

# PRENATAL AND CHILDHOOD EXPOSURE TO PESTICIDES AND NEUROBEHAVIORAL DEVELOPMENT: REVIEW OF EPIDEMIOLOGICAL STUDIES

JOANNA JUREWICZ<sup>1</sup> and WOJCIECH HANKE<sup>1,2</sup>

<sup>1</sup> Nofer Institute of Occupational Medicine, Łódź, Poland

Department of Environmental Epidemiology

<sup>2</sup> Medical University, Łódź, Poland

Department of Informatics and Medical Statistics

## Abstract

**Objectives:** Conventional pesticides comprise a diverse group of substances intended to destroy, repel or control organisms identified as pests. Compared to the studies on lead, mercury, and PCBs, few epidemiological studies have assessed the developmental neurotoxicity of pesticides. **Materials and Methods:** Epidemiological studies focused on the neurobehavioural development of children exposed to pesticides were identified by searching the PubMed, Medline, EBSCO, Agricola and TOXNET databases. **Results:** The findings of the studies reviewed imply that children's exposure to pesticides may bring about impairments in their neurobehavioral development. Children exposed to organophosphate pesticides (OP), both prenatally and during childhood, may have difficulties performing tasks that involve short-term memory, and may show increased reaction time, impaired mental development or pervasive developmental problems. In newborns, the effects of OP exposure are manifested mainly by an increased number of abnormal reflexes, while in adolescents, by mental and emotional problems. The studies investigating association between exposure to organochlorine pesticides and neurodevelopmental effects show inconsistent results. While some studies report impairments in mental and psychomotor functions, other studies do not confirm the above. **Conclusion:** The information deriving from epidemiological studies indicate a need to increase awareness among people and children exposed to pesticides about the association between the use of pesticides and neurodevelopmental impairments. Therefore, the principle of prudence should become a rule.

## Key words:

Children, Neurobehavioural development, Exposure to pesticides

## INTRODUCTION

Pesticides comprise a diverse group of substances intended to destroy, repel or control organisms identified as pests. Some are broad-spectrum biocides and others are relatively selective, targeting specific organisms such as insects, fungi or plants. In the homes and apartments, pesticides are used to control termites, roaches and rodents. In gardens and lawns as well as along highways, chemical

herbicides are applied to control the growth of unwanted plants. By controlling agricultural pests, pesticides have contributed to a substantial increase in crop yields. By controlling insect vectors, they have helped to limit the spread of disease. However, as the pesticides are intended to be toxic for insects or unwanted plants, there is a concern that they can also cause adverse health effects on humans as well as the environment.

Received: February 12, 2008. Accepted: April 11, 2008.

The study was performed under the project "Environmental and occupational exposure to pesticides among agricultural families" supported by the Ministry of Science and Higher Education, Poland, from Grant No. PBZ-MEiN-/8/2/2006; contract no. K140/P01/2007/2.1.2.

Address reprint requests to J. Jurewicz, Department of Environmental Epidemiology, Nofer Institute of Occupational Medicine, ul. Teresy 8, 91-348 Łódź, Poland (e-mail: joannaj@imp.lodz.pl).

Young children can be exposed to pesticides during their normal exploration of the environment and contact with floors and other surfaces. The children of farmers or agricultural workers are considered to have a higher risk of pesticide exposure than does the general population, because of the close proximity of their homes to the fields where pesticides are applied, and from the take-home exposure [1].

Recent studies have shown that the fetus and young child have lower than adult levels of detoxifying enzymes (paraoxonase or chlorpyrifos-oxonase) that deactivate organophosphate pesticides (OP) [2], which implies that they may be more vulnerable to exposure. The hypothetical effects of perinatal exposure to pesticides include social and emotional deficits, autism, cerebral palsy and mental retardation [3]. In addition, pesticide exposure may negatively influence the child's development while not producing any evident disease. However, compared to several studies on lead, mercury and PCBs, few epidemiological studies have assessed the developmental neurotoxicity of pesticides.

The study of behavior and its reversible or irreversible modifications by chemicals, including pesticides, is a relatively new approach in the field of neurotoxicology [4]. Specific tests are used as neuropsychological tools for the differential diagnosis of various forms of brain damage or neurobehavioral dysfunction.

