ORIGINAL ARTICLE

Adverse Effect of Pre- and Postnatal Exposure to Carbamate and Pyrethroid Pesticides on Cognition in Children at 4 and 6 Years of Age: A Prospective Study

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ABSTRACT

Objective. To determine the adverse effect of pre- and postnatal exposure to carbamate (propoxur) and pyrethroid pesticides on children's cognitive functions at 4 and 6 years of age.

Method. As part of a prospective cohort study among children with known pre- and postnatal exposure to propoxur and pyrethroids, children were examined at time points, 4 and 6 years, to determine the adverse effect of pesticide exposure on neurocognitive function, i.e., intelligence (IQ) using the WPPSI-III test. Pre- and postnatal pesticide exposures were measured by meconium and hair analysis, respectively, using gas chromatography-mass spectrometry (GCMS).

Results. Seven hundred twenty-four (724) maternal/children dyads were seen at four years and 717 at six years of age. Their mean (SD) full-scale IQ was low on average: 83.9 (10.2) at four years and 83.6 (8.6) at six years. Prenatal but not postnatal exposure to propoxur (-0.139, p=0.01) and pyrethroids (-0.097, p=0.05) were significantly correlated (negative) with full-scale IQ at four years but not at six years. The confounders that were significantly correlated to full-scale IQ at four and/or six years of age were maternal IQ, child's weight, height, head circumference, socio-economic status, child environment, and stimulation at home (HOME) violence or abuse at home. Regression analysis of pesticides and confounders showed similar results, except for weight and head circumference.

Conclusion. We conclude that prenatal exposure to propoxur and pyrethroids had a negative effect on the children's IQ at four years but no longer at six years. Thus, the ability of the child's IQ to recover from the adverse effect of intrauterine pesticide insult may be attributable to the neuronal plasticity of its brain. Similarly, confounders to these outcome measures are multiple and are essential to address when evaluating the effect of pesticides on neuro-cognitive development in children.

Keywords: carbamate, propoxur, pyrethroids, pesticide, IQ, neurocognitive development

INTRODUCTION

Due to the widespread use of pesticides, exposure of pregnant women to pesticides is of major concern since pesticides are neurotoxic to the developing and vulnerable fetal brain.[1-8] Adverse effects on the subsequent neurobehavioral, cognitive, and learning development have been described in the children prenatally exposed to organophosphates, e.g., decreased scores on the Stanford-Binet copying test, mean reaction time, short term memory, executive function, and lower MDI and PDI scores on the

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Bayley Scales of Infant Development) and to carbamates, e.g., negative trends in children's adaptive, social, language, and total average developmental quotient, and poorer cognitive function in working memory and verbal comprehension.[9-21] Subsequent ban on using organophosphates and carbamates, particularly in home pesticides, has led to their replacement with pyrethroids due to the latter's lower toxicity in children. However, adverse effects from exposure to pyrethroids have also been reported in both animals, e.g., delayed growth, increased anxiety-like behaviors, spatial learning, and memory difficulties in children, e.g., lower scores on the arithmetic test, poorer working memory, and verbal comprehension, abnormal social behavior, poorer visual-motor coordination, increased prevalence of cognitive problems/ inattention, oppositional disorders and decreased ability to discriminate colors.[22-29]

We have reported adverse neurodevelopment at age two years among children pre- and postnatally exposed to propoxur and pyrethroids.[30] At two years of age, prenatal exposure to propoxur had a significant negative effect on the child's motor development as assessed by the Griffiths Mental Development Score but not with prenatal exposure to pyrethroids.[30] We have continued to follow these children up to the age of 4 and 6 years to determine the progress of developmental deficits and the possible appearance of delayed neurodevelopmental toxicity, particularly to pyrethroids. Other factors such as demographics, socioeconomic characteristics, parental education and income, exposure to lead, and nutrition that affect neurodevelopment in children were also studied.[3,31]

The cognitive outcome of the children at 2 and 4 years of age in this extensive, longitudinal study is the subject of this report.

METHOD

Pregnant women were prospectively enrolled in the study at midgestation at the Bulacan Provincial Hospital (BPH). Bulacan is an agro-industrial province in the Philippines. The following pesticides are predominantly used at home or in the farm: cyfluthrin/propoxur (73%), chlorpyrifos (37%), cypermethrin (31%), pretilachlor (28%), bioallethrin (26%), malathion (15%), diazinon (12%) and transfluthrin (11%). This study was approved by the Human Investigation Committee at Wayne State University, the University of the Philippines, and the Bulacan Provincial Hospital. Informed consent was obtained from the mothers for themselves and their children. Prenatal exposure to pesticides in the infant was determined by analyzing maternal blood at mid-gestation and delivery, umbilical cord blood, infant hair, and meconium at birth using gas chromatography-mass spectrometry or GCMS.[32-35] Postnatal exposure of the children to pesticides were analyzed in the children's hair by GCMS.[36] Lead in children's hair and teeth was analyzed by atomic absorption spectrophotometry.[37]

Neurodevelopmental Outcomes

The infants/children were prospectively studied to test their cognitive function from birth 4 and 6. A total of 793 mother/infant dyads were initially enrolled at birth. However, six children died soon after hospital discharge, leaving a total of 787 maternal/infant dyads for follow-up at the 4- and 6-year assessments. Of the 787 children, 724 children were seen at four years and 717 children at six years. The dropout rate on follow-up of approximately 9% of the initially enrolled children was due to death, inability to locate the families who moved to other places, and voluntary withdrawal from the study.

Each infant underwent a battery of neurobehavioral and cognitive tests at two-time points of 4 and 6 years of age to assess the following domains: general cognition, attention & executive function, memory, and behavior by the Wechsler Preschool and Primary Scale of Intelligence (Third Edition) which was the WPPSI version at the time of the study.[38] The WPPSI-III assesses intelligence quotient (IQ) in children aged 2 yrs, six months through 7 years three months and provides Verbal, Performance and Full-Scale IQ and Processing Speed Quotient (known as the Processing Speed Index).

Several potential predictors of children's neurodevelopment were also studied, e.g., infant's gender, maternal age, maternal intelligence by a modified WAIS-III performance subscale, home environment and home stimulation of the child by the Home Observation for Measurement of the Environment or HOME, home violence by the Parent-Child Conflict Tactics Scale and KIDSAVE at six years, physical functional and social support by the Norbeck test, Conflict Tactile Scale, anthropometric measures (body mass index or BMI, weight, length, and head circumference), and socioeconomic status (SES) by the Roberto scale.[39-45] The Roberto scale is a short, practical scale to assess SES in the Philippines and is based on home structure and appearance. The scale ranges from A with 1 (highest score) to E with 5 (lowest score). A high numeric value on the Roberto scale is indicative of low socioeconomic status.

