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Use of the Rey-Osterrieth Complex Figure Test for neurotoxicity evaluation of mixtures in children

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ABSTRACT

The aim of this study was to assess the value of the children's version of the Rey-Osterrieth Complex Figure Test as a screening test in a population exposed to different mixtures of neurotoxicants. Copy and Immediate Recall scores were evaluated through the test. Children were recruited from three sites; an area with natural contamination by fluoride and arsenic (F–As), a mining-metallurgical area with lead and arsenic contamination (Pb–As) and a malaria zone with the evidence of fish contaminated with dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs). Children aged 6–11 years old, living in one of the three polluted sites since birth were recruited ($n = 166$). The exposure was evaluated as follows: fluoride and arsenic in urine, lead in blood and DDT, dichlorodiphenyldichloroethylene (DDE) and PCBs in serum. To evaluate the test performance, *z*-scores for Copy and Immediate Recall were calculated. The proportion of children by residence area with performance lower than expected by age (below −1 SD) for Copy and Immediate Recall was in the F–As area (88.7% and 59%) and in the DDT–PCBs area (73% and 43.8%), respectively. In the Pb–As area, the proportion was 62% for both tests. After adjustment, Copy correlated inversely with fluoride in urine ($r = -0.29$; $p < 0.001$) and Immediate Recall correlated inversely with fluoride in urine ($r = -0.27$; $p < 0.05$), lead in blood ($r = -0.72$; $p < 0.01$), arsenic in urine ($r = -0.63$; $p < 0.05$) and DDE ($r = -0.25$; $p < 0.05$). This study provided evidence that children included in this research are living in high risk areas and were exposed to neurotoxicants. Poor performance in the test could be explained in some way by F, Pb, As or DDE exposure, however social factors or the low quality of school education prevalent in the areas could be playing an important role.

1. Introduction

Several approaches can be used to identify high risk populations exposed to mixtures of pollutants, including biomonitoring. In Mexico, we have identified populations living in high risk areas exposed to either fluoride (F), arsenic (As), lead (Pb), p,p′-dichlorodiphenyltrichloroethane (p,p′-DDT) or polychlorinated biphenyls (PCBs) at levels above those recommended as safe (Rocha-Amador et al., 2007; Carrizales et al., 2006; Calderón et al., 2001a; Yáñez et al., 2002; Noreña-Barroso et al., 1998, 2004, 2007). The sources and pathways of exposure to these toxicants in each area are very different. For example, exposure to F and As occurs through naturally contaminated drinking water; for As and Pb the exposure is primarily through soil and dust deriving from pyrometallurgic nonferrous metal production or mining activity. For p,p′-DDT, intake is likely due to diet (fish consumption) or maternal milk in areas where this insecticide was used as a preventive Public Health strategy to control malaria. For PCBs, exposure is likely through the intake of fish. The assessment of health effects in these settings is very complicated due to exposure to mixtures of toxicants but also economical and social disadvantages.

A review of health effects data associated with exposure to the toxicants mentioned above concluded that the Central Nervous System (CNS) effects were the most likely adverse health outcomes. Several authors have reported reduction in Intelligence Quotient (IQ) scores (Li et al., 1995; Zhao et al., 1996; Lu et al.,

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2000; Xiang et al., 2003; Wasserman et al., 2004; Wang et al., 2007; Needleman and Gatsonis, 1990; Pocock et al., 1994; Schwartz, 1994; Rocha-Amador et al., 2007; Calderón et al., 2001a). In regard to p,p′-DDT and PCBs, the main effects reported from exposure to p,p′-DDT and PCBs were cognitive and motor deficits associated with prenatal exposure (Faroon et al., 2001; Ribas-Fitó et al., 2001, 2003, 2006; Eskenazi et al., 2006; Torres-Sánchez et al., 2007).

