August 2006; accepted 21 December 2006
Available online 10 January 2007

Abstract

We investigated whether decrements in neonatal neurodevelopment, as determined by the Brazelton neonatal behavioral assessment scale (BNBAS), were associated with *in utero* exposure to dichlorodiphenyltrichloroethane (DDT): *p*,*p*′-dichlorodiphenyl trichloroethane (*p*,*p*′-DDT), *o*,*p*′-dichlorodiphenyl trichloroethane (*o*,*p*′-DDT) and *p*,*p*′-DDT's primary breakdown product *p*,*p*′-dichlorodiphenyl dichloroethylene (*p*,*p*′-DDE) (heretofore collectively referred to as DDT/DDE). Our subjects were a birth cohort of 303 infants whose mothers were low-income Latinas living in the Salinas Valley, an agricultural community in California. We assessed neonates ≤2 months old using the seven BNBAS clusters (habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and reflex) and examined performance in relationship to DDT/DDE measures in maternal serum samples collected during pregnancy. We did not find any detrimental associations between *in utero* DDT/DDE levels and neonatal performance on the BNBAS. In this same cohort, we previously demonstrated that exposures to DDT/DDE were related to decrements in neurodevelopment at 6–24 months of age. The failure to observe effects on the BNBAS in these same children may be due to limited sensitivity of a single BNBAS assessment or a delay in the manifestations of neurodevelopmental effects of DDT/DDE until after the neonatal period.

Keywords: Brazelton; DDT; DDE; Neonatal behavioral assessment scale (NBAS); Neurobehavioral outcomes; Organochlorine; Pesticides

1. Background

Dichlorodiphenyltrichloroethane (DDT) was used worldwide as an agricultural pesticide and against mosquitoes in public health campaigns beginning in the 1940s (Rogan and Chen, 2005), until concern about toxic effects on wildlife and humans, environmental persistence, and concentration in the food supply led to restrictions in the 1970s. All uses of DDT other than for public health were prohibited in the United States by 1972, while DDT was restricted somewhat later in many other countries.

For example, agricultural use of DDT in Mexico declined starting in the mid-1970s, but use for malaria control continued until 2000 (Chanon et al., 2003). The Stockholm Convention on Persistent Organic Pollutants, put into effect in 2004, intends to phase out all uses of DDT. However, under the terms of the Convention, countries are permitted to continue the use of DDT for indoor residual spraying for malaria control until there are acceptable replacements, and such use is supported by the World Health Organization

Abbreviations: 95% CI, 95% confidence interval; β-HCH, beta-hexachlorocyclohexane; BNBAS, Brazelton neonatal behavioral assessment scale; CHAMACOS, Center for Health Analysis of Mothers and Children of Salinas; DAP, dialkyl phosphates; g, gram; HCB, hexachlorobenzene; LOD, limit of detection; *n*, number (total); ng/g lipid, nanograms per gram of lipid; NHANES, National Health & Nutrition Examination Study; *o*,*p*′-DDT, *o*,*p*′-dichlorodiphenyl trichloroethane; *p*,*p*′-DDE, *p*,*p*′-dichlorodiphenyl dichloroethylene; *p*,*p*′-DDT, *p*,*p*′-dichlorodiphenyl trichloroethane; PCBs, polychlorinated biphenyls; pg/g, picograms per gram; ppm, parts per million; S.D., standard deviation; μg/L, micrograms per liter

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0161-813X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2006.12.009

(RBM/WHO, 2005). Approximately 19, countries, most in Africa, are currently using DDT and the reported number of households or units sprayed in Africa rose from around 2.7 million in 1999 to over 4 million in 2003 (RBM/WHO and UNICEF, 2005).

DDT and its primary breakdown product, dichlorodiphenyldichloroethylene (DDE), cross the placenta and are excreted in breast milk (Rogan and Chen, 2005). In animals, DDT interferes with thyroid function (Goldman, 1981) and causes neurodevelopmental toxicity (ATSDR, 2002). Two previous human studies of the relationship between DDE levels and neonatal neurodevelopment, as assessed by the Brazelton neonatal behavioral assessment scale (BNBAS), have shown inconsistent results. In a large North Carolina birth cohort, Rogan and colleagues (Rogan et al., 1986b) found that increasing DDE levels (calculated as a weighted sum of all available maternal serum, placenta and breast milk samples and expressed as parts per million (ppm) DDE in milk fat at birth) were related to hyporeflexia in neonates, but not to the six other clusters of neurodevelopmental assessment. A smaller study in Oswego, New York, found no association between cord blood DDE levels and newborn performance on the BNBAS (Stewart et al., 2000).

