Mini review

Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes

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ABSTRACT

For the appropriate protection of human health it is necessary to accurately estimate the health effects of human exposure to toxic compounds. In the present review, epidemiological studies on the health effects of human exposure to organophosphorus (OP) and pyrethroid (PYR) insecticides have been critically assessed. This review is focused on studies where the exposure assessment was based on quantification of specific biomarkers in urine or plasma. The 49 studies reviewed used different epidemiological approaches and analytical methods as well as different exposure assessment methodologies. With regard to OP pesticides, the studies reviewed suggested negative effects of prenatal exposure to these pesticides on neurodevelopment and male reproduction. Neurologic effects on adults, DNA damage and adverse birth outcomes were also associated with exposure to OP pesticides. With regard to exposure to PYR pesticides, there are currently few studies investigating the adverse health outcomes due to these pesticides. The effects studied in relation to PYR exposure were mainly male reproductive effects (sperm quality, sperm DNA damage and reproductive hormone disorders). Studies’ findings provided evidence to support the hypothesis that PYR exposure is adversely associated with effects on the male reproductive system.

The validity of these epidemiological studies is strongly enhanced by exposure assessment based on biomarker quantification. However, for valid and reliable results and conclusions, attention should also be focused on the validity of the analytical methods used, study designs and the measured toxicants characteristics.

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Abbreviations: OP, organophosphates; PYR, pyrethroids; AChE, acetylcholinesterase; PON1, paraoxonase 1; CHAMACOS, Center for the Health Assessment of Mothers and Children of Salinas; CCCEH, Columbia Center for Children’s Environmental Health; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethylthiodiphosphate; PON1, paraoxonase 1; CHAMACOS, Center for the Health Assessment of Mothers and Children of Salinas; CCCEH, Columbia Center for Children’s Environmental Health; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethylthiodiphosphate; DETP, diethylphosphate; DEET, diethylthiophosphate; TCP, 3,5,6-trichloro-2-pyridinol; CIT, 5-chloro-1,2-dihydro-1-isopropyl[3H]-1,2,4-triazol-3-one; CMHC, 3-chloro-4-methyl-7-hydroxycoumarin; DEAMPY, 2-diethylamino-6-methyl-4-pyrimidinol; MDA, malathion diacarb oxalyl acid; PNP, para-nitrophenol; IMPY, 2-isopropyl-4-methyl-6-hydroxy pyrimidinol; 3-BPA, 3-phenoxbenzoic acid; TDCCATrans, -2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid; 4F3PBA, 4-fluoro-3-phenoxbenzoic acid; CDCAcis, -2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid; DBCAcis, -2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid; CPR, chlorpyrifos; MP, methyl-parathion; CPMcis, -permethrin; TPMtrans, -permethrin; GC–FPFD, gas chromatography–flame photometric detector; GC–GFPD, gas chromatography–pulsed flame photometric detector; GC–MS, gas chromatography–mass spectrometry; GC–MS/MS, gas chromatography–tandem mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; PADD, photodiode array detector; CBCL, Child Behavior Checklist; NEPSY, Developmental NEuroPSYcological Assessment; K–CTP, Conners’ Kiddie Continuous Performance Test; BSID, Bayley Scales of Infants development; BSID-II, Bayley Scale of infants development II; BNBAS, Brazelton Neonatal Behavioral Assessment Scale; DISC–IV, Diagnostic Interview Schedule for Children IV; PENTB, Pediatric Environmental Test Battery; WISC–III, Wechsler Intelligence Scale for Children–Third Edition; WPPSI–III, Wechsler Preschool and Primary Scale of Intelligence–Third Edition; WCST, Wisconsin Card Sorting Test; TMTA & TMTB, Trail Making Test A & B; TRF, The Teacher Report Form; MDD, Mental Development Index; NHANES, National Health and Nutrition Examination Survey; NCS, Nerve Conduction Study; CNS, central nervous system; TSH, thyrotropin; T3, thyroxine; T4, triiodothyronine; FSF, follicle stimulating hormone; LH, luteinizing hormone; 8-OHdG, 8-hydroxydeoxyguanosine; ALL, acute lymphoblastic leukemia.

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1. Introduction

A pesticide is "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. Pests can be insects, mice and other animals, unwanted plants (weeds), fungi, or microorganisms like bacteria and viruses" (U.S. EPA, 2011). In this review we focused on recent epidemiological studies investigating the impact of occupational or environmental pesticides exposure on human health. Specifically, we review epidemiological studies that used biomarkers of exposure to organophosphate (OP) and pyrethroid (PYR) insecticides, and investigated their associations with health outcomes.

OP pesticides were widely used in agriculture until recently and had gradually replaced the persistent organochlorine pesticides for many years. OP pesticides poison insects and mammals by phosphorylation of acetylcholinesterase (AChE) found in nerve endings. The effector organ becomes overstimulated by excess acetylcholine (Ach), the impulse-transmitting substance in the nerve ending, as a result of a loss of AChE activity. Acute exposure to organophosphates may cause vomiting, diarrhea, abdominal cramps, dizziness, eye pain, blurred vision, confusion, numbness, twitching, paralysis and death. A variety of health effects have been correlated with chronic low dose exposure to OP pesticides. Neurologic effects have been studied extensively. The association between OP exposure and neurobehavioral effects and delayed neurologic diseases such as Parkinson’s disease have been examined in numerous epidemiological studies (Colosio et al., 2003; Hancock et al., 2008; Manthripragada et al., 2010; Rohlman et al., 2011). Endocrine disrupting properties and genotoxicity of OP pesticides have also been investigated (Bolognesi, 2003; McKinlay et al., 2008). In addition OPs may contribute to birth deficits, childhood brain tumors, leukemia and lymphomas and may also act as liver and respiratory system toxicants.

The other important chemical class of insecticides, synthetic pyrethroids, were developed by modifying the structures of the pyrethrins, which are naturally occurring pesticides found in some members of the chrysanthemum family. This was done in order to increase photostability and simultaneously retain the insecticidal activity of pyrethrum and its relatively low acute mammalian toxicity (Elliott, 1995). Pyrethroids alter the normal function of insect nerves by modifying the kinetics of voltage-sensitive sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the rising phase of the nerve action potential. Acute symptoms resulting from PYR exposure are dyspnea, coughing and bronchospasm, nausea and vomiting, as well as dermal effects. Pyrethroids can produce local paresthesia and allergies due to inhalation of or direct dermal contact (Ujvary, 2010). The long-term effects of pyrethroid exposure are not clear. The most commonly examined effects in epidemiological and animal studies include neurobehavioral and neurodevelopmental effects as well as endocrine disrupting effects (Bolognesi, 2003; McKinlay et al., 2008; Shafer et al., 2005; Wolansky and Harrill, 2008).