## MATERIALS AND METHODS

Epidemiological studies focused on the neurodevelopment of children exposed to pesticides were identified by searching the PubMed, Medline, EBSCO, Agricola and TOXNET literature databases. The language of publication was not specified. The key words used included exposure to pesticides, children, neurobehavioral disorders, organochlorine, organophosphate, and environmental exposure. From each study, the following information was abstracted: study population, type of outcome, neurodevelopmental test used, risk estimate, type of exposure (prenatal, postnatal) and biomarkers of exposure.

Using the systematic search technique, a total of 120 articles were identified. Eighteen articles met the eligibility criteria and were included in the present review.

## RESULTS

### Neurobehavioural effects of exposure to organophosphate (OP) pesticides (prenatal and childhood exposure)

Animal studies have demonstrated that OP insecticides affect mammalian brain development [5,6]. Few studies have assessed the neurodevelopment of newborns and young children after low-level exposure to OP pesticides [7–11]. Most of the studies concerned US populations [8–12], and two were performed in Nicaragua and Ecuador [7,13]. Two studies were conducted among newborns [11,14], four among young children [7–10] and one among adolescents [13] (Table 1).

The effects of prenatal exposure to OPs on abnormal reflexes in neonates were evaluated in two studies from the United States. In the study conducted among three-day-old infants, increased prenatal levels of urinary OP metabolites were associated with an increase both in the number of abnormal reflexes and the proportion of infants with more than three abnormal reflexes [11]. Prenatal exposure to OPs and abnormal neonatal behavior and/or primitive reflexes were examined by a team from the Children's Environmental Health Center in New York City. The Brazelton Neonatal Behavioral Assessment Scale was used. Maternal urine samples were analyzed for six dialkylphosphate metabolites and malathion dicarboxylic acid. Malathion dicarboxylic acid levels above the limit of detection were associated with an increased number of abnormal reflexes [14].

Rauh et al. [9] examined the relationship between maternal blood levels of a diethyl phosphate pesticide during pregnancy and performance on the Bayley Scales of Infant Development in 254 inner-city children followed up for 3 years of age. Three-year-olds with high prenatal levels of chlorpyrifos in cord plasma ( $> 6.17$  pg/g plasma) showed significantly more delays in psychomotor and mental development, and their mothers reported more attention

**Table 1.** Exposure to organophosphate pesticides and the risk of neurodevelopmental effects in children

Study population	Tool	Biomarkers	Results	References
US, California 396 children at 6 months 395 children at 12 months 372 children at 24 months	Bayley Scales of Infant Development (Mental Development (MDI) and Psychomotor Development (PDI) indices)	6 nonspecific OP metabolites, malathion- and chlorpyrifos-specific metabolites, measured in urine	Pregnancy DAP (dialkyl phosphate metabolite) levels were negatively associated with MDI, but child measures were positively associated. At 24 months of age, these associations reached statistical significance (per 10-fold increase in prenatal DAPs: $\beta = -3.5$ points; 95% CI: -6.6 to -0.5; child DAPs: $\beta = 2.4$ points; 95% CI: 0.5-4.2). Neither prenatal nor child DAPs were associated with PDI or attention problems, but both prenatal and postnatal DAPs were associated with the risk of pervasive developmental disorder (per 10-fold increase in prenatal DAPs: odds ratio (OR) = 2.3, $p = 0.05$ ; child DAPs OR = 1.7, $p = 0.04$ ).	[12]
US, California 381 infants at $\leq 2$ months	Brazelton Neonatal Behavioral Assessment Scale	OP metabolites in urine, measured twice during pregnancy (M = 14 and 26 weeks of gestation) and once after delivery (M = 7 days postpartum)	Significant association between exposure and the reflex cluster for the entire sample and for infants > 3 days old. In the latter group, the increasing average prenatal urinary metabolite levels were associated with an increase both in the number of abnormal reflexes and the proportion of infants with more than three abnormal reflexes.	[11]
US, Mississippi and Ohio; 365 children in Mississippi (147 exposed to methyl parathion and 218 unexposed) 287 children in Ohio (104 exposed and 183 unexposed)	The Developmental Test of Visual-Motor Integration, to measure integration of visual and motor skills. The Kaufman Brief Intelligence test, to measure general intelligence, verbal ability, and nonverbal reasoning. The Purdue Pegboard Test, to measure visual-motor coordination, manual dexterity, and motor speed. The Story Memory and Story Memory Delay from Wide-Range Assessment of Memory and Learning Tests, to measure verbal memory. The Trail-Making test to assess multistep processing involving more than one cognitive function areas (visual perception, motor speed, sequential skills and symbol recognition) in children aged 9 years and above. The Verbal Cancellation Test, to measure sustained selective attention.	urinary para-nitrophenol levels and environmental wipe samples	Exposed children had more difficulties performing tasks that involved short-term memory and attention. Moreover, the parents of exposed children had more behavioral and motor skill problems than did the parents of unexposed children. There were no differences in test scores for general intelligence and integration of visual and motor skills.	[10]