We recently reported that maternal serum levels of DDT, and to a lesser extent DDE, were associated with decrements in neurodevelopment in children from Mexican-American families residing in an agricultural community in California (Eskenazi et al., 2006). Specifically, we found maternal pregnancy serum levels of DDT to be associated with a decrement in mental development when the children were 12 and 24 months old (but not at 6 months) and a decrease in psychomotor development at 6 and 12 months (but not at 24 months). For DDE, we observed a decrease in psychomotor development at 6 months only. In the current investigation, we examined whether *in utero* exposure to *p*,*p*′-DDT, *o*,*p*′-DDT and *p*,*p*′-DDE (heretofore collectively referred to as DDT/DDE) was related to neonatal performance on the BNBAS administered at ≤2 months of age in the same birth cohort.

2. Materials and methods

2.1. Participants and recruitment

The keystone project of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is a longitudinal birth cohort study aimed at assessing the exposure of pregnant women and children to pesticides and other environmental agents and to determine if these exposures are associated with health consequences (Eskenazi et al., 2004). Pregnant women entering prenatal care at Natividad Medical Center or at five clinics of Clinica de Salud del Valle de Salinas were screened for eligibility between October 1999 and October 2000. Eligible women were ≥18 years old, <20 weeks gestation, English- or Spanish-speaking, Medi-Cal (subsidized health care) eligible, and planning to deliver at Natividad. We followed 528 women to delivery of a live birth. The BNBAS was performed on 419 neonates. Of these, 8 twins, 27 preterm infants, and 5 infants whose BNBAS was administered later than 62 days after delivery were excluded from the analysis. Additionally, 76 infants whose mothers had no available DDT/DDE measurements were excluded. Thus, the final sample consisted of a total of 303 neonates and their mothers. Written informed consent was obtained from mothers and the study was approved by the Institutional Review Boards of the participating institutions.

2.2. Interview, medical record abstraction, and BNBAS procedures

Bilingual, bicultural staff interviewed participants in English or Spanish during the first and second trimesters of pregnancy and again shortly after delivery. Questionnaire data included information on demographics and lifestyle behaviors during the previous trimester. Information about previous pregnancies, medical conditions, medications, and pregnancy complications was obtained by interview and confirmed through medical record abstraction performed by a registered nurse. The BNBAS was administered once to each infant by one of four examiners blind to exposure status. The examiners were trained by a BNBAS certified trainer and tested for reliability.

2.3. DDT/DDE exposure measurement

Blood samples were collected by venipuncture from the mother at the time of the second interview during pregnancy ($n = 279$, mean gestation = 26 weeks, standard deviation (S.D.) = 3.0) or in the hospital before delivery ($n = 24$). We measured levels of *p*,*p*′-DDT, *p*,*p*′-DDE, and *o*,*p*′-DDT in the serum using gas chromatography-high resolution mass spectrometry (GC/MS) as previously described (Barr et al., 2003). We included quality-control materials and blank samples in each analytical run to ensure proper operation of the method. The average limits of detection (LODs) of the organochlorine pesticides were as follows: *p*,*p*′-DDT, 1.60 ± 1.93 picograms per gram (pg/g) serum; *o*,*p*′-DDT, 1.36 ± 2.40 pg/g; and *p*,*p*′-DDE, 3.07 ± 2.27 pg/g. Data below the LOD were assigned the value of LOD/2, following Hornung and Reed (Hornung and Reed, 1990). We measured total cholesterol and triglycerides using standard clinical enzymatic methods (Roche Chemicals, Indianapolis, IN). Total lipids were calculated using the summation method reported by Phillips et al. (Phillips et al., 1989). The laboratory and analytical methods were certified according to guidelines set forth in the Clinical Laboratory Improvement Amendment of 1988 (USDHHS, 1988).