Exposure assessment to non-persistent pesticides is challenging. Biomonitoring of exposure involves measurement of the parent compound or its metabolites in human biological samples in order to identify and quantify the internal exposure to specific chemicals. Biomarkers of pesticide exposure have been used in both occupational and non-occupational epidemiological studies, in order to detect and quantify exposure to a variety of compounds. The aim of this paper is to review the epidemiological studies that used biomonitoring techniques to assess human exposure, and associate the biomonitoring exposure measurements to health outcomes. Moreover, the article aims to examine the usefulness of OP and PYR exposure biomarkers for exposure assessment and investigate the perspectives and limitations of this approach.

2. Methods

Articles were selected by an electronic research of Scopus, Pubmed and ScienceDirect and Google Scholar databases. Key words or text word combinations involved the terms “human”, “biomarkers” or “biomonitoring”, “pesticide exposure”, “organophosphates” or “organophosphorus”, “pyrethroids”, “metabolites”, “urine” or “urinary”, “plasma”, “health effects” or “outcomes”. Epidemiological studies involving the measurement of pesticide compounds and their metabolites in human biological samples in association with adverse health effects were selected and reviewed. The use of biomarkers of exposure to OP and PYR insecticides was the main criterion for inclusion. The use of biomarkers of exposure to other chemical classes (e.g. carbamate or organochlorine pesticides) was not an inclusion criterion nor was the measurement of AChE. Studies that were published before 1991 were not included.

3. Results

3.1. Reviewed studies designs and objectives

Fifty five publications met the criteria and were included in this review. Some of the studies were presented by multiple publications. In total, we identified 49 different studies. Studies were categorized into three groups, according to the population
examined: (a) pregnant women–infant and children studies, (b) occupational studies, and (c) non-occupational studies. According to the design and methodology of the study they were further categorized as cohort (14), case-control (2) and cross-sectional (33).

3.1.1. Pregnant women–infant and children studies

Effects on children’s health were investigated in 19 studies, and ages of the children varied from neonates to 18 years old (Table 1). In 13 studies, prenatal exposure to pesticides was also assessed, in order to examine the effects of exposure during pregnancy to newborns. Effects associated with biomarkers of exposure mainly focused on neurodevelopment parameters and birth outcomes.

Large prospective cohort studies were more common when assessing the association between pesticide exposure and children’s health. Significant findings in this field were from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), which was a large prospective cohort study that investigated the association between long-term pesticide exposure and the health of pregnant women and their children living in Salinas Valley, California. A detailed description of the CHAMACOS study’s research activities was published by Castorina et al. (2003). Five studies presenting results from the CHAMACOS cohort are included in our review. A similar cohort study, conducted by the Columbia Center for Children’s Environmental Health (CCCEH) was initiated in 1997 to examine the effects of prenatal exposure to a variety of pollutants on birth outcomes, neurocognitive development, and procarcinogenic damage in mothers and infants from minority communities in New York City (Perera et al., 2003). Four studies presenting findings from the CCCEH cohort are reviewed. Mother–infant pairs were also recruited during a multiethnic study which took place at Mount Sinai Hospital in New York City. Details regarding the study design and study procedures have been published by Berkowitz et al. (2003). Four studies from the Mount Sinai cohort are included in this review.

Five cross-sectional studies were also found, and one case-control study that investigated the association between OP exposure and acute lymphoblastic leukemia. The sample size in these studies ranged from 48 to 488.

3.1.2. Occupational studies

Twenty studies (26 publications) focused on health effects resulting from occupational exposure (Table 2). Subjects included pesticide sprayers, agricultural workers, sheep dippers, and workers employed in chemical manufacturing companies. Hormonal disorders, neurologic function, DNA damage, sperm parameters and acute health symptoms were studied.

Most of the occupational studies were cross-sectional (19 studies), measuring exposure and effect simultaneously. In the majority of these studies, exposed and unexposed groups were recruited separately and biomarkers were used to examine the variation between the magnitude of exposure between groups. In some studies, seasonal variation was also estimated. One cohort study was also found. We observed a wide range in sample sizes, ranging from only 9 (Recio et al., 2001) to 772 subjects (Pilkington et al., 2001).

3.1.3. Non-occupational studies

Ten articles studied the effects of non-occupationally exposed populations (Table 3). All of these studies examined the effects of pesticide exposure on male infertility. Subjects were recruited from infertility clinics and were either seeking infertility diagnosis or were diagnosed as infertile. Studies attempting to link pesticide exposure in the general population to health outcomes were mostly cross-sectional (9 studies). One case–control study was identified. Sample size varied from 88 to 322 persons.

3.2. Assessment and monitoring of human exposure to pesticides

3.2.1. Exposure assessment

Human exposure science was defined by D. B Barr as “the study of human contact with chemical, physical, or biological agents occurring in their environments, and [advancement of] knowledge of the mechanisms and dynamics of events either causing or preventing adverse health outcomes” (Barr, 2006). Exposure can be expressed in terms of magnitude, frequency and duration. Assessing human exposure to pesticides in epidemiology is sometimes not straightforward and can be achieved using different approaches. The simultaneous estimation of exposures and doses to multiple stressors from multiple routes and sources requires a well designed exposure framework. Self-reported exposure derived from questionnaires is the most common way of assessing exposure in epidemiology. Environmental and ambient monitoring also have applications in epidemiological studies, and exposure models combining different types of data can be used to estimate the magnitude, the timing and the frequency of exposure.

All of the studies included in this review used biomonitoring techniques to assess exposure to pesticides. It is notable that in some occupational exposure studies the exposure status was clear from the beginning of the study, and the exposed-non exposed groups were classified by occupation. In these studies, biomarkers were used to confirm the larger extent of exposure in the exposed group and to estimate the variations in exposure magnitude between groups.

Biomonitoring data increase their utility when they are combined with other methods for estimating the magnitude, duration and frequency of exposure. Biomarkers of exposure to OP and PYR insecticides in urine or plasma lack the ability to estimate exposure history, since they are acute or short-term dosimeters providing values that reflect only acute or short-term exposure and thus, should be combined with other methods to better describe the exposure continuum.