Before study inclusion, a pilot test was conducted to determine the validity of the WAIS-III to test maternal intelligence and to determine subtests that were suitable for the study population.[39] A group of 30 females that were not among the current subjects but from the same people were administered the performance subtest of the WAIS-III. Based on their performance scores, the following subtests were selected to comprise the short form for computing the performance IQ: picture completion, matrix reasoning, picture arrangement and object assembly (unpublished).

Statistical analysis

Mean (standard deviation) and frequency distribution were calculated to describe the demographic and socioenvironmental characteristics of the study population. Pearson's correlation coefficient correlated predictors to the WPPSI

III Full-scale IQ scores to maternal intelligence and children, pre- and postnatal pesticide exposure, lead exposure, anthropometric measures, HOME score, gender, and exposure to violence at home. Pesticides with an exposure rate of <1% were excluded from the correlation analysis. However, due to a similar mechanism of action of the pyrethroids (transfluthrin, cypermethrin, bioallethrin), they were grouped and analyzed as grouped pyrethroids (2.5%) despite low individual exposure rates and concentration. Hierarchical regressions further examined factors that significantly correlated to the full-scale IQ by initially forcing in all the covariates and prenatal pesticides, then stepwise entry of lead, 2, 4, and 6-year pesticides, and finally stepwise entry of head circumference, weight, and length at 4 and 6 years of age.

Structural equation modeling using LISERL 8.72 was also performed to determine the effects of fetal and postnatal exposure to pesticides on cognitive development at 4 and 6 years as assessed by WPPSI III while controlling for the following confounders: socioeconomic status, maternal intelligence, and HOME, domestic violence. Due to non-normality in the data, the Satorra-Bentler scaled chi-square was used.[45-47] Paths that were not significant at $p \leq 0.05$ were removed from the model unless there were strong theoretical reasons for including them. Modifications to the model were based on both substantive clinical and statistical grounds. For the final model, results are reported as standardized regression coefficients. Before performing the path analysis, SPSS version 19 was used to assess the assumptions of linearity and multivariate normality. To identify multivariate outliers, Mahalanobis distance, which is a measure of the multivariate distance of a case from the centroid of all other cases, was used.[48] Mahalanobis distance can be evaluated using a χ^2 statistic. The probability of the χ^2 statistic indicates the probability that the values for that case come from the same multivariate distribution of the other cases. A criterion of $p<0.001$ was used to indicate multivariate outliers. Cases with $p<0.001$ have a minimal probability of coming from the same multivariate distribution as the other cases. Using this criterion, multivariate outliers were removed from the data set. An iterative process was used to clean the data. Univariate outliers were winsorized to reduce the influence of outlying cases, and highly skewed variables were transformed.

RESULTS

At the two time points of 4 and 6 years of age, a total of 724 mother/child dyads were tested at four years and 717 dyads at six years. The demographic characteristics of the study population were as follows: Based on the Roberto scale, the majority of the subjects belonged to the middle to low socioeconomic status (Class C = 51%, D=36.7%, E=4%). The highest level of parental education was up to high school for the father (50.7%) and the mother (57%). 67% of the fathers were non-skilled laborers for their parent's occupation, and 61.9% of the mothers were homemakers.

The prevalence of exposure to pesticides is shown in Tables 1 and 2. The specimens were not evenly available for analysis which explains the difference in sample sizes.

The exposure rate by meconium analysis was 21.3% and 2.5% to grouped pyrethroids. The prenatal exposure rate to some pesticides were low (diazinon 0.1%, lindane 0%, malathion 0.3%, chlorpyrifos 0% and DDT 0.5%, and were not included in the table. Meconium analysis was the most sensitive test to detect prenatal exposure compared to cord blood, infant hair, and maternal hair.

Postnatal exposure rates at 4 and 6 years of age to propoxur and pyrethroids by hair analysis are shown in Table 2.[36]

The exposure rate to propoxur decreased from 12.4% at four years to 1.7% at six years. On the other hand, the exposure rate to bioallethrin increased from 12.4% at four years to 18.4% at six years, and transfluthrin from 1% at four years to 9.2% at six years.

WPPSI-III

The IQ scores based on the WPPSI-III test at 4 and 6 years of age are shown in Table 3. The fewer Processing Speed Composite scores at age four years (N=517) were due

Table 1. Prenatal exposure rate to propoxur and pyrethroids

	Meconium (N=717)	Cord blood (N=630)	Infant hair (N=730)	Maternal hair (N=740)
Propoxur	153 (21.3%)	12 (1.9%)	1 (0.1%)	156 (21.1%)
Grouped Pyrethroids	18 (2.5%)	0 (0%)	0 (0%)	103 (13.9%)

Table 2. Comparison of pesticide prevalence and median concentration (ng/g) for children's hair at 4 and 6 years of age)[a]

Pesticide	Prevalence (%) and concentration (ng/g)		Comparing prevalence	Comparing concentration
	Four years (N=711)	Six years (N=294)	Four vs. six (N=292)	Four vs. six
Propoxur	24.1 (29.5)	1.7 (28.6)	<0.001	<0.001
Transfluthrin	1.0 (107.9)	9.2 (102.4)	<0.001	0.001
Bioallethrin	12.4 (271.4)	18.4 (347.2)	0.016	0.049

[a] *Values given are prevalence and, for positive samples, their median concentrations.*

Table 3. WPPSI-III scores at 4 and 6 years of age

	N	Mean	SD	N	Mean	SD
		(4 years)			(6 years)	
Full IQ	720	83.9	10.0	714	83.6	8.6
Verbal Composite	721	80.8	8.2	714	79.7	6.3
Performance Speed Composite	724	88.4	12.0	715	87.8	11.2
Processing Speed Composite	517	104.3	15.0	714	104.3	13.0
Language Composite	724	76.5	9.1	714	79.3	7.3

Table 4. Pearson correlation coefficients of factors that were significantly (*p <0.05; **p<0.01) related (2-tailed test) to the WPPSI-III full scale IQ scores at 4 and 6 years of age