2.4. Outcome definition

The BNBAS is a valid and reliable instrument and can detect subtle deviations in performance related to environmental exposures (Tronick, 1987). It is used to evaluate the neonate's capacity to regulate its internal state and to respond to the environment. The BNBAS consists of a set of 27 behavioral and 18 reflex items used to assess the critical domains of neonatal functioning (Brazelton and Nugent, 1995). We formed seven

clusters from the behavioral scores and reflex items following the scoring method of Lester et al. (Lester et al., 1982). The habituation cluster is comprised of 4 items (bell, pinprick, rattle, and light); the orientation cluster has seven items (animate visual, animate auditory, animate visual-auditory, inanimate visual, inanimate auditory, inanimate visual-auditory, and alertness); the motor performance cluster is made up of five items (defense, maturity, tonus, activity, pull-to-sit); the range of state cluster has four items (irritability, peak of excitement, lability of state, rapidity of build-up); the regulation of state cluster is comprised of 4 items (self-quieting, consolability, cuddliness, hand-to-mouth); and the autonomic stability cluster is made up of three items (skin color, tremors, startles). The reflex cluster has 18 items (passive resistance-arms, passive resistance-legs, tonic deviation of head and eyes, rooting, sucking, glabella, nystagmus, tonic neck reflex, Moro reflex, plantar, Babinski, ankle clonus, palmar, placing, standing, walking, crawling, and incurvation). Higher scores represent more optimal functioning for the six behavioral cluster scores. In contrast, higher scores on the reflex cluster indicate less optimal functioning.

For each of the six behavioral clusters, a subject's cluster score was calculated by following two steps. First, some items that were originally scaled such that lower scores meant more optimal performance were re-coded such that a higher score meant more optimal performance. Second, the cluster score was derived by averaging the scores of the items comprising the cluster. For the reflex cluster, a subject's score was derived by simply summing the number of abnormal reflexes.

2.5. Data analysis

Missing values frequently occur when administering the BNBAS, since the neonates are required to be in a particular state to be in accordance with the examination guidelines. When all of a cluster's items were missing, then the cluster score was set to missing. More than half the infants (53%) were missing all items in the habituation cluster due to the requirement that the infant be asleep immediately prior to the assessment, and 22% were missing at least one item. For the regulation of state cluster, 40% of the sample was missing at least one of the four items comprising the cluster. The individual items of the regulation of state cluster with the most missing values were consolability (40% missing) and self-quieting (25% missing). Both of these items required the infant to be in a crying state for at least 15 seconds. Approximately 5% or less of the sample was missing at least one item for the orientation, motor, and autonomic stability clusters. Roughly 10% of the sample was missing range of state cluster items. Incurvation, tonic neck reflex, and nystagmus were the individual reflex cluster items with the most missing values, with 9, 6, and 5% missing, respectively. None of the other reflex items had more than 3% of the sample with missing values. Approximately 26% of the neonates had one or more of the 18 reflex items missing.

We ran a separate regression model for each of the seven Lester clusters to examine the association between serum DDT/DDE levels and neonatal performance on the BNBAS. To predict performance on the six behavioral clusters, we used least squares regression models. Because the reflex cluster data is comprised of counts of abnormal reflexes and because there was evidence of over-dispersion (relative to the Poisson distribution), we used negative binomial regression to examine the relationship between DDT/DDE levels and the reflex cluster score. DDT/DDE levels were lipid-adjusted (ng/g lipid) and treated as continuous variables on a $\log_{10}$ scale. Each organochlorine (p,p'-DDT, p,p'-DDE, and o,p'-DDT) was modeled separately, because the levels of these organochlorines were highly correlated.

We examined potential confounding by the following risk factors reported in the literature: maternal age, pre-pregnancy body mass index, any smoking, alcohol, or drug use during pregnancy, gestational age at which prenatal care was initiated, parity, method of delivery, anesthesia used during delivery, breastfeeding, and poverty level. Poverty level was calculated by dividing household income by the number of people supported by that income and comparing this value to federal poverty thresholds (USCB/HHES, 2005). Other potential confounders examined were related to the infant and the administration of the BNBAS, and included infant sex, age in days at BNBAS, minutes since the infant was last fed prior to administration of BNBAS, and BNBAS examiner (four different examiners).