Besides biomonitoring, we identified numerous other approaches for exposure assessment, varying from single questionnaires to sophisticated exposure models. All studies, in addition to biomarker measurement, used data derived from questionnaires. However, in 17 studies questions related to exposure could not be found. Data derived from questionnaires were limited to demographic, lifestyle and medical factors and provided no information on exposure parameters. In the other studies, questionnaires included data on occupational and environmental exposure history, work practices and use of personal protective equipment.

Ten studies extended their exposure assessment framework to involve ambient and environmental measurements and exposure models. Pilkington developed an empirical model based on urinary OP biomarker measurements and combined it with an exposure history questionnaire in order to estimate cumulative exposure (Buchanan et al., 2001; Pilkington et al., 2001). Albers also estimated exposure retrospectively using hygiene records and air monitoring data combined with urinary TCP excretion to assess and quantify exposure history (Albers et al., 2004a,c). In the CCCEH study regarding pesticides, exposure was assessed with maternal personal air measurements of chlorpyrifos and diazinon (Whyyatt et al., 2004) and of cis-, trans-permethrin, and piperonyl butoxide (Horton et al., 2011) during pregnancy. Subjects were asked to wear a small backpack holding a personal ambient air monitor during daytime hours, and to place the monitor near the bed at night, for a period of 48 h. Six other studies combined biomonitoring with environmental and ambient monitoring in different media such as house dust, personal air, vehicle samples and skin patch samples (Bouchard et al., 2010; Padungtod et al., 2000; Rauh et al., 2006; Rothlein et al., 2006; Ruckart et al., 2004; Strong et al., 2004).
### Table 1

Studies on pregnant women-infants and children.

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Project</th>
<th>Type of study</th>
<th>Target population</th>
<th>Health effect examined</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouchard et al. (2011)</td>
<td>CHAMACOS</td>
<td>Prospective, cohort</td>
<td>329 Mother–children pairs</td>
<td>Neurodevelopment</td>
<td>Salinas Valley, California, United States</td>
</tr>
<tr>
<td>Marks et al. (2010)</td>
<td>CHAMACOS</td>
<td>Prospective, cohort</td>
<td>348 Mothers–children pairs</td>
<td>Neurodevelopment</td>
<td>Salinas Valley, California, United States</td>
</tr>
<tr>
<td>Eskanazi et al. (2007)</td>
<td>CHAMACOS</td>
<td>Prospective, cohort</td>
<td>396 Mother–children pairs</td>
<td>Neurodevelopment</td>
<td>Salinas Valley, California, United States</td>
</tr>
<tr>
<td>Young et al. (2005)</td>
<td>CHAMACOS</td>
<td>Prospective cohort</td>
<td>381 Mother–children pairs</td>
<td>Neurodevelopment</td>
<td>Salinas Valley, California, United States</td>
</tr>
<tr>
<td>Eskanazi et al. (2004)</td>
<td>CHAMACOS</td>
<td>Prospective, cohort</td>
<td>488 Mother–newborn pairs</td>
<td>Birth outcomes</td>
<td>Salinas Valley, California, United States</td>
</tr>
<tr>
<td>Horton et al. (2011)</td>
<td>CCCEH</td>
<td>Prospective, cohort</td>
<td>346 Mother–children pairs</td>
<td>Neurodevelopment</td>
<td>New York, United States</td>
</tr>
<tr>
<td>Rauh et al. (2006)</td>
<td>CCCEH</td>
<td>Prospective cohort</td>
<td>254 Mother–children pairs</td>
<td>Neurodevelopment</td>
<td>New York, United States</td>
</tr>
<tr>
<td>Whyatt et al. (2004)</td>
<td>CCCEH</td>
<td>Prospective, cohort</td>
<td>314 Mother–newborn pairs</td>
<td>Birth outcomes</td>
<td>New York, United States</td>
</tr>
<tr>
<td>Perera et al. (2003)</td>
<td>Mt. Sinai</td>
<td>Prospective, cohort</td>
<td>360 Mother–children pairs</td>
<td>Neurodevelopment</td>
<td>New York, United States</td>
</tr>
<tr>
<td>Engel et al. (2011)</td>
<td>Mt. Sinai</td>
<td>Prospective cohort</td>
<td>404 Mother–newborn pairs</td>
<td>Birth outcomes</td>
<td>New York, United States</td>
</tr>
<tr>
<td>Wolff et al. (2007)</td>
<td>Mt. Sinai</td>
<td>Prospective, cohort</td>
<td>311 Mother–newborn pairs</td>
<td>Neurodevelopment</td>
<td>New York, United States</td>
</tr>
<tr>
<td>Engel et al. (2007)</td>
<td>Mt. Sinai</td>
<td>Prospective, cohort</td>
<td>404 Mother–newborn pairs</td>
<td>Birth outcomes</td>
<td>New York, United States</td>
</tr>
<tr>
<td>Berkowitz et al. (2004)</td>
<td>Mt Sinai</td>
<td>Prospective, cohort</td>
<td>1139 Children</td>
<td>Neurodevelopment</td>
<td>United States</td>
</tr>
<tr>
<td>Bouchard et al. (2010)</td>
<td>NHANES</td>
<td>Cross sectional</td>
<td>279 Children</td>
<td>Neurodevelopment</td>
<td>Mississippi and Ohio</td>
</tr>
<tr>
<td>Ruckart et al. (2004)</td>
<td>–</td>
<td>Cross sectional</td>
<td>72 Primary school children</td>
<td>Neurodevelopment</td>
<td>Tabacundo, Ecuador</td>
</tr>
<tr>
<td>Grandjean et al. (2006)</td>
<td>–</td>
<td>Cross sectional</td>
<td>84 Primary school children</td>
<td>Neurodevelopment</td>
<td>Tabacundo, Ecuador</td>
</tr>
<tr>
<td>Harari et al. (2010)</td>
<td>–</td>
<td>Cross sectional</td>
<td>48 Children</td>
<td>Neurodevelopment</td>
<td>Arizona, United States</td>
</tr>
<tr>
<td>Sanchez-Lizardi et al. (2008)</td>
<td>–</td>
<td>Children</td>
<td>Neurodevelopment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soldin et al. (2009)</td>
<td>–</td>
<td>Case–control</td>
<td>41 Mother–children/41 control pairs</td>
<td>Acute lymphoblastic leukemia</td>
<td>Washington, United States</td>
</tr>
</tbody>
</table>