	Descriptives (Mean ± SD or Frequency %)	Full scale IQ score at 4 years	Full scale IQ score at 6 years
Maternal IQ / Index Score	75.6 (10.5)	0.283**	0.333**
Head circumference (cm) 4 years	48.5 (1.4)	0.184**	0.237**
Head circumference (cm) 6 years	49.5 (1.4)	0.226**	0.241**
Weight (kg) 4 years	15.9 (2.6)	0.184**	0.212**
Weight (kg) 6 years	19.7 (3.8)	0.229**	0.247**
Length (cm) 4 years	103.2 (4.9)	0.199**	0.265**
Length (cm) 6 years	114.2 (5.7)	0.248**	0.292**
Child report of overall physical violence	89.80%	-0.086*	-0.048
Frequency child traumatic violence	22.70%	-0.133**	-0.077*
Frequency child physical/verbal abuse	47.70%	0.032	-0.098
SES (Roberto Scale)	2.67	-0.167**	-0.195**
Total HOME score	22.2	0.223**	0.259**
Propoxur (meconium) μg/g	0.076 (0.206)	-0.139**	-0.029
Pyrethroids (meconium) μg/g	0.025 (0.157)	-0.097*	-0.057

to some children who could not adequately hold the pencil to take the test correctly. The number of children increased to N=714 at six years.

At four years of age, the children's scores were below average. Scores on both Full IQ subtest and Verbal IQ (mean full IQ = 83.9, 18^{th} percentile and mean Verbal IQ = 80.8, 13th percentile, respectively) were more than 1 SD below the normative scores (normative mean IQ = 100; SD=15).[38] Although the children had higher scores on the Performance subtest (t=19.32, df=719, p<.001) the Performance IQ scores (88.4, 27^{th} percentile) were still below normal. Children performed the worst on the General Language IQ subtest (mean Language Composite score =76.5). However, higher scores were seen in their Processing Speed performance (Mean Processing Speed IQ score = 104.3), slightly above the normative mean of 100. At six years of age, except for Processing Speed Performance, the children again scored below average. Their full IQ score (mean = 83.6, 17^{th} percentile) was virtually identical to the age four assessment (mean = 83.9, 18^{th} percentile) and was more than 1 SD below the mean normative IQ score of 100 ± 15.[38] Similar to the age 4-assessment, the children scored significantly better on the Performance portion of the WPPSI in comparison to the Verbal (t=22.69, df=713, p<.001). However, the Performance IQ score at age 6 was still well below normative scores (87.8, 25^{th} percentile).

Correlation and regression

The factors that were significantly correlated to the WPPSI-III full IQ scores are shown in Table 4.

The WPPSI-III full IQ scores at 4 and 6 years of age were positively correlated to maternal IQ, head circumference, weight, length, physical functional and social support (r=0.131 at four years and r=0.188 at six years), total HOME score and negatively correlated to traumatic violence at home, child report of overall physical violence, and the Roberto scale of socioeconomic status. Prenatal exposure to propoxur and pyrethroids by meconium analysis were negatively correlated to full scale IQ scores at four years but not at six years. Infant's gender, maternal age, children's postnatal exposure to pyrethroids and propoxur as determined by hair analysis, postnatal exposure to environmental lead from analysis of children's hair and deciduous teeth, and BMI did not correlate to WPPSI-III full IQ scores at 4 and 6 years of age and are not included in the table. We did not extract blood for lead analysis in the children at 4 or 6 years

Table 5. Standardized regression coefficients of WPPSI-III Full Scale IQ in children at 4 and 6 years

	Standardized coefficients 4 years	Sig.	Standardized coefficients 6 years	Sig.
	Beta		Beta	
IQ / Index Score	0.188	0.000	0.232	0.000
KID-SAVE frequency traumatic violence	-0.116	0.015	-0.124	0.008
Socioeconomic (Roberto Scale)	-0.135	0.006	-0.089	0.063
Total HOME score	0.117	0.023	0.104	0.039
Propoxur (meconium) µg/g	-0.138	0.002	-0.052	0.230
Pyrethroids (meconium) µg/g	-0.141	0.002	-0.025	0.563
Length (cm)	0.151	0.001	0.214	0.000

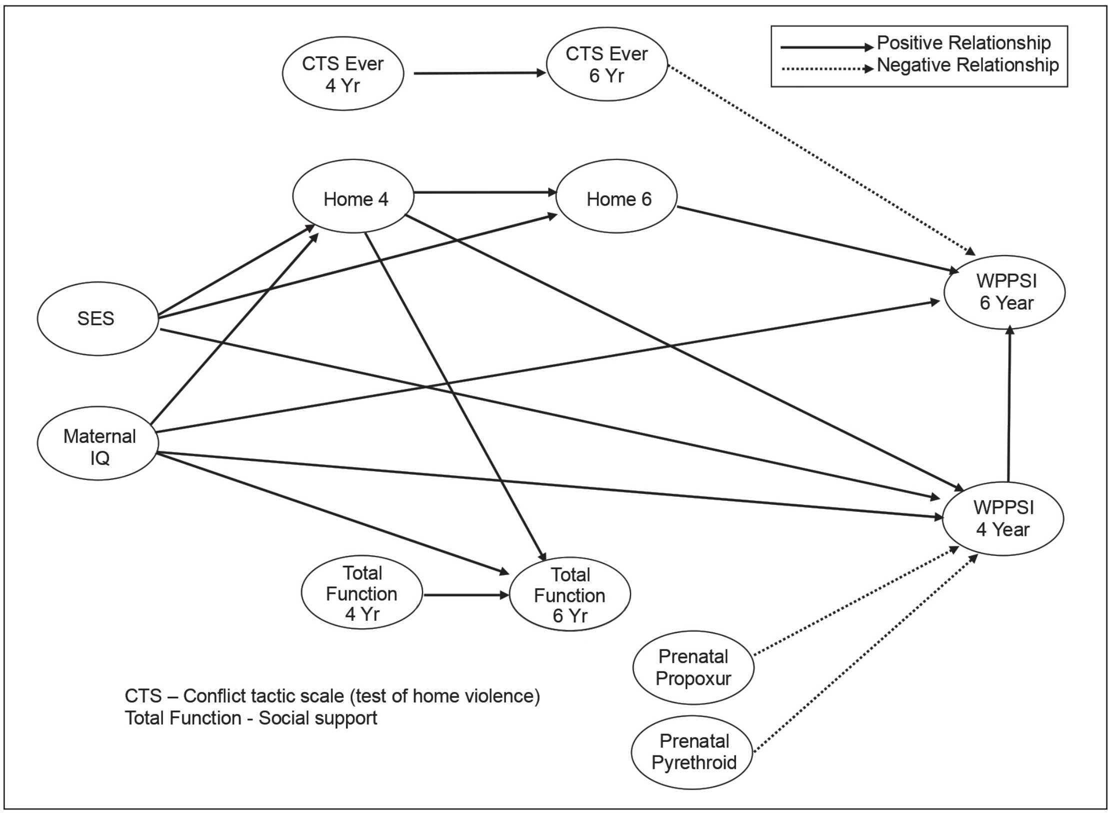

Figure 1. Path analysis showing the relationship of prenatal exposure to propoxur and pyrethroids and covariates to the WPPSI III IQ outcome at 4 and 6 years of age.