**Table 1.** Exposure to organophosphate pesticides and the risk of neurodevelopmental effects in children — cont.

Study population	Tool	Biomarkers	Results	References
US 254 children evaluated over the first 3 years of life (12, 24, 36 months of life)	Bayley Scales of Infant Development II; Child Behaviour Checklist.	Chlorpyrifos levels in umbilical cord plasma	Highly exposed children scored on average 6.5 points lower on the Bayley Psychomotor Development Index and 3.3 points lower on the Bayley Mental Development Index at 3 years of age than did the children with lower levels of exposure.	[9]
Northern Ecuador 79 elementary school children aged 7 years	Physical examination; Neurobehavioral Tests: Santa Ana Form Board, to measure dexterity, Wechsler Intelligence Scale for Children; Stanford-Binet copying subtest, finger tapping. Reaction time test	Recent and current pesticide exposure was assessed by determining erythrocyte acetylcholine esterase activity and urinary excretion of OP metabolites	OP metabolite levels were associated with increased reaction time, but not with other domains of neurobehavior.	[7]
New York City, US 311 neonates before hospital discharge	Brazelton Neonatal Behavioral Assessment Scale	Maternal urine samples were analyzed for OP metabolites. Maternal peripheral blood samples were analyzed for polychlorinated biphenyls and 1,1'-dichloro-2,2'-bis(4-chlorophenyl)ethylene	Malathion dicarboxylic acid levels above the limit of detection were associated with increased number of abnormal reflexes OR = 2.24; 95% CI = 1.55–3.24	[14]
US 78 children aged 48–71 months	Neurobehavioural battery consisted of 5 tests from the computerized Behavioral Assessment and Research System and 3 non-computerized tests: Object Memory, Purdue Pegboard, and Visual Motor Integration	No biomarkers were determined	Male children from agricultural area performed significantly worse than male children from non-agricultural communities on right hand Finger Tapping. Also, the male children from agricultural communities had significantly longer latencies on the Match-to-Sample test.	[8]
Nicaragua 100 residents in a rural community (residence of 10 years and over) and 100 residents in an urban community	Acute symptoms, neurotoxic symptoms questionnaire	Health effects of aerial spraying in the last 15 days assessed via questionnaire. Erythrocyte cholinesterase level.	Prevalence odds ratios significantly elevated for nervous system, mental and emotional symptoms. Adjusted prevalence odds ratios for residence in exposed community by symptom categories: Non-specific OR = 1.6, 95% CI (0.8–3.2) Possible OR = 4.1, 95% CI (1.7–10.2) Probable OR = 9.9, 95% CI (2.9–34.4)	[13]

problems and symptoms of pervasive developmental disorders at three years of age.

Mental and psychomotor development was also investigated in a cohort of children of farm worker families living in the Salinas Valley, California [12]. Adverse association of prenatal OP metabolites with mental development as well as pervasive developmental disorders at 24 months of age could be observed.

In four studies, the effects of postnatal pesticide exposure on neurodevelopment were studied among young children. Recently, an investigation has been performed among 6- to 9-year-old Ecuadorians ( $n = 72$ ) attending the 2 lowest grades of a public school. Maternal occupational history revealed that 37 children had been prenatally exposed to pesticides. After adjustment for confounders, prenatal pesticide exposure was associated with a lower score for design copying on the Stanford-Binet Copying Test. Postnatal exposure to OPs, as assessed by the children's urinary levels of dialkyl phosphate (DAP) metabolites, was associated with an increased reaction time, but not with the other domains of neurobehavior [7].