Intuitively, though it might seem that an IQ test would be an ideal measure, this assumption would be ill founded, because some toxicants could affect only specific functions, such as attention, memory, language, or visuospatial abilities without clear decrements on IQ scores (Calderón et al., 2001b). Furthermore, the exposure dose as well as mixtures of toxicants are important factors that also need to be considered. For example, decrements on Full IQ scores of children exposed to drinking water at F (levels above 5 mg/L) and As (levels above 150 μg/dL) were observed (Rocha-Amador et al., 2007), whereas in children exposed to F levels in drinking water lower than 3 mg/L and As lower than 30 μg/dL only associations with reaction time and visuospatial organization scores were observed and no influence of F on IQ scores (Calderón et al., 2001b). In this context, it is imperative to have a tool for rapid risk assessment to quantitatively measure health effects. In neuropsychology there are several tests that can be used for this purpose but many of them have issues including lack of validation and standardized values for the Mexican population, furthermore the influence of cultural factors also limits their usefulness. These issues could be solved in part by the Rey-Osterrieth Complex Figure (ROCF) Test. This test is one of the most widely used in neuropsychology for the evaluation of visuospatial constructional ability and non-verbal memory skills in both clinical and research settings (Weil et al., 2005; Schwartz et al., 2000; Park et al., 2006; Bowler et al., 2007; Haut et al., 1999). The objective of this study was to explore the potential usefulness of the ROCF test as a tool for a rapid assessment to evaluate visuospatial organization (Copy) and visual memory (Immediate Recall) in children living in areas of Mexico exposed to different mixtures of neurotoxic agents including F, As, Pb, p,p′-DDT or PCBs.

2. Materials and methods

2.1. Sites selection

Three sites with antecedents of environmental pollution to mixtures of either F–As, Pb–As or DDT–PCBs were selected (Fig. 1). The first was an area in Durango state (5 de Febrero) where drinking water is polluted naturally with F and As at levels exceeding 6 and 19 times, respectively, the World Health Organization (WHO) limits (WHO, 2008). Biological monitoring data in children demonstrated exposure to F and As (Rocha-Amador et al., 2007; Calderón et al., 2001a). The second was a mining-metallurgical area located in the state of San Luis Potosí (Morales). Previous data reported that 90% of soil samples had values of Pb and As above the guidelines recommended by the United States Environmental Protection Agency (USEPA) (Pb–As), also Pb in blood (PbB) and As in urine (AsU) levels were over the safe values for children (Carrizales et al., 2006; Calderón et al., 2001a). Finally, a malaria area in the state of Quintana Roo (El Ramonal) was selected, where there is evidence of environmental pollution of p,p′-DDT and PCBs (DDT–PCBs) (Noreña-Barroso et al., 1998, 2004, 2007) and children's exposure to p,p′-DDT and PCBs at high levels (Pérez-Maldonado et al., 2006).

2.2. Study population

Children attending public schools were screened through personal interviews for study eligibility. Inclusion criteria were children between 6 and 11 years old, living in the study area since birth and whose parents signed the agreement to participate. Children with neurological disease diagnosed by a physician and reported by the mother were excluded from the study. The final sample for each site was F–As ($n = 80$), Pb–As ($n = 13$) and DDT–PCBs ($n = 73$).

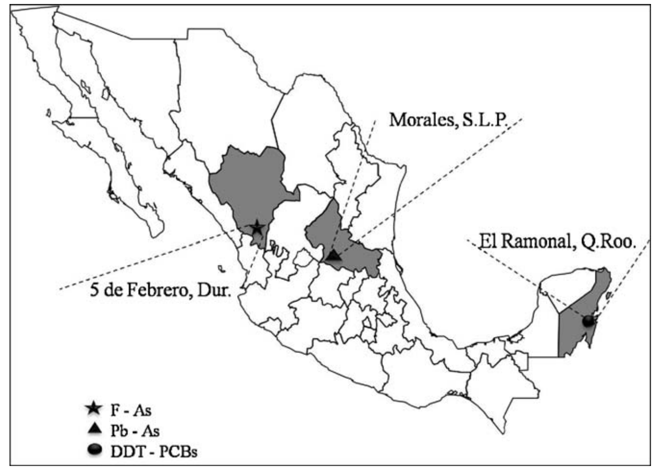

Fig. 1. Location of populations included in this study.