### Table 2

Occupational studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Type of study</th>
<th>Target population</th>
<th>Health effect examined</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanchez-Pena et al. (2004)</td>
<td>Cross sectional</td>
<td>33 Workers with different agricultural activities</td>
<td>Sperm chromatin structure</td>
<td>Villa Juarez, State of Durango, Mexico</td>
</tr>
<tr>
<td>Recio-Vega et al. (2008)</td>
<td>Cross sectional</td>
<td>52 Workers with different agricultural activities</td>
<td>Sperm quality</td>
<td>Villa Juarez, Durango, Mexico</td>
</tr>
<tr>
<td>Recio et al. (2001)</td>
<td>Cross sectional</td>
<td>4 Sprayers/5 agricultural workers (not sprayers)</td>
<td>Sperm sex null aneuploidy</td>
<td>Villa Juarez, Durango, Mexico</td>
</tr>
<tr>
<td>Recio et al. (2005)</td>
<td>Cross sectional</td>
<td>64 Agricultural workers</td>
<td>Hormone profile</td>
<td>Villa Juarez, Durango, Mexico</td>
</tr>
<tr>
<td>Blanco-Munoz et al. (2010)</td>
<td>Cross sectional</td>
<td>104 Floriculturists</td>
<td>Hormone Profile</td>
<td>Morelos-Mexico</td>
</tr>
<tr>
<td>Lacasana et al. (2010a,b)</td>
<td>Cross sectional</td>
<td>136 Floriculturists</td>
<td>Thyroid function</td>
<td>Morelos and Mexico, Mexico</td>
</tr>
<tr>
<td>Muniz et al. (2008)</td>
<td>Cross sectional</td>
<td>20 Agricultural workers</td>
<td>DNA Damage</td>
<td>Oregon, United States</td>
</tr>
<tr>
<td>McCauley et al. (2008)</td>
<td>Cross sectional</td>
<td>132 Agricultural worker/54 controls</td>
<td>DNA Damage</td>
<td>Oregon, United States</td>
</tr>
<tr>
<td>Rotlein et al. (2006)</td>
<td>Cross sectional</td>
<td>96 Farmworkers/45 controls</td>
<td>Neurologic effects</td>
<td>Oregon, United States</td>
</tr>
<tr>
<td>Albers et al. (2004a,b,c)</td>
<td>Prospective, cohort</td>
<td>53 Chlorpyrifos manufacture workers/60 controls</td>
<td>Neurologic effects</td>
<td>Dow Chemical Company, Midland, Michigan, United States</td>
</tr>
<tr>
<td>Dick et al. (2001), Steenland et al. (2000)</td>
<td>Cross sectional</td>
<td>106 Termiticide workers/52 controls</td>
<td>Neurologic effects</td>
<td>North Carolina, United States</td>
</tr>
<tr>
<td>Strong et al. (2004)</td>
<td>Cross sectional</td>
<td>211 Farm workers</td>
<td>Acute health symptoms</td>
<td>Lower Yakima Valley, United States</td>
</tr>
<tr>
<td>Buchanan et al. (2001), Pilkington et al. (2001)</td>
<td>Cross sectional</td>
<td>612 Sheep dippers, 53 farmers who were not sheep dippers, and 107 ceramic workers</td>
<td>Neurologic effects</td>
<td>Hereford and Worcester, United Kingdom</td>
</tr>
<tr>
<td>Rees (1996)</td>
<td>Cross sectional</td>
<td>38 Sheep dippers</td>
<td>Acute health symptoms</td>
<td>Wales, United Kingdom</td>
</tr>
<tr>
<td>Stephens and Sreenivasan (2004)</td>
<td>Cross sectional</td>
<td>31 Orchard sprayers, 26 pig farm workers, 33 construction workers</td>
<td>Neurologic effects</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>Stephens et al. (1995)</td>
<td>Cross sectional</td>
<td>146 Sheep dippers/143 quary workers</td>
<td>Neurologic effects</td>
<td>Devon, Cumbria, north Wales, United Kingdom</td>
</tr>
<tr>
<td>Lee et al. (2007)</td>
<td>Cross sectional</td>
<td>18 Indoor insecticide workers/18 controls</td>
<td>Oxidative DNA damage (8-OH-dG)</td>
<td>Nagoya, Japan</td>
</tr>
<tr>
<td>Yucra et al. (2008)</td>
<td>Cross sectional</td>
<td>31 Pesticide applicators/31 controls</td>
<td>Sperm quality</td>
<td>Caylloma, Arequipa, Peru</td>
</tr>
<tr>
<td>Padunzgod et al. (2000)</td>
<td>Cross sectional</td>
<td>32 OP manufacture workers/43 non exposed</td>
<td>Sperm quality</td>
<td>Anqing, China</td>
</tr>
<tr>
<td>Atherton et al. (2009)</td>
<td>Cross sectional</td>
<td>17 Horticultural workers/7 controls</td>
<td>DNA Damage</td>
<td>Almeria, Spain</td>
</tr>
</tbody>
</table>
3.2.2. Biomarkers of exposure to OP and PYR pesticides and their detection and quantification

3.2.2.1. OP and PYR metabolism. Organophosphates are derivatives of phosphoric or thiophosphoric acids. Metabolism of thiophosphorin analogues by oxidative desulfuration in humans transforms sulfur to oxygen giving the oxon form, a potent cholinesterase inhibitor. Another important metabolic reaction is hydrolysis of OP to a specific metabolite moiety and a dialkyl phosphate (DAP) moiety (Fig. 1). This reaction results in a decrease in toxicity, as the leaving group and dialkyl phosphate metabolites do not inhibit cholinesterase enzymes. These metabolites and hydrolysis products are then excreted in the urine, either in free form or bound to glucuronic acid or sulfates.

Six dialkyl phosphate (DAP) metabolites are the most commonly measured general biomarkers of OP insecticides. These metabolites reflect exposure to OP but do not verify the presence of a particular OP compound. The six common DAP metabolites measured are dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP) (Fig. 1).

![Dialkylphosphates](image)

Fig. 1. Chemical structure of the non-specific metabolites of organophosphorus pesticides (DAPs).