of age because we were concerned about the traumatic effect of blood extraction on their performance while being tested on various neurobehavioral tests, which required their full cooperation and attention. Instead, we determined lead in fallen, deciduous teeth of the children as an index of lead deposition in tissues.

Regression analysis showed that maternal IQ, traumatic violence at home, socioeconomic status, and child's height remained significantly correlated to full IQ scores at 4 and 6 years of age (Table 5). Pre- but not postnatal, exposure to propoxur and pyrethroids was also negatively associated with full IQ scores at four years but not at six years.

Structural equation modeling (path analysis) was also performed to determine the effects of fetal and postnatal exposure to pesticides on cognitive development at 4 and 6 years as assessed by WPPSI III while controlling for the following covariates, viz., socioeconomic status, maternal intelligence, home violence (C TS), physical and functional social support and HOME. The path analysis model is shown in Figure 1.

Prenatal propoxur and pyrethroids were negatively related to WPPSI III at four but not at six years of age. Home stimulation of the child and home environment (HOME), socioeconomic status, and maternal IQ were positively related to IQ at 4 and 6 years. Violence at home (CTS) at four years and six years was negatively related to WPPSI III IQ, which indicates the adverse effect of the continuing pattern of violence at home. Physical and functional social support were mutually and positively associated with maternal IQ and HOME.

DISCUSSION

Previous reports have shown that exposure to organophosphates during prenatal, postnatal, or both were associated with poor neurodevelopmental scores in children on the Stanford-Binet copying test, mean reaction time, short-term memory, executive function, and mental and physical development index scores on the Bayley Scales of Infant Development.[11-16] Consequently, organophosphates for residential use have been replaced by carbamates (e.g., propoxur) and pyrethroids due to their lower toxicity and shorter half-life. However, despite substituting organophosphates with carbamates and pyrethroids in home pesticides, neurodevelopmental toxicity from exposure to these pesticides has been reported in animals and humans, particularly in children.[20-30,49,50]

In this report, we present the cognitive development by the WPPSI-III test of intelligence of a large cohort of children who were pre- and postnatally exposed to pesticides and extensively studied from birth to 4 and 6 years of age with a high (>90%) follow-up rate. This study is significant because it utilized extensive methods to detect pre- and postnatal pesticide exposure by analyzing various matrices, including maternal hair, cord blood, infant hair, meconium, and children's hair. Furthermore, essential confounders to the child's neurodevelopment were studied, including infant's gender, maternal age, maternal intelligence, status of the home environment and home stimulation of the child, environmental lead, home violence, anthropometric measures (body mass index or BMI, weight, length, and head circumference), physical functional and social support and socioeconomic status.

The study showed that meconium analysis was the most sensitive method to detect prenatal pesticide exposure in newborn infants (Table 1). Among various pesticides commonly used in the area, propoxur (21.3%) and pyrethroids (2.5%), the main ingredients of home pesticides, were the leading prenatal pesticides found. This provides strong evidence that home pesticides are the most critical source of pesticide exposure in the mother and her children. The postnatal exposure of the children to pesticides, by hair analysis, showed an increase in exposure rate to propoxur at 24.1% at four years, and then a decrease to 1.7% at six years due principally to the subsequent ban in 2007 on using propoxur as a component of home pesticides because of its potential toxicity in children.[18] On the other hand, the exposure rate to bioallethrin increased from 12.4%% at four years to 18.4% at six years and to transfluthrin (1.0% at four years and to 9.2% at six years) due predominantly to widespread use in the houses of slow-burning mosquito coils which contained bioallethrin due to the fear of dengue and to a change in the formulation of home pesticide sprays from propoxur to transfluthrin due to the propoxur ban.

Regarding the WPPSI-III scores of the children (Table 3) at four years of age, the mean scores were below average. Scores on both full-scale composite IQ (mean score =83.9) and Verbal IQ (mean score = 80.8) were more than 1 SD below the normative mean IQ of 100 ± SD=15).[38] The Performance IQ scores (mean score = 88.4) were still below normal. Children performed the worst on the General Language IQ subtest (mean score=76.5), likely because English was not a common language for the children. On the other hand, higher scores were seen in their Processing Speed performance (mean score = 104.3). The WPPSI-III scores at six years of age did not improve and were virtually identical to the age four assessment. Like the age four assessment, the children scored significantly better on the Performance portion of the WPPSI in comparison to the Verbal ($t=22.69$, $df=713$, $p<.001$). However, the Performance IQ score at age 6 was still well below normative scores (mean score = 87.8, 25th percentile). The low mean full scale composite IQ scores of Filipino children at 4 and 6 years of age in this study are problematic since these scores have shown no improvement since 1970 based on a published report in 2002 on the national mean IQ of children worldwide.[51] The report showed that the mean IQ of 12-13-year-old Filipino children in1970 and 1979 was equal to 86. Based on this report, our study's mean IQ score of 4 to 6-year-old Filipino children remained low (mean IQ=83.6) and showed no improvement. It was considered that children's IQ theoretically increases by 2 points every ten years, known as the Flynn effect.[52] The mean IQ of Filipino children has not improved for the past 40 years! The result is reflected in the low average scores of grade-6 and high school Filipino children in a National Achievement Test in 2010-2011. Among 1,608,520 examinees in grade 6, the mean scores in Mathematics were 68.41%, in English, 65.11%, and in science, 60.35%. Among 1,544,006 examinees in high school, the mean scores in Mathematics were 42%, in English, 46.45%, and in science, 39.35%.[53] The direct relationship of low IQ scores to poor performance in national standardized tests has been demonstrated worldwide.[54]

Unfortunately, there is also a direct relationship between the IQ scores of a country's populations and its economic per capita income.[55] Thus, a nation's economic prosperity and productivity hinge significantly on the IQ of its population.