2.3. The Rey-Osterrieth Complex Figure Test application

The children's version of the ROCF test was administered at school to all participants by a trained neuropsychologist who was blinded to the type or levels of pollutants. The test is composed of nine perceptual units, in which the child has to integrate into one perceptual unit (Fig. 2). To measure visuoconstructional abilities, the child is asked to copy the ROCF (Copy). After the figure has been copied, the copy is removed and 3 min are allowed to pass before the child is asked to reproduce it from memory to evaluate the amount and quality of the original information retained in the spatial memory and visual memory (Immediate Recall). To score the ROCF, criteria defined by Galindo y Villa were followed (Cortes et al., 1997). Each of the 9 perceptual units was evaluated separately for a maximum score of 18 points. A four-point scale ranging from 0 to 2 was assigned to each unit depending upon the degree to which the units were correctly drawn and placed. The qualitative scores include Presence, Accuracy, Placement, Fragmentation, Planning, Neatness, Vertical Expansion, Horizontal Expansion, Reduction, Rotation, Perseveration, Confabulation and Asymmetry for each condition. Asymmetry receives a categorical rating (N = no Asymmetry, L = left-sided Asymmetry, R = right-sided Asymmetry).

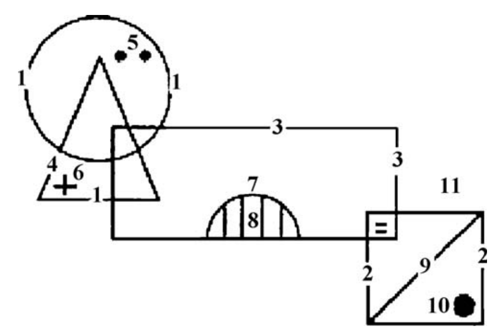

Fig. 2. The Rey-Osterrieth Complex Figure Test (ROCFT) children's version. Reproducing the ROCF is a complex cognitive task, which involves the ability to organize the figure into a meaningful perceptual unit (Rey, 1999; Shin et al., 2006). Due to the complexity at the time of drawing the figure, many authors consider the ROCF as a useful method to assess executive functions that are mediated by the prefrontal lobe, which are required for strategic planning and organizing (Shin et al., 2006; Watanabe et al., 2005).

2.4. Biological monitoring

2.4.1. Determination of fluoride and arsenic in urine

Fluoride in urine (FU) was analyzed according to method 8308 ("fluoride in urine") from the National Institute of Occupational Safety and Health (NIOSH, 1984) with a sensitive specific ion electrode. As a quality control check, reference standard "fluoride in freeze dried urine" (NIST SRM 2671a) was analyzed. The accuracy was 97.0 ± 6.0%. As in urine (AsU) was analyzed according to Cox (1980) by atomic absorption spectrophotometer coupled to a hydride system (Perkin-Elmer model Aanalyst 100). As quality control, reference standard "toxic metals in freeze-dried urine" (NIST SRM 2670) was analyzed. The accuracy was 98.0 ± 4.0%. Levels of FU and AsU were adjusted for urinary creatinine, which was analyzed by a colorimetric method (Bayer Diagnostic Kit, Sera-Pak® Plus).

2.4.2. Determination of lead in blood

To analyze lead, blood samples were obtained by venipuncture using lead-free Vacutainer tubes containing EDTA as anticoagulant. Lead in blood (PbB) was analyzed according to Subramanian (1987) with a Perkin-Elmer 3110 atomic absorption spectrophotometer using a graphite furnace. At the time of analysis, our laboratory participated in the PbB proficiency testing program conducted by the Centers for Disease Control and Prevention. The accuracy was 99.0 ± 9.0%.

2.4.3. Determination of p,p′-dichlorodiphenyltrichloroethane (p,p′-DDT), p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE) and polychlorinated biphenyls (PCBs).

Blood samples were collected by venipuncture using tubes containing heparin as anticoagulant. Quantification of organochlorine compounds was performed as reported by Trejo-Acevedo et al. (2009). p,p′-DDT, p,p′-DDE and PCBs congeners (28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187) were quantified using a HP 6890 gas chromatograph coupled with a HP 5973 mass spectrometer. As quality control, α-hexachlorocyclohexane-C13, endrin-C13 and PCB-141-C13 were added as internal standards to all samples. Our accuracy for all tested compounds was 80–120%. PCBs levels were reported as ∑PCBs.