The results of the study conducted among preschool children showed poorer neurobehavioral performance among children from agricultural communities exposed to OP, compared to the performance of those from non-agricultural communities, as measured by response speed (Finger Tapping) and number correct and latency (Match-to-Sample Test). Children from agricultural areas performed significantly worse on right hand Finger Tapping than the children from non-agricultural communities. The children from agricultural communities had significantly longer latencies on the Match-to-Sample test as well [8]. The result was adjusted for maternal age and education.

In a study of 2- to 12-year-olds living in Mississippi and Ohio where methyl parathion was illegally sprayed in the homes, the children were classified as exposed or unexposed based on urinary para-nitrophenol levels and environmental wipe samples. The exposed children had more difficulties performing tasks that involved short-term memory and attention. Moreover, the parents of the exposed children had more behavioral and motor skill problems than did the parents of the unexposed children.

No differences were found in the tests of general intelligence, or integration of visual and motor skills [10].

The study performed among adolescents in Nicaragua showed a higher prevalence of mental and emotional symptoms in youngsters exposed to aerial spraying of pesticides (organophosphate and pyrethroid compounds) in a rural community, compared to the community of unexposed controls living far away from agricultural spraying areas [13].

### **Neurobehavioral effects of prenatal exposure to organochlorine compounds**

Organochlorine compounds are persistent environmental contaminants that were intensively used in the past. Although today DDT is banned from use and production in most Western countries, it is still sprayed in some developing countries for disease vector control [15]. Organochlorine chemicals are resistant to degradation and they bioaccumulate in the food chain. Exposure occurs in utero and because of their lipophilicity also via breastfeeding. Prenatal DDE exposure in relation to children's neurodevelopment has been examined in two cohorts [16–18]. No association between performance on Bayley Scales and DDE breast milk level was found in a study conducted in North Carolina [16]. Likewise, there was no statistically significant relationship between the poorest scores on McCarthy scale and DDE exposure [17]. Neither transplacental nor breastfeeding exposure to DDE affected McCarthy scores in children aged 3, 4, and 5 years. Also, in more recent studies, no association was found between cord blood DDE levels and the scores on Fagan Test of Infant Intelligence at ages 6 or 12 months [18] (Table 2).

Another study investigated an association between prenatal exposure to p,p-DDE and mental and psychomotor development in children aged 1 year who lived in a rural village in the vicinity of an electrochemical factory where DDT, hexachlorobenzene and other chlorinated solvents were produced. The findings revealed that p,p'-DDE cord serum levels were negatively associated with a lower psychomotor development index at 13 months of age, as measured by Bayley Scales of Infant Development

**Table 2.** Exposure to organochlorine pesticides and the risk of neurodevelopmental effects in children

Study population	Tool	Biomarkers	Results	References
US, North Carolina 230 children tested at 6 months of age and again 216 children tested at 12 months	Fagan Test of Infant Intelligence (FTII)	Cord blood DDE level	No significant associations between FTII performance and DDE cord blood level.	[18]
US, North Carolina 676 children tested at 18 months and 670 at 24 months	Bayley Scales of Infant Development	Breast milk DDE level	No associations between performance on Bayley Scales and DDE breast milk level.	[16]
US, North Carolina 712 children examined at age 3, 4, 5 years	McCarthy Scales of Children's Abilities	Breast milk DDE level and transplacental DDE level	Neither transplacental nor breastfeeding exposure to DDE affected scores on McCarthy Scales at ages 3, 4, 5 years.	[17]
Spain 92 mother-infant pairs, 1-year old infants	Bayley and Griffiths Scales of Infant Development	Organochlorine compounds: DDE, hexachlorobenzene (HCB) in cord serum	DDE cord serum levels were negatively associated with both mental and psychomotor development impairments. HCB had no effect on child neurodevelopment.	[19]
Spain 475 infants at age 4 years	McCarthy Scales of Children's Abilities	DDT and DDE in cord serum	Children whose DDT concentrations in cord serum were > 0.20 ng/ml had mean decreases of 7.86 points on the verbal scale and 10.86 points on the memory scale, as compared with the children whose concentrations were < 0.05 ng/ml.	[20]
Mexico 244 children at 1, 3, 6 and 12 months of age	Bayley Scales of In- fant Development: Psychomotor Develop- ment Index (PDI), Mental Development Index (MDI)	Serum level of DDE before pregnancy and during each trimester of pregnancy	DDE levels during the first trimester of pregnancy were associated with a significant decrease in Psychomotor Development Index (PDI); DDE levels were not associated with Mental Development Index (MDI).	[21]
US, California 360 singletons tested at 6, 12, 24 months	Bayley Scales of Infant Development	Maternal serum measures of p,p'-DDT, o,p'-DDT and p,p'-DDE	2-point decrease in Psychomotor Development Index scores with each 10-fold increase in p,p'-DDT levels at 6 and 12 months and p,p'-DDE levels at 6 months.	[22]