To determine whether the above potential covariates should be included in final multivariate models, we ran separate univariate regressions for each of the seven cluster scores against each of the potential covariates. The final models for a particular cluster included covariates associated with that cluster and separately associated with DDT/DDE levels at p-values less than 0.15. We also examined several environmental exposures as potential confounders, because of evidence linking them to decrements in neurodevelopment (polychlorinated biphenyls (PCBs), lead and organophosphate pesticides), or because the CHAMACOS cohort had higher exposure levels than women of reproductive age participating in the U.S. National Health and Nutrition Examination Survey: (β-hexachlorocyclohexane (β-HCH) and hexachlorobenzene (HCB)) (Bradman et al., 2006). CHAMACOS mothers had low total PCB levels (geometric mean (95% CI) = 12.5 (11.4, 13.7) ng/g lipid) and low lead levels (geometric mean (95% CI) = 0.9 (0.8, 1.0) μg/dL) compared to women of childbearing age in the United States (Eskenazi et al., 2006).

Serum PCBs, HCB, and β-HCH were measured concurrently with DDT/DDE and were lipid-adjusted, (see Fenster et al. (Fenster et al., 2006) for calculation of total PCBs and further details). We measured total dialkyl phosphate (DAP) metabolites of organophosphate pesticides in maternal urine collected twice during pregnancy, and averaged these two measurements for data analysis; details of collection, analysis, and quality control are presented elsewhere (Bradman et al., 2005). Lead was measured in maternal and cord blood at the California Department of Health Services, Environmental Health Laboratory (LOD = 1.0 μg/dl) (Eskenazi et al., 2006). All additional exposure measures except for lead were $\log_{10}$-transformed for data analysis.

Final models were re-estimated twice, once controlling for birth weight and once controlling for gestational age, because either of these fetal growth factors may be on the causal pathway between exposure and neurobehavioral outcome. Our results did not appreciably change (data not shown), and so neither birth weight nor gestational age were included in our final models. We also re-ran models excluding outliers (>3 S.D. from mean) for *p,p′*-DDT ($n = 5$), *o,p′*-DDT ($n = 5$), and *p,p′*-DDE ($n = 4$). We found no significant differences and chose to present results with outliers included. Finally, we re-ran our analyses including an interaction term between sex and serum DDT/DDE levels. These analyses were prompted because organochlorines have been reported to affect males and females differently (Dewailly et al., 1993). We did not observe evidence of any gender differences related to DDT/DDE levels and, therefore, interaction terms were not retained in the final models.

Because previous investigators have restricted BNBAS assessment to the time between the early neonatal period to the third day of life (Rogan et al., 1986b), we also performed analyses where separate regressions were run for those infants who were assessed within the first 3 days of life ($n = 152$) and those who were assessed after 3 days ($n = 151$). We chose to present the unstratified model results, because the results of the stratified models were essentially the same as those from our unstratified models in which we adjusted for age of assessment. Analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC) and S-Plus version 6.0 (Insightful Corporation, Seattle, WA).

3. Results

Table 1 summarizes the sociodemographic characteristics of the sample. The women averaged 26.2 years of age (S.D. = 5.0), over two-thirds were parous, about 83% were married, and 19% had graduated high school. Approximately 87% of the women were born in Mexico, with approximately 55% residing in the U.S. for 5 years or less. About 66% of the women were living at or below the federal poverty level. Few women reported smoking (5%), illicit drug use (1.3%), or consuming alcohol (14%) during pregnancy. Cesarean delivery was carried out in about 24% of the births with almost 3% using general anesthesia. There were slightly more female than male neonates, and almost all of the mothers (97%) initiated breastfeeding. The median age at administration of the BNBAS was 3 days, with an interquartile range of 1–28 days post-delivery.

Table 2 presents the medians, percentiles, geometric means and 95% confidence intervals (CI) for the geometric means for DDT/DDE. All *p,p′*-DDT and *p,p′*-DDE, and 95.4% of *o,p′*-DDT levels were above the LOD. The range of *p,p′*-DDT was 1.6–33174 ng/g lipid, for *o,p′*-DDT the range was 0.1–1878 ng/g lipid, and *p,p′*-DDE ranged from 48.8 to 159303 ng/g lipid.