2.5. Anthropometric measurements

Each child's height and weight were taken to calculate height-for-age *z*-scores (HAZ) and weight-for-age *z*-scores (WAZ) based on the WHO/National Center for Health Statistics (NCHS) growth reference, as indexes of chronic and acute undernutrition (WHO, 1986). All the calculations were done with Nut Stat of EPI-info™ version 3.4.3. Demographic and socioeconomic information was obtained by questionnaire.

2.6. Statistical analysis

Log transformed values of FU, AsU, p,p′-DDT, p,p′-DDE and ∑PCBs were used for statistical analysis. Descriptive statistics (mean, standard deviation, minimum, maximum) were calculated for sociodemographic variables and for the levels of FU, AsU, p,p′-DDE, p,p′-DDT and ∑PCBs. ROCF scores are highly influenced by age (meaning that older children perform better than young children). To compare the proportion of children between areas, *z*-scores were generated for Copy and Immediate Recall (raw score − expected mean by age/expected standard deviation by age). The ROCF reference values reported for Mexican children were used. Three cut points were defined; low performance (*z*-score values <−1 SD), normal performance (*z*-score values between ≤−1 SD to ≤1 SD) and high performance (*z*-score values >1 SD). For example, the expected value in copy performance for 6 years old children is 9.94 ± 2.28 points (Galindo y Villa et al., 1997). Children classified in the low performance category had less than 7.6 points in the test. A Construction/Memory score was calculated (Immediate Recall/Copy × 100). Finally, to evaluate the association between FU, AsU, PbB, p,p′-DDT, p,p′-DDE and ∑PCBs in serum and ROCF scores, crude and partial correlations were calculated by each contaminant individually. Confounding factors tested in the analysis included PbB, age, gender, and height-for-age *z*-scores, only age had significant associations and was included in the final analysis. Significance level was fixed at 0.05. All analysis was done with SPSS statistical software, package version 16.0.

3. Results

Sociodemographic characteristics of children in each area are presented in Table 1. The mean values for age ranged from 6.5 ± 0.6 to 8.4 ± 1.4 years. The mean values of years of children's education ranged from 1.2 ± 0.5 to 2.5 ± 1.3. The proportion of boys was lower than proportion of girls for F–As (49%) and Pb–As (43%) areas in contrast with the low girls' proportion in the DDT–PCBs area (38%). The proportion of children below −2 standard deviations for height-age *z*-score was 18.2 for the Pb–As area and 5.9 for DDT–PCBs area and 1.6% for F–As area. In regard to the father's education, the DDT–PCBs area had the low mean value (4.8 ± 3.2), compared to the Pb–As area (6.6 ± 2.6).

Data from biological monitoring are presented in Table 2. In the F–As area, the mean level f FU was 5.6 ± 1.7 mg/gcrt. The proportion of children with FU values above the reference value was 97.5%. The mean of AsU was 64.5 ± 2.3 and 59% of the children had values above the reference value of 50 μg/L. The mean PbB was 5.2 ± 3.2 μg/dL and 8% of the children had values above the reference value of 10 μg/dL. In the Pb–As area, the mean PbB value was 12.7 ± 6.1 μg/dL and 50% of the children had values above 10 μg/dL. For AsU, the mean value was 64.2 ± 2.3 μg/gcrt and 58% had levels above 50 μg/L. For DDT and PCBs area mean values for DDT, DDE and ∑PCBs were 5.5 ± 6.4 ng/mL, 53.7 ± 6.6 ng/mL and 8.3 ± 2.9 ng/mL, respectively. For PbB, none of the children had levels above 10 μg/dL.

Table 3 shows the proportion of *z*-score categories for Copy and Immediate Recall. The greatest number of children with Copy *z*-scores below −1 SD was observed in the F–As area (88.7%) followed by 73% from DDT–PCBs area, the lowest value was for the Pb–As area (62%). The highest proportion of children with low scores in Immediate Recall (<−1 SD) was found in the Pb–As area (62%), followed by 59% in the F–As area and the lowest value was for the

Table 1
Sociodemographic characteristics by study area.