and Griffiths Mental Development Scales [19]. Long-term breastfeeding was found to be protective against this effect. On the other hand, no such association was found for exposure to hexachlorobenzene. The same authors evaluated the association of cord serum level of DDT and its metabolite DDE with neurodevelopment at four years of age [20]. The infants were examined using McCarthy Scales of Children's Abilities. DDT cord serum concentration at birth inversely correlated with verbal,

memory, quantitative and perceptual performance skills at age 4 years.

Torres-Sanchez [21] found that DDE levels during the first trimester of pregnancy were associated with a significant decrease in the psychomotor development index (PDI) but not with the mental development index (MDI).

The effects of in utero exposure to DDT and DDE on infant neurodevelopment were also investigated among the children of Mexican farm workers in California. The

birth cohort study included 360 singletons with maternal serum measures of p,p'-DDT, o,p'-DDT, and p,p'-DDE. Psychomotor development and mental development were assessed with the Bayley Scales of Infant Development at 6, 12, and 24 months. The study revealed a 2-point decrease in Psychomotor Development Index scores with each 10-fold increase in p,p'-DDT levels at 6 and 12 months (but not 24 months) and p,p'-DDE levels only at 6 months. Breastfeeding was positively associated with the score on Bayley Scales [22]. The studies reviewed focused on the prenatal exposure to organochlorines and its neurobehavioural effects. We are not aware of any studies of children who were postnatally exposed to this pesticide category.

### Neurobehavioural effects of exposure to pesticides in general (information about specific compounds not available)

In two studies that evaluated the risk of neurodevelopmental effects in populations exposed to multiple pesticides, the data on the quantitative assessment of exposure to specific pesticides were not available. One study was conducted in Mexico [23] and the other in the United States [24] (Table 3).

In the study by Guilette et al. 1998 [23], performance on psychomotor skill tests was compared in a group of 33 Mexican 4- to 5-year-olds living in a valley where pesticides, including OPs and organochlorines, were used and in 17 children inhabiting an area where no pesticides

were applied. The exposed children showed a lower performance score with respect to gross motor skills, eye-hand coordination, ability to draw a person, and delayed recall. Maternal in utero and early childhood exposures were associated with neurodevelopmental effects among pre-school children. The children with pesticide exposure demonstrated a decrease in stamina, 30-minute memory test, and ability to draw a person [23].

Neurobehavioral tests selected from the computerized Behavioral Assessment and Research System were performed on 96 Hispanic adolescents currently working in agriculture (AG) and 51 adolescents who were not currently working in agriculture (Non-AG). As regards the cognitive tests, AG performance was significantly below the non-AG performance. However, the authors did not control for educational and cultural differences between the examined groups [24].