The main objective of this paper was to determine the relationship of pre- and postnatal exposure to pesticides in children's to its IQ since pesticides are known neurotoxicants to the developing and vulnerable fetal and children's brains.[4-6] By extensive analysis of various matrices to detect prenatal pesticide exposure and the long-term follow-up of pesticide exposure during childhood by hair analysis, these neurotoxicants to children's cognitive function were demonstrated. Interestingly, prenatal exposure to propoxur and pyrethroids significantly negatively affected the children's IQ at four years. Still, the effect was no longer evident at six years of age (Table 4). Of further note from a previous publication from the same cohort[30] that pyrethroids showed no adverse effect on the neurologic function of the children at age two years but became evident at four years of age. The latter is consistent with the phenomenon of delayed neurotoxicity. The delayed manifestation of neurobehavioral abnormalities (delayed neurotoxicity) stemming from fetal exposure to neurotoxic agents is well recognized and has been attributed to three possible causes[56,57]: (1) The ontogeny of a particular neurologic function may occur late in development; thus, the manifestation of pathological changes is not revealed until the function in normal animals is apparent; (2) The anatomical and functional effects of the developmental insult may be masked or attenuated due to neuronal compensation (neuronal plasticity), resulting in transient effects that may nonetheless have later sequelae. Delayed neurotoxicity has been shown to occur in primates and rodents experimentally exposed to environmental toxicants, including methyl mercury and chlorpyrifos.[57-59]; (3) One of the prominent features of the CNS concerning developmental neurotoxicity is the sheer length of time over which development of the CNS proceeds. Thus, delayed neurotoxic effects secondary to prenatal exposure to neurotoxicants may extend into later life (long latency delayed neurotoxicity). For instance, developmental exposure of rodents to triethyltin produced damage to cortical structures of the nervous system and transient deficits in spatial learning.[56] Apparent compensation at the anatomical level was evident, with some reactive synaptogenesis from surviving cells in the entorhinal cortex and recovery from spatial learning deficits during adulthood. However, when littermates were examined morphologically and functionally in later life, decreases in synaptic densities and deficits in spatial learning became apparent. Delayed neurotoxicity is, therefore, a plausible explanation for the delayed emergence of toxic manifes-tations in children who have been prenatally exposed to neurotoxic agents, such as pesticides.

On the other hand, in this study, the disappearance of the adverse effect of prenatal propoxur and pyrethroids on the children's IQ at age six years, compared to 4 years, may be due to compensatory mechanism attributable to neuronal plasticity. Neuronal plasticity has been observed in several species and acts as a compensating mechanism in brain development.[57-60] The nervous system continues to remodel and change early in development and throughout the entire period of growth in response to environmental influences and genetically programmed events. As a result, the toxic effects on the CNS observed at an early age may no longer become evident in later life.

Another interesting observation in this study was the adverse effect on the children's IQ of prenatal but not postnatal exposure to propoxur and pyrethroids. This phenomenon is not unique and has been observed by other investigators with organophosphates and lead. In a study of 7-year-old Ecuadorian children, prenatal but not postnatal exposure to organophosphates was associated with decreased scores on the Stanford-Binet copying test.[15] Bellinger also reported in a longitudinal analysis of prenatal and postnatal lead exposure and early cognitive development and found that the Bayley scores were not related to the infant's postnatal blood lead levels.[61] The most significant neurotoxicant effect on the brain occurs during fetal life when brain development is in a state of rapid growth and development and, therefore, is highly vulnerable to the adverse effect of the toxicants.[4-6] Although there could have been some adverse effects on the neurobehavioral development of postnatal exposure to pesticides, its outcome could have been masked by the magnitude of the effect of prenatal exposure.

Lead in hair and teeth did not correlate with IQ scores at 4 and 6 years. The exposure to lead was high in the area.[36] In a pilot study examining sources of lead exposure in 150 children in a study population, the children's hair was 91.3% lead positive (median concentration or MC = 8.9 µg/mg, IQR = 5.5 – 13.4) – Table 6.

The teeth were lead positive in 42.6% of the samples analyzed, and the soil was 100% positive for Pb. The highest lead level in the soil was 1155.80 mg/kg. The most likely cause of the high lead content in the soil was the overflow of lead-contaminated water from rivers surrounding the region (Ostrea et al., 2015). The air (N=19) was 21% positive for lead, and home faucet water (N=150) was 4% positive for lead. There was a significant correlation between Pb in

Table 6. Prevalence and concentration of lead (Pb) in children's hair, teeth, house soil, environmental air, home faucet water, and river water

	N	% positive	Median	Interquartile Range	Minimum	Maximum
Pb in hair (ppm)	150	91.3%	8.92	5.48 – 13.42	0.00	38.29
Pb in teeth (µg/mg)	143	46.2%	0.0	0.00 – 0.005	0.00	0.02
Pb in soil (mg/kg)	150	100.0%	27.06	14.72 – 55.97	3.05	1155.80
Pb in air (µg/Ncm)	19	21.1%	0.0	0.0 – 0.0	0	0.10
Pb in faucet water (ppm)	150	4.0%	0.0	0.0 – 0.0	0	40
Water in river (ppb)	7	100.0%	70.00	60 – 70	30	90

children's hair and soil (Spearman's rho = 0.195; p=0.017) and between lead in faucet water and outdoor air (Spearman's rho = 0.616; p=0.005). On the other hand, there was no significant correlation between Pb in children's hair and teeth (Spearman's rho = -0.001; p=0.993) and between Pb in teeth and soil, faucet water, and outdoor air. (Ostrea et al., 2015). Although the children's exposure to lead from the environment, predominantly from the soil, was high, we did not observe a significant correlation between lead in hair and teeth and the children's IQ at 4 and 6 years. It might be that the ingestion of lead was low, as evidenced by the low concentration of lead in teeth (MC = 0.000 μg/mg in positive samples; range = 0.00–0.020). Unfortunately, blood lead levels were not examined to confirm this.

By regression analysis of the various factors that correlated to children's IQ scores, maternal IQ, socioeconomic status, home stimulation of the child, home violence, and nutrition (represented by its effect on children's length) had a more lasting adverse effect on the children's IQ at both 4 and 6 years than pesticide exposure. Thus, interventions to improve cognitive function in children must include prevention of exposure to environmental toxicants and improvement in other vital areas, such as violence at home,[62] home stimulation of the children, and nutrition.