Area	*n*	Age[a] (years)	Children's education[a] (years)	Gender (%)		Height-for-age *z*-score	Father's education[a] (years)	School type (urban/rural)
				Boys	Girls	(% <−2 SD[b])		
F–As	80	8.2 ± 1.1 (6–11)	2.0 ± 1.0 (1–5)	49	51	1.6	5.1 ± 3.0 (0–16)	Rural
Pb–As	13	6.5 ± 0.6 (6–8)	1.2 ± 0.5 (1–2)	43	57	18	6.6 ± 2.6 (3–11)	Urban
DDT–PCBs	73	8.4 ± 1.4 (6–11)	2.5 ± 1.3 (0.5–5)	62	38	5.9	4.8 ± 3.2 (0–12)	Rural

[a] Values are expressed as arithmetic mean ± standard deviation and minimum and maximum in parenthesis.
[b] World Health Organization and Center for Disease Control and Prevention cutoff point for severe chronic under nutrition.

Table 2
Levels of contaminants in different biologic matrixes by study area.

	$\bar{x} \pm$ SD	Minimum–maximum	Proportion of children > reference value
F–As (n = 80)			
Pb blood (μg/dL)	5.2 ± 3.2	0.1–15.7	8.0[a]
F urine (mg/gcrt)	5.6 ± 1.7[b]	1.2–24.9	97.5[c]
As urine (μg/gcrt)	64.2 ± 2.3[b]	10.0–325.3	59.0[d]
Pb–As (n = 13)			
Pb blood (μg/dL)	12.7 ± 6.1	5.0–26.0	50.0[a]
As urine (μg/gcrt)	57.5 ± 1.5[b]	27.9–142.2	58.0[d]
DDT–PCBs (n = 73)			
Pb blood (μg/dL)	4.2 ± 1.5	0.1–7.6	0.0[a]
p,p′-DDT plasma (ng/mL)	5.5 ± 6.4[b]	0.3–89.1	–[e]
p,p′-DDE plasma (ng/mL)	53.7 ± 6.6[b]	0.3–598.6	–[e]
∑PCBs plasma (ng/mL)	8.3 ± 2.9[b]	0.8–50.5	–

[a] 10 μg Pb/dL—Centers for Disease Control and Prevention reference value.
[b] Geometric mean ± standard deviation (SD).
[c] 2 mgF/gcrt—value quantified in areas with levels of fluoride in drinking water less than 1 mg/L.
[d] 50 μg/L—Centers for Disease Control and Prevention limit for environmental intervention for As in urine.
[e] No reference values report.

Table 3
Proportion of children by z-score categories and Construction/Memory score for Copy and Immediate Recall by study area.

Area	z-Score[a] > −1 SD[b]	Mean[c] CMS	z-Score[a] ≤ −1 to ≤ 1 SD[b]	Mean[c] CMS	z-Score[a] > 1 SD[b]	Mean[c] CMS
Copy						
F–As	88.7 (n = 71)	74%	10 (n = 8)	67%	1.3 (n = 1)	89%
Pb–As	62 (n = 8)	66%	38 (n = 5)	51%	0	0
DDT–PCBs	73 (n = 53)	78.6%	26 (n = 19)	75%	1.4 (n = 1)	40%
Immediate Recall						
F–As	59 (n = 47)	63%	39 (n = 31)	70%	2.5 (n = 2)	89%
Pb–As	62 (n = 8)	51%	38 (n = 5)	76%	0	0
DDT–PCBs	43.8 (n = 32)	62%	54.8 (n = 40)	88%	1.4 (n = 1)	129%

[a] Values to calculate z-scores were taken from ROCF test reference values reported for Mexican children in Cortes et al. (1997).
[b] SD: standard deviation.
[c] CMS = Construction/Memory score = (Immediate Recall/Copy) × 100.

Table 4
Associations between fluoride and arsenic in urine, lead in blood, p,p′-DTT, p,p′-DDE and ∑PCBs in plasma and Copy and Immediate Recall scores.

Area		Rey-Osterrieth Complex Figure Test			
		Copy		Immediate Recall	
		r[a]	r[b]	r[a]	r[b]
F–As	F[c] (mg/gcrt)	−0.33**	−0.29**	−0.29**	−0.27*
	As[c] (μg/gcrt)	−0.09	−0.05	0.005	0.02
Pb–As	Pb (μg/dL)	0.06	0.08	−0.78**	−0.72**
	As[c] (μg/gcrt)	−0.34	−0.35	−0.61*	−0.63*
DDT–PCBs	p,p′-DDT[c] (ng/mL)	0.06	0.07	0.05	0.07
	p,p′-DDE[c] (ng/mL)	0.01	0.09	−0.24**	−0.25*
	∑PCBs[c] (ng/mL)	−0.02	−0.01	0.03	0.05

[a] Pearson correlation.
[b] Partial correlation adjusted by age.
[c] Values were log transformed.
* $p < 0.05$.
** $p < 0.01$.