## DISCUSSION

### Effects of pesticide exposure on neurobehavioral development of children

Neonates demonstrated a higher proportion of abnormal reflexes on the Brazelton Neonatal Behavioural Assessment Scale [14,11]. Young children exposed to OP pesticides had a poorer performance on measures of response speed and latency [8], difficulties when performing tasks that involved short-term memory and attention

**Table 3.** Exposure to pesticides in general and the risk of neurodevelopmental effects in children

Study population	Tool	Exposure assessment	Results	References
Yaqui Valley in northwestern Mexico 33 children from an area where lots of pesticides were applied, and 17 control children from an area where pesticides were not used; both populations aged 48 and 62 months	Rapid Assessment Tool for Preschool Children: Bayley Scales of Infant Development and McCarthy Scales of Children's Abilities	Annual DDT spraying and mixed organochlorine, organophosphate and pyrethroid compounds, 2 crops/year, up to 45 applications/crop, daily household bug spraying	No differences were found in growth patterns. Functionally, the exposed children demonstrated decrease in stamina, gross and fine eye-hand coordination, 30-minute memory, and ability to draw a person.	[23]
US 96 adolescents currently working in agriculture (AG) and 51 currently not working in agriculture (Non-AG)	Neurobehavioral tests were selected from the computerized Behavioral Assessment and Research System	No biomarkers were determined.	AG test performance was significantly below non-AG performance on the cognitive function tests.	[24]

[10], increased reaction time, and lower score for design copying [7] on a battery of different tests. Bayley Scales of Infant Development showed delays in psychomotor and mental development [12,9] that was associated with exposure to OP pesticides.

Exposure to organochlorine pesticides adversely affected the psychomotor development index on the Bayley Scale of Infant Development [21,22] and on McCarthy Scales of Children's Abilities [19]. Ribas-Fito et al. [20] also found a deterioration of the verbal, memory, quantitative, and perceptual performance after exposure to organochlorine pesticides. The children from agricultural areas, whose exposure to pesticides was assumed but not measured, showed a lower performance with respect to gross motor skills, eye-hand coordination, ability to draw a person, and delayed recall [23]. They also scored lower on the neurobehavioral test compared with the children from the non-agricultural area.

In two studies on organochlorine pesticide exposure that were performed in the United States [22] and Spain [19], long-term breastfeeding was found to be protective against poorer psychomotor development as assessed on Bayley Scales of Infant Development.

Recently, Garry et al. (2002) [25] in a follow-up of children born to applicators of the fumigant phosphine and/or herbicide glyphosate found that these children were more likely to display adverse neurological and neurobehavioral developmental effects: attention deficit disorder (ADD), attention-deficit hyperactivity disorder (ADHD), or autism [25].

### Exposure assessment

In several studies, human exposure to pesticides was documented based on information received from parents [23,8] on the number of sprayings, dose of chemicals used (annual DDT spraying; organochlorine, organophosphate, pyrethroid compounds used) [23] and on 'work in agriculture' as a job title [8]. Only Garry et al. [25], in a study on applicators from Minnesota, assessed exposure to specific pesticides: herbicide glyphosate and fumigant phosphine, but the exposure was not confirmed by specific biomarkers

of exposure, and the neurological disorders were reported by parents.

Several other studies included biological markers of absorbed dose in urine [10,11], whole blood [14,18], serum [20,21] or milk [16,17].

### Critical window of exposure

A number of studies on organochlorine exposure have examined whether there is a critical window of exposure during fetal development that would account for the disruption of neurodevelopment. Torres-Sanchez [21] examined exposure to DDE before pregnancy and during each trimester of pregnancy. Ribas-Fito [19,20], Rogan [16], Dravill [18] and Gladen [17] assessed prenatal exposure to DDT at the time of delivery. Ribas-Fito found evidence that pesticides may impair neurodevelopment, but the other authors did not.

With regard to organophosphate exposure, Young et al. [11] found that the prenatal level of urinary OP metabolites was associated with abnormal reflexes among 3-day-old infants. Postnatal exposure to OP pesticides was related to mental and emotional symptoms in adolescents exposed to aerial spraying [13].

Maternal exposure to OP pesticides in pregnancy (occupational history: 'floriculture') was associated with a lower drawing score for design copying in children from Ecuador [7]. The children's current pesticide exposure, as indicated by increased total urinary metabolites of OPs, was associated with increased simple reaction time. These findings suggest that the effects of prenatal pesticide exposure may be lasting and distinct from the effects of postnatal exposure.

Contradictory findings were reported by Eskanazi and co-workers [12]: maternal urinary levels of dialkylphosphate metabolites were negatively associated with the child's Mental Development Index, but respective child levels, as the measures of exposure, were positively associated.