The most frequently measured specific metabolite is 3,5,6-trichloropyridinol (TCP) which is a metabolite of the widely used OP insecticide, chlorpyrifos. Other specific OP biomarkers include malathion metabolites (malathion dicarboxylic acid, α and β isomers of malathion monocarboxylic acid), parathion and methyl parathion metabolites (para-nitrophenol) (Barr, 2008).

3.2.2.1.2. Pyrethroids. Pyrethroids are rapidly metabolized into the corresponding carboxylic acids and by hydrolytic cleavage of the ester bond, followed by oxidation and mainly glucuronization, being eliminated in urine as conjugates (Margariti et al., 2007). Because of pyrethroids rapid metabolism the concentrations of intact pyrethroids in serum or plasma are much lower than urinary metabolites, and therefore urine samples are preferred to blood samples (Margariti et al., 2007).

The most commonly measured pyrethroid metabolites are cis- and trans-isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (cis- and trans-DCCA) which are metabolites of permethrin, cypermethrin, and cyfluthrin; cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA) which is a metabolite of deltamethrin; and 4-fluoro-3-phenoxybenzoic acid (4F3PBA) which is a metabolite of cyfluthrin. The most frequently measured general pyrethroid metabolite is 3-phenoxybenzoic acid (3PBA) which is a metabolite that is common to as many as 20 synthetic pyrethroids (Barr, 2008) (Fig. 2).

![Pyrethroid Metabolites](image)

Fig. 2. Chemical structure of the most commonly measured pyrethroid metabolites; cis- and trans-isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (cis/trans-DCCA), cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA), 3-Phenoxybenzoic acid (3PBA) and 4-fluoro-3-phenoxybenzoic acid (4F3PBA).
3.2.2. Reviewed studies: Analytical approaches. For the detection and quantification of OP and PVR exposure biomarkers, urine is by far the most frequent matrix used. Forty-five out of 49 studies analyzed urine samples and 4 determined pesticide levels in plasma. DAP urinary metabolites of OP pesticides were measured in 30 studies, and among specific metabolites, TCP in urine was the most common measurement (9 studies). Only 6 studies measured PVR metabolites and mostly urinary 3-PBA. All studies measuring PVR metabolites were published after 2004 indicating the increased interest in PVR biomonitoring and this obviously correlates with the increase in residential and recreational PVR applications.

The methods used for metabolite quantification were mainly gas chromatography combined with mass spectrometry (25 studies, Table 4) or flame photometric detection (15 studies, Table 4). For simultaneous multicomponent monitoring of small chemical compounds, capillary GC based methods are most appropriate due to the high resolving power and high peak capacity of capillary columns usually used. MS or FPD detectors ensure low detection limits that are needed for monitoring human exposure to pesticides. Six studies used LC–MS analytical approaches. One study used HPLC with Photodiode Array Detector (PAD) for monitoring 3-PBA and TCP.

It should be underlined that in some studies, information on the analytical approaches used was not clear or was incomplete, the validity of the method was not demonstrated or traceable (e.g. reference to the adopted method and adjustments done) and this sometimes questioned the validity of the whole study. In addition, the dispersion in concentration units used sometimes made the comparison of results presented by different studies difficult. Regarding urinary analysis, results were presented in nmol/L, µg/L and µg/g creatinine. Accurate conversion of nmol to µg in order to compare the results was not always possible, because in some studies the results were presented as aggregates of different substances (e.g. DMP, ΣDEP or ΣDAP) in nmol/L. Moreover, in some studies the concentrations found in urine were expressed as µg/g creatinine, in others creatinine was measured but not used for normalization or was not even measured.

Therefore, in future studies the analytical approaches used and their validity should be better described.

3.3. Epidemiological findings: Pesticides exposure and health outcomes

Specific effects and pathological parameters have been studied in relation to levels of pesticides and their metabolites in human

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Analytical approaches used in the reviewed studies.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical approach</td>
<td>Studies</td>
</tr>
<tr>
<td>GC–MS or GC–MS/MS 25 Studies</td>
<td>(Horton et al., 2011; Perera et al., 2003; Rauh et al., 2006; Soldin et al., 2009; Whyatt et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>(Bouchard et al., 2011; Eskenazi et al., 2004, 2007; Grandjean et al., 2006; Harari et al., 2010; Marks et al., 2010; Young et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>(Bouchard et al., 2010; Engel et al., 2007, 2011; Wolff et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>(Meeker et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>(Swan et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>(Meeker et al., 2004a,b)</td>
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<td></td>
<td>(Han et al., 2008)</td>
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<td></td>
<td>(Lee et al., 2007)</td>
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<tr>
<td></td>
<td>(Ji et al., 2011; Xia et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>(Dick et al., 2001; Steenland et al., 2000)</td>
</tr>
<tr>
<td>GC-FPD or GC-FPD 15 studies</td>
<td>(Recio et al., 2001, 2005; Recio-Vega et al., 2008; Sanchez-Pena et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>(McCauley et al., 2008; Muniz et al., 2008; Rothlein et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>(Blanco-Munoz et al., 2010; Lacasana et al., 2010a)</td>
</tr>
<tr>
<td></td>
<td>(Buchanan et al., 2001; Pilkington et al., 2001; Rees, 1996; Stephens et al., 1995; Stephens and Sreenivasan, 2004)</td>
</tr>
<tr>
<td></td>
<td>(Sanchez-Lizardi et al., 2008; Yucra et al., 2008)</td>
</tr>
<tr>
<td>LC–MS or LC–MS/MS 6 studies</td>
<td>(Meeker et al., 2008, 2009)</td>
</tr>
<tr>
<td></td>
<td>(Atherton et al., 2009)</td>
</tr>
<tr>
<td>HPLC 1 study</td>
<td>(Engel et al., 2007; Eskenazi et al., 2004, 2007)</td>
</tr>
<tr>
<td></td>
<td>(Berkowitz et al., 2004)</td>
</tr>
<tr>
<td>Undefined methods 5 studies</td>
<td>(Albers et al., 2004a,b,c, 2007; Padungtod et al., 2000; Perry et al., 2007; Ruckart et al., 2004; Strong et al., 2004)</td>
</tr>
</tbody>
</table>