Table 3 shows the sample means, standard deviations, and ranges for each of the seven cluster scores.

Table 4 presents parameter estimates and 95% confidence intervals for adjusted models of each of the seven cluster scores versus DDT/DDE levels. No significant associations between DDT/DDE levels and BNBAS performance were observed. Instead of a detrimental impact of DDT/DDE exposure on reflex functioning, we observed non-significant negative associations. For example, we observed approximately 0.08 fewer abnormal reflexes for each 10-fold increase in *p,p′*-DDE (adjusted $\beta = -0.08$ abnormal reflexes; 95% CI, −0.25–0.08; $p = 0.31$).

In our multivariate DDT/DDE models, we also examined the independent relationship between the potential environmental confounders and performance on the BNBAS Lester cluster scores. Only β-HCH levels were significantly associated with a decrement in performance; increasing β-HCH levels in each of

Table 1
Demographic characteristics of analysis sample (CHAMACOS study, Salinas Valley, California, 2000–2001), $n = 303$[a]

	n	%
Parity		
0	95	31.3
1+	208	68.7
Marital status		
Married/living as married	250	82.5
Single	53	17.5
Maternal education		
Less than 6th grade	132	43.6
7th through 12th grade	113	37.3
Completed high school	58	19.1
Country of birth		
Mexico	263	86.8
U.S.	32	10.6
Other	8	2.6
Poverty		
At or below poverty level	187	66.1
200% poverty level	85	30.0
Above 200% poverty level	11	3.9
Smoking during pregnancy		
Yes	15	5.0
No	286	95.0
Drug use during pregnancy		
Yes	4	1.3
No	298	98.7
Alcohol use during pregnancy		
Yes	41	13.6
No	261	86.4
Cesarean delivery		
Yes	72	23.8
No	231	76.2
General anesthesia		
Yes	8	2.6
No	295	97.4
Infant sex		
Female	161	53.1
Male	142	46.9
Breastfeeding initiated after delivery		
Yes	294	97.3
No	8	2.7

[a] Total number of observations vary due to missing data.

Table 2
Range of LOD, percent detectable, percentiles and geometric means and 95% CIs of organochlorines measured in serum during pregnancy (CHAMACOS study, Salinas Valley, CA, 2000–2001)

Marker of exposure (ng/g lipid)	*n*	LOD range	%> LOD	5th Percentile	Median	95th Percentile	Geometric mean	95% CI
p,p′-DDT	303	0.06–4.70	100.0	3.53	14.1	825.6	23.2	(19.1, 28.2)
o,p′-DDT	301	0.04–6.05	95.4	0.21	1.4	34.5	1.8	(1.5, 2.1)
p,p′-DDE	303	0.06–4.83	100.0	278.13	1,103.7	18,160.3	1,464.2	(1,268, 1,691)

the DDT/DDE models was associated with decreased performance on the autonomic stability cluster (data not shown). For example, in models where *p,p′*-DDT was the exposure of interest, we found an approximate decrease of 0.20 in the autonomic stability score for each 10-fold increase in β-HCH (adjusted $\beta = -0.20$; 95% CI, −0.38 to −0.03, $p = 0.02$). This corresponds to a reduction of 0.34 in the autonomic stability score between the 5th and 95th percentiles (3.90 and 185.7 ng/g lipid, respectively) of β-HCH exposure in our cohort or about a third of a standard deviation.

4. Discussion

In this study of a predominantly Mexican-American population residing in California, *in utero* exposure to DDT/DDE was not associated with decreased performance on the BNBAS, although levels of *p,p′*-DDE and, *p,p′*-DDT were significantly higher in our sample than in a sample of women of childbearing age in the U.S. population (Bradman et al., 2006). Overall, our results are consistent with the findings of Stewart et al. (Stewart et al., 2000), who did not observe an association of cord blood DDE and newborn performance on the BNBAS in their Oswego cohort. However, our results failed to confirm the findings of hyporeflexia on the BNBAS observed in the North Carolina birth cohort followed by Rogan and colleagues (Rogan et al., 1986b).