DDT–PCBs area (43.8%). For Construction/Memory score, the proportion across z-score categories by area increased only for Immediate Recall. For the F–As area, values were 63%, 70% and 89%. In the DDT–PCBs area, values were 62%, 88% and 129%, whereas for Pb–As areas the values were 51% and 76%. This pattern was not observed in the Construction/Memory score for Copy.

Table 4 displays crude and partial correlations. Significant adjusted correlations were observed in the F–As area between FU and Copy and Immediate Recall ($r = -0.29$, $p < 0.001$ and $r = -0.27$, $p < 0.001$), respectively. In the Pb–As area, Immediate Recall and PbB and AsU levels were correlated ($r = -0.72$, $p < 0.01$ and $r = -0.63$, $p < 0.05$), respectively. Finally, in the DDT–PCBs area, only p,p′-DDE and Immediate Recall correlated ($r = -0.25$, $p < 0.05$).

4. Discussion

This study provides evidence that children living in high risk areas were exposed to either F, As, Pb, DDT or PCBs and these contaminants could contribute, to some degree, to children's low performance observed in the tests of the Rey-Osterrieth Complex Figure. The highest proportion of children (89%) with Copy performance below −1 SD was observed in children from F–As area. Approximately 9 out of 10 children were unable to copy the ROCF as expected for their age. For example, the expected score on Copy for a 6-year-old child is 9.94 ± 2.28 points. A child classified in the category below −1 SD means that his score was lower than 7.66. In the F–As area children had *z*-scores as low as −5 SD (scoring only two points on the test). For Immediate Recall, the proportion of children in the lowest category was 59% and almost 6 out of 10 children were unable to draw the figure as expected for their age after 3 min had elapsed. Following the same example of a 6-year-old child, the expected value for drawing the figure from memory is 7.26 ± 2.45. One child classified in the −1 SD category had a score below 4.81 points. Fluoride correlated inversely with Copy and Immediate Recall $r = -0.29$ and $r = -0.27$ (adjusted values). In the F–As area, the mean of FU was 5.6 ± 1.7 and the proportion of children with FU levels over 2 mg/gcrt was 97.5%. All children had some degree of dental fluorosis as an indicator of chronic exposure to fluoride. In the Pb–As and DDT–PCBs, none of the children had dental fluorosis (data not shown). Previous data have reported a similar association between Copy scores and FU in children ($r = -0.27$; $p < 0.05$) at mean values of 4.3 ± 1.5 mg/gcrt (Calderón et al., 2001b). We have reported previous studies with similar data. For example, in children exposed to F via drinking water (with FU levels above 5 mg/gcrt) we observed an inverse association with Performance IQ scores ($\beta = -13$, $p < 0.001$) (Rocha-Amador et al., 2007). Other epidemiological and experimental data support the potential of F to damage the Central Nervous System (CNS). For example, Full IQ score reductions were found in children exposed to F in water above 2.0 mg/L (Li et al., 1995; Lu et al., 2000; Zhao et al., 1996; Xiang et al., 2003). In experimental animal studies, alterations in nicotinic acetylcholine receptors, brain membrane lipids and oxidative stress after fluoride exposure have been reported (Mullenix et al., 1995; Shivarajashankara et al., 2002a,b; Long et al., 2002; Guan et al., 1998). No associations were observed with either Copy or Immediate Recall scores for AsU. However, when AsU and FU were included simultaneously in the partial correlation analysis there was a small increment in the *r* value (data not shown). For Copy, the correlation increased from $r = -0.29$ to $r = -0.32$, and for Immediate Recall, it was from $r = -0.27$ to $r = -0.34$. However, it is interesting to discuss the inverse associations in the Pb–As area between AsU and Immediate Recall ($r = -0.63$; $p < 0.05$) and Copy ($r = -0.35$) but that was not significant, probably because of the low sample size. The mean AsU levels were similar between areas (57.5 ± 1.5 vs. 64.2 ± 2.3) for F–As and Pb–As areas, respectively, and the proportion of children with values above 50 μg/L was also similar at 59% and 58%, respectively. PbB in the Pb–As area was inversely associated with Immediate Recall ($r = -0.72$; $p < 0.01$). It is important to note that the Pb–As area had the highest PbB values (12.7 ± 6.1 μg/dL) and 50% of the children had levels above 10 μg/dL compared to 8% in the F–As area and none in the PCBs–DDT area. A previous study conducted in children living in the same area, using the categories of Wechsler Intelligence Test Revised version for Mexican children (WISC-RM), reported a negative association between PbB and sequential factor ($r = -0.36$; $p<0.05$) (related to mental agility, sequential organization, short-term memory and visual memory) (Calderón et al., 2001a). There are data reporting visual memory deficits at concentrations of PbB as low as 5 μg/dL (Lidsky and Schneider, 2006). In occupational studies there is also evidence of the influence of Pb exposure and visual memory effects, evaluated through ROCF test adult version (Schwartz et al., 2000). In the Pb–As area, the proportion of children with scores below −1 SD was 62% for both Copy and Immediate Recall. For the Pb–As area, As in urine correlated inversely with memory scores ($r = -0.63$, $p < 0.05$) and for copy scores the association was also inverse but non-significant ($r = -0.35$, $p < 0.3$), which could be explained by the small sample size of children evaluated ($n = 13$). We were unable to increase the sample size in this area because children finished the school year before the project was concluded. Two children in this area had values below −2 SD for height-for-age-*z*-score. The controversial data for AsU and ROCF scores suggests that the test is unspecific for As effects. Toxicological mechanisms for the F–As mixture are probably different compared to the Pb–As mixture. Experimental data where central monoamines were analyzed in adult mice exposed to As-Pb mixture suggests an interaction between both contaminants (Mejía et al., 1997). Whereas for experimental data of F and As and oxidative stress damage, the conclusion is that F and As by itself are toxic but the mixture is less toxic and may provide protective value against F or As induced oxidative stress (Mittal and Flora, 2006). As expected, because of the low PbB values, we did not observe any relationship between exposure to PbB and Copy or Immediate Recall scores in any of the other two areas (data not shown). It is important to remember that the PbB levels were very low because they were not exposed to significant levels of environmental Pb. The mean concentration ranged from 4.2 ± 1.5 to 5.2 ± 3.2 μg/dL for DDT–PCBs and F–As areas respectively, compared to 12.7 ± 6.1 for the Pb–As area, where there is an important source of Pb environmental pollution (Carrizales et al., 2006).