1. dimethylphosphate (DMP); 2. dimethylphosphate (DMP); 3. dimethylthiophosphate (DMTDP); 4. diethylphosphate (DEP); 5. diethylthiophosphate (DETP); 6. diethylthiophosphate (DEPT); 7. 3,5,6-trichloro-2-pyridinyl (TCP, methyl/ethyl chlorpyrifos metabolite); 8. 5-chloro-1,2-dihydro-1-isopropyl-[3H]-1,2,4-triazol-3-one (CIT, isozofos methyl/ethyl metabolite); 9. 3-chloro-4-methyl-7-hydroxycoumarin (CMHC coumaphos metabolite); 10. 2-diethylamino-5-methyl-4-pyrimidinol (DEAMY, primiphos methyl metabolite); 11. malathion dicarboxylic acid (MDA, malathion metabolite); 12. para-nitrophenol (PNP, methyl/ethyl parathion metabolite); 13. 2-isopropyl-5-ethyl-6-hydroxypropirimine (IMP, diazinon metabolite); 14. 3-PBA; 15. TDCCA; 16. 4-FPBA; 17. CDDCA; 18. BDDCA; 19. Chlorpyrifos (CPF); 20. Diazinon; 21. 4-nitrophenol; 22. cis-permethrin (CPM); 23. trans-permethrin (TPM).

* Three out of five studies do not present the limits of detection.
biological samples. Biomarkers of organophosphate and pyrethroid insecticides have mainly been used to investigate the relationship between pesticides exposure, expressed as levels of parent compounds or metabolites with: (a) birth outcomes, (b) neuro-behavioral and neurophysiologic parameters, (c) sperm quality parameters and sperm DNA damage, (d) hormone disorders (e) DNA damage, (f) other health outcomes.

3.3.1. Birth outcome studies

The hypothesis that pesticide exposure during pregnancy is associated with fetal development was examined in five of the publications included in this review. Articles presented the findings from studies that investigated prenatal pesticide exposure and the potential effects on birth outcomes. Results were derived from three large cohort studies, the CHAMACOS, the CCCEH cohort (Perera et al., 2003; Whyatt et al., 2004) and the Mount Sinai cohort (Berkowitz et al., 2004; Wolff et al., 2007). In the CHAMACOS (Eskenazi et al., 2004) cohort, the effects of pesticide exposure, estimated as metabolite levels in maternal urine, on fetal growth and length of gestation were examined. The results showed an association between urinary DMP metabolites and decreased gestation duration. However, no adverse relationships were found between any of the biomarker levels (DAPs, TCP, MDMA, PNP) and fetal growth. In the Mount Sinai cohort, Berkowitz et al. evaluated the effects of pesticides exposure on birth weight, birth length, head circumference and gestational length. Exposure was assessed by questionnaire data and urinary TCP and 3-PBA measurements in mothers. No association was found between exposure and fetal growth or gestational length. Maternal levels of TCP were associated with a small reduction in head circumference only when PON1 was considered. The relationship between maternal DAP’s and birth outcomes was investigated on the same population sample in a later published study (Wolff et al., 2007). Birth weight was marginally associated with prenatal DEP’s and birth length was affected by prenatal DMP’s but only when individual susceptibility (PON1) was considered. During the CCEH study, Perera et al. found significant associations between CPF levels in plasma and decreased birth weight and birth length (Perera et al., 2003). Whyatt et al. expanded this research to include an analysis of personal air during pregnancy and additional metabolite measurements. Decreased birth weight and birth length were again associated with plasma OP metabolites (Whyatt et al., 2004).

The results were not consistent throughout the studies. In the CCEH study, the associations between exposure and fetal growth were significant, in contrast to the CHAMACOS study where no association was found. In the Mount Sinai study, weak associations between birth weight, birth length and head circumference with biomarker levels were reported and only when paraoxonase activity was taken into account. Gestational age was associated with maternal biomarker measurements only in the CHAMACOS study, while the other two studies reported no association.

3.3.2. Studies that examined neurologic effects

Studies that investigated the neurologic effects of pesticides can be subcategorized into studies that focused on children's neurodevelopment and studies that measured the effects on adults, occupationally exposed.

3.3.2.1. Neurodevelopment and neurobehavioral studies in children.

Children are more vulnerable to pesticide neurotoxicity because their brains develop rapidly (Eskenazi et al., 1999; Rice and Barone, 2000). In addition, the magnitude of exposure in children is greater than in adults because children ingest and inhale larger quantities of food and air, respectively, per body weight unit. Animal studies have shown that prenatal exposure to OP pesticides is also related to neurodevelopment (Eskenazi et al., 1999).

Behavioral changes can indicate disruptions in nervous system function, and can result from low-dose pesticide exposure. Neurobehavioral tests are non-invasive, quantitative methods that can measure a wide variety of sensor, motor and cognitive functions. Methods used in the studies presented in this review, included numerous tests to assess the subject’s performance on a wide variety of functions.

During the CHAMACOS cohort study, Young et al. assessed the relationship between levels of DAP metabolites in urine in pregnant women (during pregnancy and after delivery) and behavioral effects as well as abnormal reflexes in neonates (Young et al., 2005). The Brazelton Neonatal Behavioral Assessment Scale (BNBAS) was used for the evaluation of neurobehavioral performance. Measured effects were divided into seven clusters including habituation, orientation, motor performance, range of state, regulation of state, autonomic stability and reflexes. A significant association was found between exposure and the reflex cluster. No significant association was reported between DAP levels and other clusters. A similar assessment scheme was adopted in the Mount Sinai Children’s Environmental Health Cohort study (Engel et al., 2007). The same seven cluster scoring method was used to reduce the dimensionality of data derived from the BNBAS. Pre-natal measurements of DAP metabolites and MDMA were used to quantify maternal exposure to OP pesticides. The results were consistent with Young et al. indicating strong associations between prenatal OP exposure and abnormal reflexes.