The interrelationship of the pesticides and various confounders are more graphically shown in the path analysis model. Maternal IQ, socioeconomic status, home stimulation of the child was all related to affect the child's IQ at 4 and 6 years and were interrelated to one another, which indicates that many factors interacted and contributed to the low average IQ of the children.

There are some limitations in the study, e.g., using the WPPSI-III in Filipino children, the uneven sample size of specimens analyzed, the number of children tested, and the time interval of the study to the date of this report. The low average cognitive performance of the children in the WPPSI-III test may have been influenced by WPPSI-III items, especially in general language, that is not culturally fit the Filipino language and the children's learning experiences. The latter is shown in the HOME total score, and many of its subscales are significantly lower in the study population than in the US normative sample (Table 7).

The HOME measures parenting quality, stimulation quantity, and the amount of support available to a child in their home environment. It focuses on the child in the background, as a recipient of inputs from objects, events, and transactions with the family surroundings. Although cultural and environmental differences would impact the WPPSI-III, this impact applies to all children in the study population. Thus, the WPPSI may not be valid to compare children in the Philippines to children in other countries (between-group differences). Still, it remains useful as a test when looking at within-group differences.

Furthermore, the WPPSI-III had components not affected by cultural differences wherein the Filipino children performed well (see Processing Speed in Table 3). It should also be noted that the WPPSI-II scores were also low in the other subtests, such as Verbal composite (mean=80.8) and Performance speed (88.4). The Verbal IQ (VIQ) tests acquired knowledge, verbal reasoning, and comprehension, and attention to verbal stimuli, and Performance IQ (PIQ) tests for a fluid reason, spatial processing, attentiveness to detail, and visual-motor integration (Block Design, Matrix Reasoning, Picture Concepts). These subtests are not necessarily culture-dependent. Furthermore, the fact that the WPPSI-III scores in the study were unchanged at 4 (mean=83.9) and six years (mean=83.6) of age when typically most children score slightly higher the second time due to the "practice effect" and lastly, the low IQ scores of the children in the current study which are similar to the IQ scores (mean=86) of Filipino children in the years 1970 and 1979 indicate that the low average IQ scores of the Filipino children cannot be ignored solely because of cultural differences.

The interval in the timing of this report to the end of the study of approximately ten years may affect the conclusions of this report. However, the study results are still valid, considering that poverty (SES) strongly determines

Table 7. HOME test of children in the study (Philippines) compared to USA norms (Student "t" test)

	Philippines (n=717)		USA Norms (n=124)		t	p
	Mean	SD	Mean	SD		
Responsivity	7.87	1.86	8.4	2.3	-2.43	0.016
Encouragement Maturity	5.07	1.77	4.8	1.6	1.71	0.090
Emotional Climate	5.38	1.37	6.0	1.6	-4.07	<0.001
Learning Materials	3.22	1.81	5.2	2.0	-10.32	<0.001
Enrichment	2.80	1.42	3.4	2.2	-2.93	0.004
Family Companionship	3.49	1.25	4.1	1.4	-4.55	<0.001
Family Integration	2.80	0.84	2.4	1.2	3.57	<0.001
Physical Environment	4.84	2.56	6.8	1.7	-10.88	<0.001
Total Score	35.47	8.56	41.6	9.0	-7.05	<0.001

children's IQ. As of 2018, the poverty level was still high in the Philippines: 16.6% of the population was living below the national poverty level, the proportion of employed population below $1.90 purchasing power parity a day, which is a strong index of poverty, was 2.7% and for every 1000 infants born, 28 die before their 5th birth.[63-65]

The "p" value in some of the correlation and regression coefficients was significant since the values were at or below the level set for statistical significance. A value of $p<.05$ indicates evidence against the null hypothesis, a 5% probability that the null hypothesis is correct, and a ≥ 95% probability that the alternative hypothesis is true. However, one should be careful in interpreting the "p" value as it only indicates an "association" but not a "causal" relationship between a variable and the outcome measure. The *p*-value is also conditional upon the null hypothesis being true but unrelated to the alternative hypothesis' truth or falsity. Instead, it merely indicates that our results "provide support for" or "give evidence for" our research hypothesis. We, therefore, state that there was a significant trend towards the association of the variable and outcome measure based on the significant "p" value.

For an ethical reason, the information from this study will be adequately utilized by a planned virtual conference in 2022 with important stakeholders in government, education, environmental agencies, social work, child welfare, nutrition, and medical and public health to provide information that will be useful in developing evidence-based national intervention programs to improve neurobehavioral and cognitive outcomes in Filipino children.

We conclude that based on a robust and large study population, a significant follow-up rate of the subjects, and an extensive analysis of various matrices to detect pesticide exposure, that prenatal exposure of children to propoxur and pyrethroids have associated adverse effects on their IQ at four but not at six years of age. Other factors such as maternal IQ, socioeconomic status, home stimulation of the child, home violence, and nutrition (represented by its effect on children's length) are also associated with adverse effects on children's IQ. Thus, interventions to improve cognitive function in children must include not only prevention of exposure to environmental toxicants but improvement in other associated areas, as well.[3]

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Statement of Authorship

All authors contributed in the conceptualization of work, acquisition and analysis of data, drafting and revising and approved the final version submitted.

Author Disclosure

All authors declared no conflicts of interest.