For the DDT–PCB area, we observed an inverse association only between p,p′-DDE levels and memory scores ($r = -0.25$, $p < 0.05$). These children had 77 times higher values than children living in non-exposed areas (53.7 ng/mL vs. 0.7 ng/mL) (Pérez-Maldonado et al., 2004). The low performance on the test (73% for Copy and 43.8 for Immediate Recall) was not explained by either DDT or PCBs exposure. Other factors not evaluated in this research could explain the results. Nonetheless, 73% of our children had values exceeding 5.1 ng/mL mean value of total PCBs reported in children breast-fed by intoxicated women (Jacobson et al., 1990). The neurological effects associated with p,p′-DDT or p,p′-DDE exposures are psychomotor and mental development in young children and these neurological alterations are not evaluated by the ROCF test (Ribas-Fitó et al., 2001, 2003, 2006; Eskenazi et al., 2006; Torres-Sánchez et al., 2007; Fenster et al., 2007).

In conclusion, children evaluated in this study were highly exposed to either F, As, Pb, DDT, DDE or PCBs. They had very poor performance in Copy and Immediate Recall tests and in general they recalled less information on the Construction/Memory score. Although these results could be explained in some way by F, Pb, As or DDE exposure, needs to be interpreted with caution because of the low sample size. Social factors and the low quality of education in the schools that are prevalent in the areas could be playing an important role on test performance.

Conflict of interest

There are no conflicts of interest.

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