It is not clear why our results differ from those of Rogan et al. On average, the median maternal serum levels of DDE reported for the Rogan et al. cohort in a separate manuscript (Rogan et al., 1986a) were higher than levels reported in our cohort (12.60 ppb versus 9.08 ppb), but the 95th percentile and maximum were much lower in the North Carolina sample (34.60 ppb versus 151.92 ppb, and 180.00 ppb versus 1219.73 ppb, respectively). Our sample size was considerably smaller: 303 neonates in contrast with Rogan's 867. Our cohort had a higher prevalence of abnormal reflexes; 20.8% had four or more abnormal reflexes, whereas this was true for only 13.7% of the infants in the Rogan et al. study. The Rogan et al. population had much higher maternal serum PCB levels (Rogan et al., 1986a) than did ours (median = 9.06 ppb versus 0.10 ppb); in addition, Rogan et al. did not control for PCBs in their analyses of DDE and the BNBAS (Rogan et al., 1986b).

Table 3
BNBAS Lester cluster scores for study sample (CHAMACOS study, Salinas Valley, California, 2000–2001)

	n	Mean	S.D.	Range
Habituation	143	6.3	1.7	1.0–9.0
Orientation	301	7.5	1.4	1.0–9.0
Motor	303	5.8	0.7	2.0–7.2
Range of state	303	3.4	1.0	1.2–5.2
Regulation of state	303	5.7	1.6	1.0–9.0
Autonomic stability	303	7.0	0.8	4.3–9.0
Reflex	303	2.2	1.8	0.0–10.0

Rogan et al. categorized milk fat DDE levels into seven groups and found that the proportion of infants with four or more abnormal reflexes gradually increased with increasing DDE categories. For comparison, we also categorized maternal DDE serum levels into seven groups for the CHAMACOS cohort. In contrast to the Rogan et al. sample, the lowest DDE category in CHAMACOS had the highest percentage of infants with four or more abnormal reflexes (36.4%), and this percent generally decreased with increasing DDE level (data not shown).

We do not know whether the adverse association of β-HCH and autonomic stability is real, perhaps related to its ability to alter estrogen (Li, 1999) or thyroid hormone (Ribas-Fito et al., 2003), or a spurious result of multiple testing. β-HCH is one of eight isomers of the insecticide HCH, which has not been used in the U.S. for over 20 years and was phased out in Mexico in 2000 (Pardio et al., 2003). To our knowledge, there have been no prior studies of neurodevelopmental effects of β-HCH in humans or animals (ATSDR, 2005). Because of the results in this study, we examined the relationship of maternal β-HCH in these same children on the Bayley Scales of Infant Development at 6, 12 or 24 months of age and did not see any association (data not shown). Thus, these observations from the newborn period do not appear to be indicative of longer-term functioning.

Strengths of our study include analysis of a longitudinal birth cohort, considerable information on additional environmental exposures and potential confounders, and relatively high average levels of serum DDT/DDE; for example, CHAMACOS women had approximately eight times the DDE levels compared to women of reproductive age participating in the National Health and Nutrition Examination Survey of the U.S. (Bradman et al., 2006). The CHAMACOS population also had relatively low levels of *in utero* exposure to other known developmental neurotoxicants such as PCBs and lead. In addition, our population was homogeneous for factors such as diet, breastfeeding, country of origin and socioeconomic status, thereby reducing uncontrolled confounding. One limitation of our study is that we performed a BNBAS assessment on each neonate only once. Neonatal behavior is inherently unstable; when repeated tests are performed the

Table 4
Adjusted associations between OC levels[a] in maternal serum during pregnancy and seven BNBAS Lester cluster scores (CHAMACOS study, Salinas Valley, California, 2000–2001)