### Transient effect of exposure associated with age

In a cohort study performed in North Carolina, the children exposed to organochlorine pesticides were tested on Bayley Scales of Infant Development at 18, 24 months [16]

and later at 3, 4, 5 years of age [17]. The children at 18 and 24 months had poorer scores on Bayley test but the children at 3, 4, 5 years did not. The deficits seen in those children over two years of age were no longer apparent. No DDE exposure was associated with lower scores on infant intelligence scale. These findings suggests that in children who were 5 years of age or younger when exposed, the neurobehavioral effects of exposure may be transient.

#### Tests for measuring specific neurobehavioural effects

To find a suitable test to measure different endpoints is a very challenging task. The investigator has to choose from a large number of potential endpoints that can be assessed in a prospective study on any given pesticide or a mixture of compounds to which the fetus or the child may be exposed. Ruckart et al. [10] used the Pediatric Environmental Neurobehavioral Test Battery (PENTB) to assess the neurobehavioral functions in children. The PENTB consists of the performance-based and informant-based tests (Table 4). Guillette et al. 1998 [23] used the tests based on a play approach to evaluate physical stamina and coordination along with mental processes. Special test was used for specific neurodevelopmental disorders.

Bayley Scales of Infant Development (Mental Development (MDI) and Psychomotor Development (PDI) Indexes) are the most common tests used for evaluating the neurodevelopmental effects of pesticide exposure in young children [22,12,9,16,19,21]. For newborns, the Brazelton Neonatal Behavioral Assessment Scale was used [14,11]. Other tests used in neurodevelopmental studies include Fagan Test of Infant Intelligence (FTII) [18], McCarthy Scales of Children's Abilities [17,20] and Griffiths Mental Development Scales [19].

Since most of the tests cover the mental and psychomotor aspects, a wide spectrum of infant development can be assessed based on these tests.

#### Study designs

In most of the studies reviewed, prospective cohorts were used to assess the neurobehavioural effects of exposure to pesticides, but Guillette et al. [23], Keifer et al. [13], and Roldan et al. [16] performed cross-sectional studies. The administration of neurobehavioral tests covering cognitive and sensorimotor functions in children has significantly contributed to documenting the risk for neurobehavioural adversity associated with environmental exposure to low levels of pesticides.

#### Confounding

Confounders are variables exhibiting association with both exposure and outcome. If not considered adequately, their presence may give rise to spurious associations between the exposure variable and the target variable. Particularly important confounders are parental intelligence, to account for genetic background, and quality of the home environment, to account for the differences in environmental stimulation during development. Other confounders which were considered in the analysis were: age, sex, education, and drug or vitamin intake during pregnancy. Although most of the studies evaluated pesticide exposure, a number of different variables, such as diet, exposure to metals and other environmental contaminants, can have influence on the neurodevelopment in children. The great number of variables did not allow for controlling them in epidemiological studies; thus they may affect the study results.

**Table 4.** Tests used for assessment of neurobehavioral disorders in children

Test used	Endpoint measured	References
Simple reaction time	Reaction time after visual and auditory stimulus	[7,24]
Santa Ana Form Board	Motor coordination	[7,24]
Wechsler Intelligence Scale for Children-Revised. Digital Span Test	IQ test — children repeat strings of digits and the score is the number of correct trials	[7]
Stanford-Binet Copying Test	Visuospatial and visuoconstructional function	[7]