Eskenazi et al. studied the association between levels of different biomarkers (Table 5) during pregnancy and offspring early childhood with effects on neurodevelopment (Eskenazi et al., 2007). Children were followed up to age 24 months. Cognitive abilities, muscle and motor coordination and emotional/behavioral problems were measured at age 6, 12 and 24 months. Pervasive development disorder was (correlated) associated with prenatal and postnatal levels of urinary DAP. DAP levels were also associated with decreased cognitive abilities. However, the opposite result was found in children when the DAP levels were associated with increased cognitive abilities. This result is difficult to interpret. An interesting explanation for these contradictory results was suggested by the authors. In particular, they supposed that the higher levels of exposure may result from increased cognitive functioning, because children with increased mental development interact more with the environment. However, these results were not repeated or confirmed by other studies. Marks et al., continuing the study of Eskenazi et al., examined the effects of prenatal and childhood exposure to OPs on children’s attention at age 3.5 and 5 years (Marks et al., 2010). Attention was assessed using maternal reports, psychometrical observations and direct assessment. The authors reported positive associations between attention problems and biomarker levels both in pregnant women and offspring. However, the relationship between prenatal exposure and attention problems at the age of 5 years was strongest. In the latest published study from the CHAMACOS cohort children from the same population sample was examined at the age of 7. Maternal DAP’s were associated with poorer intellectual development at 7-year-old children (Bouchard et al., 2011). Additionally, recent published evidence from the Mt Sinai cohort demonstrate an association of prenatal DAP’s with a decrement in mental development at 12 months age and slight decrements in later childhood (6–9 years old) (Engel et al., 2011).

Children’s neurodevelopment in response to prenatal pesticide exposure was also studied in the CCEH cohort study. Chlorpyrifos exposure was assessed by analysis of pregnant women’s plasma, and motor, cognitive and behavioral function was examined in children at age 12, 24 and 36 months. The results showed significant associations between prenatal chlorpyrifos exposure and both mental and motor delays, attention problems, attention
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Biomarkers</th>
<th>Target population</th>
<th>Neurobehavioral tests</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouchard et al. (2011)</td>
<td>Urinary DAP metabolites (prenatal and children measurements)</td>
<td>329 Mother–children pairs</td>
<td>Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV);</td>
<td>Prenatal DAP's were associated with poorer intellectual development in 7-year-old children, Postnatal DAP's were not associated with cognitive scores</td>
</tr>
<tr>
<td>Marks et al. (2010)</td>
<td>Urinary DAP metabolites (prenatal and children measurements)</td>
<td>348 Mother–children pairs</td>
<td>Child Behavior Checklist (CBCL); Developmental NEuroloPSYcological Assessment (NEPSY II); Conners' Kiddie Continuous Performance Test (K-CPT)</td>
<td>Positive association of prenatal DAP with attention problems, Weaker association between child DAP and attention problems</td>
</tr>
<tr>
<td>Eskenazi et al. (2007)</td>
<td>Urinary DAP metabolites, TCP, MDA (prenatal and children measurements)</td>
<td>396 Mother–children pairs</td>
<td>Child Behavior Checklist (CBCL); Baleys Scale of infants development (BSID)</td>
<td>Prenatal DAP's were negatively associated with Mental Development, Child DAP's were positively associated with Mental development, No associations were found between pre natal or child measures with pervasive development disorder</td>
</tr>
<tr>
<td>Young et al. (2005)</td>
<td>Urinary DAP (prenatal and postnatal measurements)</td>
<td>381 Mother–children pairs</td>
<td>Brazelton Neonatal Behavioral Assessment Scale (BNBAS)</td>
<td>Prenatal DAP's were associated with abnormal reflexes, Postnatal DAP's were not associated with neurodevelopment effects</td>
</tr>
<tr>
<td>Horton et al. (2011)</td>
<td>Plasma cis- and trans permethrin and CPF (at delivery)</td>
<td>346 Mother–children pairs</td>
<td>Baleys Scale of infants development (BSID-II)</td>
<td>No association between prenatal and child PYR metabolites with neurodevelopment reported</td>
</tr>
<tr>
<td>Rauh et al. (2006)</td>
<td>Plasma CPF (at delivery)</td>
<td>254 Mother–children pairs</td>
<td>Baleys Scale of infants development (BSID); Child Behavior Checklist (CBCL)</td>
<td>Higher levels of prenatal chlorpyrifos were associated with psychomotor and mental development delays, attention problems and attention deficit/hyperactivity disorders</td>
</tr>
<tr>
<td>Engel et al. (2011)</td>
<td>Urinary DAP metabolites (prenatal measurements)</td>
<td>360 Mother–children pairs</td>
<td>Baleys Scale of infants development (BSID-II); Wechsler Preschool and Primary Scale of Intelligence – Third Edition (WPPSI-III), Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV)</td>
<td>Prenatal DAP's were associated with a decrement in mental development at 12 months, Prenatal DAP's were associated with decrements in perceptual reasoning at 6–9 years old (only when paraoxonase Q192R Effect was taken into account)</td>
</tr>
<tr>
<td>Engel et al. (2007)</td>
<td>Urinary DAP metabolites, MDA (prenatal measurements)</td>
<td>311 Mothers–newborn pairs</td>
<td>Brazelton Neonatal Behavioral Assessment Scale (BNBAS)</td>
<td>Prenatal MDA levels were associated with an increase in abnormal reflexes, Higher levels of total DAP's were associated with an increase in abnormal reflexes, Weaker associations were associated with Attention</td>
</tr>
<tr>
<td>Bouchard et al. (2010)</td>
<td>Urinary DAP metabolites (children measurements)</td>
<td>1139 Children</td>
<td>Diagnostic Interview Schedule for Children IV (DISC-IV)</td>
<td>Child DAP's levels were associated with Attention</td>
</tr>
<tr>
<td>Ruckart et al. (2004)</td>
<td>Urinary PNP (children measurements)</td>
<td>279 Children</td>
<td>Pediatric Environmental Test Battery (PENTB)</td>
<td>Deficit/Hyperactivity Disorder Exposed children (classified by urinary PNP and MP household samples) had increased short memory, attention problems, behavioral and motor skill problems. However the results were not consistent in both sites of the study</td>
</tr>
<tr>
<td>Harari et al. (2010)</td>
<td>Urinary DAP metabolites (children measurements)</td>
<td>84 Primary School Children</td>
<td>Wechsler Intelligence Scale for Children-revised (WISC), Raven's Colored Progressive Matrices, Stanford-Binet Copying test, Conners' Kiddie Continuous Performance Test (K-CPT)</td>
<td>Borderline statistically significant association of child exposure with reaction time</td>
</tr>
<tr>
<td>Grandjean et al. (2006)</td>
<td>Urinary DAP (children measurements)</td>
<td>72 Primary School Children</td>
<td>Santa Ana Pegboard; Wechsler Intelligence Scale for Children, Stanford-Binet copying subtest</td>
<td>Child DAP's were associated with increased reaction time only</td>
</tr>
<tr>
<td>Sanchez-Lizardi et al. (2008)</td>
<td>Urinary DAP (children measurements)</td>
<td>48 Children</td>
<td>Wechsler Intelligence Scale for Children – Third Edition (WISC-III); Children's Memory Scale (CMS); Wisconsin Card Sorting Test (WCST); Trail Making Test A &amp; B (TMTA &amp; TMTB); Child Behavior Checklist (CBCL); The Teacher Report Form (TRF)</td>
<td>Child DAP's levels were associated with effects of speed of attention, sequencing, mental flexibility, visual search, conceptual formation and conceptual flexibility</td>
</tr>
</tbody>
</table>