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REFERENCES

1. U.S. EPA. Pesticides Reregistration [Internet]. [cited 2007 Apr 25]. Available from: http://www.epa.gov/fedrgstr/EPA-PEST/2007/April/Day-25/p7769.htm).
2. Waliszewski SM, Aguirre AA, Infanzon RM, Rivera J, Infanzon R. Time Trend of Organochlorine Pesticide Residues in Human Adipose Tissue in Veracruz, Mexico: 1988–1997 survey. Sci Total Environ 1998;221:201–4.
3. Dórea J. Exposure to Environmental Neurotoxic Substances and Neurodevelopment in Children from Latin America and the Caribbean. Environ Res 2021;192,1-20
4. Bruckner JV. Differences in Sensitivity of Children and Adults to Chemical Toxicity: The NAS Panel Report. Regul Toxicol Pharmacol. 2000;31:280-5.
5. Eriksson P. Developmental Neurotoxicity of Environmental Agents in the Neonate. Neurotoxicology 1997;18:719–26.
6. Barone S Jr, Das KP, Lassiter TL, White LD. Vulnerable Processes of Nervous System Development: A Review of Markers and Methods. Neurotoxicology 2000;21:15–36.
7. Weiss B. Vulnerability of Children and the Developing Brain to Neurotoxic Hazards. Environ Health Perspect 2000;108(Suppl. 3):375–81.
8. Holland N, Furlong C, Bastaki M, Richter R, Bradman A, Huen K, Beckman K, et al. Paraoxonase Polymorphisms, Haplotypes, and Enzyme Activity in Latino Mothers and Newborns. Environ Health Perspect 2006;114:985–91.
9. Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, et al. Prenatal Organophosphate Metabolite and Organochlorine Levels and Performance on the Brazelton Neonatal Behavioral Assessment Scale in a Multiethnic Pregnancy Cohort. Am J Epidemiol 2007;165:1397–404.
10. Young JG, Eskenazi B, Gladstone EA, Bradman A, Pedersen L, Johnson C, et al. Association between in Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates. Neurotoxicology. 2005;26:199–209.
11. Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, et al. Organophosphate Pesticide Exposure and Neurodevelopment in Young Mexican–American Children. Environ Health Perspect 2007;115:792–8.
12. Handal AJ, Lozoff B, Breilh J, Harlow SD. Neurobehavioral Development in Children with Potential Exposure to Pesticides. Epidemiology 2007;18:312–20.
13. Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, et al. Impact of Prenatal Chlorpyrifos Exposure on Neurodevelopment in the First 3 Years of Life among Inner-city Children. Pediatrics. 2006;118:e1845–59.
14. Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. Attention-deficit/hyperactivity Disorder and Urinary Metabolites of Organophosphate Pesticides. Pediatrics. 2010;125:e1270-7.
15. Grandjean P, Harari R, Barr D, Debes F. Pesticide Exposure and Stunting as Independent Predictors of Neurobehavioral Deficits in Ecuadorian School Children. Pediatrics. 2006;117:546–56.
16. Ruckart PZ, Kakolewski K, Bove FJ, Kaye WE. Long-term Neurobehavioral Health Effects of Methyl Parathion Exposure