**Table 4.** Tests used for assessment of neurobehavioral disorders in children — cont.

Test used	Endpoint measured	References
The Developmental Test of Visual-Motor Integration	Integration of visual and motor skills	[10]
The Kaufman Brief Intelligence Test	General intelligence, verbal ability and nonverbal reasoning	[10]
The Purdue Pegboard Test	Visual-motor coordination, manual dexterity, and motor speed	[10,24]
The Story Memory and Story Memory-Delay from Wide Range Assessment of Memory and Learning	Verbal memory: immediate and delayed recall of both specific and general items	[10]
The Trail-Making Test — Part A and Part B	Visual perception, motor speed, sequential skills and symbol recognition; multistep processing involving more than one cognitive function area	[10]
The Verbal Cancellation Test	Sustained selective attention	[10]
The Parenting Stress Index	Occurrence of common signs and symptoms of child and family dysfunction	[10]
The Personality Inventory for Children	Child's behaviour and cognitive status	[10]
The Vineland Adaptive Behavior Scales	Communication, daily living skills such as eating and dressing, household tasks, time and money skills, socialization and motor skills	[10]
Finger Tapping	Motor speed and coordination	[24]
Pursuit Aiming	Motor speed and coordination	[24]
Continuous Performance	Sustained attention and information processing speed	[24]
Symbol-Digit	Sustained attention and information processing speed	[24]
McCarthy Scales of Children's Abilities (MCSA)	General cognitive, verbal, perceptual performance, and quantitative, memory, and motor skills; MCSA consists of 18 items derived from 6 different scales as above	[20,23,17]
Bayley Scales of Infant Development	Mental Development Index — a variety of cognitive abilities. Psychomotor Development Index — characterizes large muscle and fine motor coordination.	[12,23,16,21,9,22,19]
Battelle Developmental Inventory	Multiple aspects of body growth and functional abilities for normal childhood activities	[23]
Fagan Test of Infant Intelligence	Visual recognition memory, assessed using a 10-trial habituation format	[18]
Questionnaire	Chronic neurobehavioral symptoms	[13]
The Brazelton Neonatal Behavioural Assessment Scale	Infant behavior divided into seven domains: Habituation — ability to respond to and inhibit discrete stimuli while asleep. Orientation — attention to visual and auditory stimuli and quality of overall alertness. Motor — motor performance and quality of movement and tone. Range of state — measure of infant arousal and state lability. Regulation of state — ability to regulate state in the face of increasing levels of stimulation. Automatic stability — signs of stress related to homeostatic adjustment of the central nervous system. Number and type of abnormal primitive reflexes.	[14,11]
Griffiths Scales of Infant Development	Locomotion, personal-social skills, hearing and language, eye-hand coordination, performance	[19]

### Challenges for future studies on neurobehavioural effects of pesticide exposure

The choice of a given test should be determined by what is known about the impact of a particular pesticide on the development of specific cognitive, neuromotor or behavioural functions. Animal studies can be a key in identifying the functional dominants that are affected by exposure and would be relevant to assess in human populations. A good strategy would be to develop tests that would include both the board-based measures of cognitive and neuromotor status and more fine-grained assessments of specific skills [26].

Long-term studies, sufficient to follow the participants into adolescence and early adulthood, are essential to assess the full range of neurodevelopmental consequences of pesticide exposure.

### CONCLUSIONS

The findings of the studies reviewed indicate that children's exposure to pesticides may bring about impairments in the neurodevelopmental processes. In all the studies, exposure to OP pesticides was associated with neurodevelopmental disorders reflected by a significantly worse score on right hand Finger Tapping and longer latencies on the Match-to-Sample test [24]. Children exposed to methyl parathion and chlorpyrifos had more difficulties performing tasks that involved short-term memory and attention [10,9]. An association was found between prenatal levels of OP metabolites and the problems in mental development and pervasive developmental disorders at 24 months of age [12] as well as with increased reaction time [7] and increased number of abnormal reflexes [14,11] in newborns, and mental and emotional symptoms in adolescents [13].

Four studies investigating the relationship between exposure to organochlorine pesticides and neurobehavioural effects indicated that organochlorine exposure may bring about impaired neurodevelopment [19–22], whereas in three studies, no such association was found [16–17]. However, the finding of no effect on the infant's behaviour cannot be regarded as conclusive evidence that the toxic

agent has had no health impact at all and we have to be aware of this.

Chronic exposure due to parental occupation and proximity of the pesticide-treated fields was assumed based on the evidence of bringing pesticide exposure home from active agricultural area in two studies, but has not been confirmed thus far [23,8]. In both the studies, pesticide exposure did affect the neurodevelopmental functions of the children [23].

The information deriving from epidemiological studies indicate a need to increase awareness among the adults and children exposed to pesticides about the association between the use of pesticides and the neurodevelopmental impairments. Therefore, the principle of prudence [27] should become a rule.

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