		p,p′-DDT		*o,p′*-DDT		*p,p′*-DDE	
	n	β^*	(95% CI)	β	(95% CI)	β	(95% CI)
Habituation[b]	143	0.13	(−0.22, 0.48)	0.12	(−0.27, 0.50)	0.23	(−0.26, 0.72)
Orientation[c]	254	−0.01	(−0.25, 0.24)	−0.04	(−0.30, 0.22)	0.10	(−0.23, 0.42)
Motor[d]	303	0.02	(−0.08, 0.12)	0.03	(−0.08, 0.14)	0.08	(−0.05, 0.22)
Range of state[e]	256	−0.03	(−0.21, 0.15)	−0.06	(−0.25, 0.13)	−0.16	(−0.40, 0.07)
Regulation of state[f]	302	0.01	(−0.20, 0.23)	0.03	(−0.21, 0.26)	0.05	(−0.24, 0.34)
Autonomic stability[g]	301	−0.08	(−0.20, 0.05)	−0.07	(−0.20, 0.07)	−0.11	(−0.28, 0.06)
Reflex[h,i]	302	−0.03	(−0.14, 0.09)	−0.08	(−0.21, 0.05)	−0.08	(−0.25, 0.08)

$^*p < 0.05$.
[a] ng/g lipid, $\log_{10}$ scale.
[b] Adjusted for age at administration of BNBAS, cesarean section, HCB, interviewer.
[c] Adjusted for age at administration of BNBAS, maternal blood lead level, interviewer.
[d] Adjusted for age at administration of BNBAS, interviewer.
[e] Adjusted for age at administration of BNBAS, mother's education, drug use, maternal blood lead level, interviewer.
[f] Adjusted for age at administration of BNBAS, breastfeeding, cesarean section, total DAPs, interviewer.
[g] Adjusted for age at administration of BNBAS, mother's age at delivery, total DAPs, β-HCH, interviewer.
[h] Adjusted for age at administration of BNBAS, mother's age at delivery, total DAPs, total PCBs, interviewer.
[i] Negative binomial regression used for Reflex cluster models.

BNBAS has achieved predictive validity (Brazelton, 1991). Also, approximately half of the infants were assessed after the first 3 days of life, but our results were not appreciably different when we restricted our analyses to those assessed within the first 3 days of life.

In summary, maternal serum levels of DDT/DDE were not related to neonatal performance on the BNBAS. However, in this same cohort, we previously demonstrated that exposures to DDT were related to decrements in mental development on the Bayley Scales when the children were 12 and 24 months but not at 6 months, and in psychomotor development at 6 months and 12 months but not at 24 months (Eskenazi et al., 2006). In the present study, the failure to observe effects in these same children as neonates may be due to the limited capability of a single BNBAS assessment to detect decrements in neurobehavior or to a delay in the manifestations of neurodevelopmental effects of DDT until after the neonatal period. Additionally, the associations seen on the Bayley Scales at later ages may not have a counterpart that is detectable in neonates. While research has focused on the predictive value of positive findings of the BNBAS (Brazelton and Nugent, 1995), much less attention has been given to the interpretation of negative findings. Subsequent follow up of the North Carolina (Rogan and Chen, 2005) and Oswego (Stewart et al., 2005) cohorts found no significant associations between DDE and neurodevelopmental performance in children 8 through 10 years of age. A study of 475 four-year-old children from Spain found an inverse association between DDT cord serum concentration and verbal, memory, quantitative, and perceptual-performance skills (Ribas-Fito et al., 2006); however, these children were not assessed when they were neonates. We plan to follow the CHAMACOS cohort for persistent deleterious effects of DDT/DDE and other environmental exposures on neurodevelopment.

Acknowledgements

This work was supported by the National Institute of Occupational Safety & Health (NIOSH) (RO1-OH007400), the US Environmental Protection Agency (USEPA) (RD-83171001), and the National Institute of Environmental Health Science (NIEHS) (PO1-ES009605). The ideas expressed are those of the authors' and do not necessarily reflect the positions of the California Department of Health Services, NIOSH, USEPA or NIEHS. All authors have disclosed that there exist no potential conflicts of interest regarding this manuscript. We are indebted to Jessica Young for her previous work on organophosphate pesticide exposure and Brazelton assessments in CHAMACOS subjects. We gratefully acknowledge Caroline Johnson for her contribution to the neurobehavioral assessments, Judy Withers for training on the Brazelton assessments, and Lesley Pederson, Michelle Trinidad, and Virginia Hernandez for performing them. We thank the CHAMACOS staff, students, community partners, participants and their families; Jackie Schwartz and Amy Marks for manuscript review; and Theresa Saunders for manuscript preparation.

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