in Children in Mississippi and Ohio. Environ Health Perspect. 2004;112:46-51.
17. Bjørling-Poulsen M, Andersen HR, Grandjean P. Potential Developmental Neurotoxicity of Pesticides Used in Europe. Environ Health. 2008;22:7:50.
18. Rotenberg M, Shefi M, Dany S, Dore I, Tirosh M, Almog S. Differentiation between Organophosphate and Carbamate Poisoning. Clin Chim Acta. 1995;234:11–21.
19. US Environmental Protection Agency. Pesticide Industry Sales and Usage Report: 2000-2001 Market Estimates [Internet]. [cited 2009 Aug]. Available from: http://www.epa.gov/oppbead1/pestsales
20. Zhang J, Wu C, Lv S, Lu D, Qi X, Jiang S, et al. Exposure to Carbamate and Neurodevelopment in children: Evidence from the SMBCS Cohort in China. Environ Res. 2019;177:108590.
21. Rowe C, Gunier R, Bradman A, Harley KG, Kogut K, Parra K, et al. Residential Proximity to Organophosphate and Carbamate Pesticide Use during Pregnancy, Poverty during Childhood, and Cognitive Functioning in 10-year-old Children. Environ Res. 2016 Oct;150: 128-37.
22. Narahashi T. Nerve Membrane Ionic Channels as the Target of Toxicants. Arch Toxicol Suppl. 1986;9:3-13.
23. Gunier R, Bradman A, Harley K, Kogut K, Eskenazi B. Prenatal Residential Proximity to Agricultural Pesticide Use and IQ in 7-year-old Children. Environ Health Perspect. 2017;125(5):057002. doi: 10.1289/EHP504.
24. Liu JJ, Guo C, Wang B, Shi M, Yang Y, Yu Z, et al. Maternal Fenvalerate Exposure during Pregnancy Impairs Growth and Neurobehavioral Development in Mouse Offspring. PLoS ONE. 2018; 13: e0205403. https://doi.org/ 10.1371.
25. Wang N, Huang M, Guo X, Lin P. Urinary Metabolites of Organophosphate and Pyrethroid Pesticides and Neurobehavioral Effects in Chinese Children. Environ Sci Technol. 2016; 50:9627−9635 doi: 10.1021/acs.est.6b01219.
26. Viel J, Warembourg C, Maner-Idrissi G, LaCroix A, Limon G, Rouget F, et al. Pyrethroid Insecticide Exposure and Cognitive Developmental Disabilities in Children: The PELAGIE Mother-child Cohort. Environ Int. 2015;82:69-75.
27. Viel J, Rouget F, Warembourg C, Monfort C, Limon G, Cordier S, et al. Behavioural Disorders in 6-year-old Children and Pyrethroid Insecticide Exposure: The PELAGIE Mother-child Cohort. Occup Environ Med. 2017;74:275–281.
28. Watkins D, Fortenberry G, Sanchez B, Barr D, Panuwet P, Schnaas L, et al. Urinary 3-phenoxybenzoic Acid (3-PBA) Levels among Pregnant Women in Mexico City: Distribution and. Relationships with Child Neurodevelopment. Environ Res. 2016;147:307–13.
29. Wendel de Joode B, Mora A, Lindh C, Hernandez Bonilla D, Cordoba L, Wesseling C, et al. Pesticide Exposure and Neurodevelopment in Children Aged 6-9 Years from Talamanca, Costa Rica. Cortex. 2016;85: 137e150.
30. Ostrea EM Jr, Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, et al. Fetal Exposure to Propoxur and Abnormal Child Neurodevelopment at 2 Years of Age. Neurotoxicology. 2012;33: 669-75.
31. Wehby G, McCarthy A. Economic Gradients in Early Child Neurodevelopment: A Multi-country Study. Soc Sci Med. 2013;78:86-95.
32. Bielawski D, Ostrea E, Posecion N, Corrion M, Seagraves J. Detection of Several Classes of Pesticides and Metabolites in Meconium by Gas Chromatography/Mass Spectrometry. Chromatographia. 2005;62:623–9.
33. Corrion ML, Ostrea EM Jr, Bielawski DM, Posecion NC Jr, Seagraves JJ. Detection of Prenatal Exposure to Several Classes of Environmental Toxicants and Their Metabolites by Gas Chromatography-mass Spectrometry in Maternal and Umbilical Cord Blood. J Chromatogr B Analyt Technol Biomed Life Sci. 2005;882:221–9.
34. Ostrea EM Jr, Villanueva-Uy E, Bielawski DM, Posecion NC Jr, Corrion ML, Jin Y, et al. Maternal Hair – An Appropriate Matrix for Detecting Maternal Exposure to Pesticides during Pregnancy. Environ Res. 2006;101:312–22.
35. Posecion N, Ostrea E, Bielawski D, Corrion M, Seagraves J, Jan Y. Detection of Exposure to Environmental Pesticides during Pregnancy by the Analysis of Maternal Hair Using GC–MS. Chromatographia. 2006;64:681–7.
36. Ostrea EM Jr, Villanueva-Uy E, Bielawski D, Birn S, Janisse JJ. Trends in Long Term Exposure to Propoxur and Pyrethroids in Young Children in the Philippines. Environ Res. 2014;131:13-6.
37. Ostrea EM, Ostrea A, Villanueva-Uy E, Chiodo L, Janisse J. Alluvial and Riparian Soils as Major Sources of Lead Exposure in Young Children in the Philippines: The Role of Floods. Environ Sci Pollut Res Int. 2015;22:5082-91.
38. Wechsler D. Wechsler Intelligence Scale for Children–Fourth Edition Administration and Scoring Manual. San Antonio, Texas: Harcourt Assessment. 2003.
39. Weschler D. Manual for the Wechsler Adult Intelligence Scale-Revised. San Antonio, TX: The Psychological Corporation; 1981.
40. Caldwell BM, Bradley RH. Administration Manual, Revised Edition: Home Observation for Measurement of the Environment. In: Little Rock. University of Arkansas at Little Rock; 1984.
41. Straus MA. Conflict Tactics Scales. in Encyclopedia of Domestic Violence, N. A. Jackson. New York: Routledge: Taylor & Francis Group. 2007 pp. 190-197.
42. Flowers A, Lanclos NF, Kelley ML. Validation of a Screening Instrument for Exposure to Violence in African American children. J Pediatr Psychol. 2002;27:351-61.
43. Norbeck JS. Social Support: A Model for Clinical Research and Application. ANS Adv Nurs Sci. 1981 Jul;3(4):43-59.
44. Strauss JS. Social and Cultural Influences on Psychopathology. Annu Rev Psychol. 1979;30:397-415.
45. Roberto N. The Marketer's Guide to Socioeconomic Classification of Consumers: Insights and Challenges of Target Marketing in the Philippines Using SEC Indicators. Thousand Oaks CA: Sage Publications; 2002.
46. Hu L, Bentler P. Cutoff Criteria for Fit Indexes in Covariance Structure Analysis: Conventional Criteria versus New Alternatives. Struct Eq Model. 1999;6:1–55.
47. Satorra A, Bentler PM. Corrections to Test Statistics and Standard Errors in Covariance Structure Analysis. In: von Eye A, Clogg CC, editors. Latent variables analysis applications for developmental research, Thousand Oaks, CA: Sage; 1994. p. 399–419.
48. Tabachnick BG, Fidell LS. Using Multivariate Statistics. 5th ed. Allyn & Bacon; 2000, ISBN: 0205459382.
49. Bradberry SM, Cage SA, Proudfoot AT, Vale JA. Poisoning due to Pyrethroids. Toxicol Rev. 2005;24:93-106.
50. Xue Z, Li X, Su Q, Xu L, Zhang P, Kong Z, et al. Effect of Synthetic Pyrethroid Pesticide Exposure during Pregnancy on the Growth and Development of Infants. Asia Pac J Public Health. 2013;25(Suppl): 72S-9S.
51. Lynn R, Vanhanen T. IQ and The Wealth of Nations. Westport, CT: Praeger 2002.
52. Baker D, Eslinger P, Benavides M, Peters E, Dieckmann N, Leon J. The Cognitive Impact of the Education Revolution: A Possible cause of the Flynn Effect on Population IQ. Intelligence. 2015;49: 144–58 doi:10.1016/j.intell.2015.01.003. ISSN 0160-2896.
53. Philippine Statistical Yearbook (National Statistical Coordination Board) 2012.
54. Lynn R, Meisenberg G, Mikk J, Williams A. National IQs Predict Differences in Scholastic Achievement in 67 Countries. J Biosoc Sci. 2007;39(6):861-874
55. Lynn R, Vanhanen T. National IQs: A Review of Their Educational, Cognitive, Economic, Political, Demographic, Sociological, Epidemiological, Geographic and Climatic Correlates. Intelligence. 2012;40(2):226–34.
56. Barone S Jr, Stanton ME, Mundy WR. Neurotoxic Effects of Neonatal Triethyltin (TET) Exposure are Exacerbated with Aging. Neurobiol Aging. 1995;16:723-35.
57. Rice D, Barone S Jr. Critical Periods of Vulnerability for the Developing Nervous System: Evidence from Humans and Animal Models. Environ Health Perspect. 2000;108(Suppl. 3):511–33.

58. Bourgeois JP, Rakic P. Changes of Synaptic Density in the Primary Visual Cortex of the Macaque Monkey from Fetal to Adult Stage. J Neurosci. 1993;13:2801–20.
59. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent Overproduction of Synapses in Diverse Regions of the Primate Cerebral Cortex. Science. 1986;232:232–5.
60. King AJ, Moore DR. Plasticity of Auditory Maps in the Brain. Trends Neurosci. 1991;14:31–7.
61. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Longitudinal Analyses of Prenatal and Postnatal Lead Exposure and Early Cognitive Development. N Engl J Med. 1987;316:1037-43.
62. Martin J, Langley J, Millichamp J. Domestic Violence as Witnessed by New Zealand Children. N Z Med J. 2006; 119;U1817.
63. Perou R, Robinson L, Danielson M, Claussen A, Visser S, Scott K, et al. The Legacy for Children™ Randomized Control Trial: Effects on Cognition through Third Grade for Young Children Experiencing Poverty. J Dev Behav Pediatr. 2019 May; 40(4):275–84.
64. Asian Development Bank Poverty Data: Philippines. Asian Development Bank Basic Statistics Report. 2021.
65. Philippine Statistical Yearbook 2012. National Statistical Coordination Board (NSCB). Table 10.8.