Table 5: Neurobehavioral studies on children and infants exposed to pesticides.
3.3.2.2. Neuropathy

Neuropathy cannot be associated with increased reaction time in a cross-sectional study conducted in Ecuador (Grandjean et al., 2006). No association resulted from the statistical analysis on other examined neurobehavioral outcomes. In a similar cross-sectional study examining 84 primary school children a borderline significant association between DAP’s and reaction time was reported (Harari et al., 2010). Adverse associations were also found between attention-related performance and DAP metabolites in a small study involving 48 children living in an agricultural community (Sanchez-Lizardi et al., 2008).

A recently published large cross-sectional study examined the effect of exposure to OP in children aged 8–15 years (n = 1139). The exposure data were extracted from the National Health and Nutrition Examination Survey (NHANES) and correlated with attention deficit/hyperactivity disorder status assessed from interviews with parents. This study reported that children with higher DAP metabolite levels were more likely to be diagnosed with attention deficit–hyperactivity disorder problems (Bouchard et al., 2010).

In only the study that examined PYR exposure, no association was found between prenatal exposure and neurodevelopment (Horton et al., 2011). However, an association was found between prenatal piperonyl butoxide (a pyrethroid synergist) in personal air samples and the Mental Development Index (MDI) scores.

In general, these studies produced significant evidence that women exposure to OP during pregnancy is correlated with neurodevelopmental effects in children. However, when exposure was determined by the analysis of children’s samples, the results were not conclusive. Although all nine publications reported some association, concerns have been raised regarding contradiction (Eskenazi et al., 2007), inconsistency (Ruckart et al., 2004), and decreased significance of the findings compared to the prenatal exposure–effect relationship (Marks et al., 2010). In general, from the existing literature we can assume that exposure of children to OP pesticides might affect neurodevelopment, but safe conclusions cannot be made.

3.3.2.2. Neurological studies on occupationally exposed males

Six studies have examined the relationship between exposure to pesticides and neurological effects in adults. Behavioral tests, motor and sensory tests, clinical examinations and nerve conduction studies were used to assess neurological effects. All studies focused on OP exposure.

A prospective cohort study used TCP measurements and Nerve Conduction Study (NCS) examinations along with clinical evaluations to determine whether chronic occupational exposure to chlorpyrifos was related to peripheral neuropathy or central nervous system function (Albers et al., 2004a,b,c, 2007). This study combined biomonitoring with air monitoring data derived from hygiene records and exposure history questionnaires, to estimate chronic exposure. There was no evidence that central nervous system (CNS) dysfunction and clinical or subclinical peripheral neuropathy were associated with chlorpyrifos exposure (Albers et al., 2004b,c). Few statistically significant associations between chlorpyrifos exposure and NCS results were found, providing little support for the hypothesis that chronic chlorpyrifos exposure affects peripheral nerve electrophysiology (Albers et al., 2007).

Cumulative exposure to OPs was estimated using an empirical exposure model derived from DAP metabolite measurements and an exposure history questionnaire, in a cross-sectional exposure–response study conducted on sheep dippers working with OP pesticides (Buchanan et al., 2001; Pilkinson et al., 2001). Neurological assessments included a symptoms questionnaire and quantitative sensory tests. A higher prevalence of neurologic symptoms was reported in the exposed group (sheep dippers) and a weak positive association was found between the estimated cumulative exposure and symptoms. Quantitative sensory thresholds were not associated with the estimated cumulative exposure.

Steenland et al. studied the neurologic effects of chlorpyrifos exposure in termiticide applicators. Urinary TCP was used as a biomarker of exposure (Steenland et al., 2000). Neurologic tests, self-reported symptoms, and clinical examinations were used. Steenland et al. reported few significant differences in neurologic test results between exposed and non-exposed groups, but significantly more self-reported symptoms in the exposed group. Dick et al. provided more detailed analysis of the results of this study, focusing on the acute neurologic effects measured by motor and sensory tests (Dick et al., 2001). A statistically significant effect on postural sway was reported, but correlations between TCP levels and other motor–sensory functions were limited or absent.

Three other studies examined neurobehavioral effects resulting from OP exposure (Stephens et al., 1995; Stephens and Sreenivasan, 2004; Rothlein et al., 2006). These studies reported increased neurobehavioral deficits in the highly exposed groups, but only Rothlein et al. reported a correlation between urinary OP metabolite levels and poorer performance on neurobehavioral tests. It should be noted that Stephens et al. (1995) did not attempt to correlate biomarker levels with the measured effect but used the measurement to confirm absence of recent OP exposure.

In two of these studies, exposure to OP was associated with neurologic symptoms assessed using a questionnaire. Moreover, when the outcome was measured using neurobehavioral tests, a correlation between neurobehavioral deficits and OP pesticide exposure was identified.

However, limited evidence was provided for neurologic effects when the outcome was measured using neurologic tests.

3.3.3. Sperm quality parameters and sperm DNA damage studies

There has been growing concern regarding the progressive decline in semen quality of persons exposed to pesticides, especially sperm concentration. Animal studies indicate that PYR exposure can affect sperm concentration, motility and morphology (Elbeteiha et al., 2001; Kumar et al., 2004; Mani et al., 2002).

Perry summarized and reviewed studies that examined the effects of occupational and environmental pesticide exposure on human sperm and suggested that there was a correlation between sperm parameters and pesticide exposure (Perry, 2008). However, findings regarding the effects on sperm DNA damage and aneuploidy were not conclusive.

In this review, twelve studies are included which focused on the effects of PYR and OP exposure on semen quality parameters and sperm DNA. Study subjects, biomarkers measured, parameters investigated, and study findings are summarized in Table 6. Among the twelve studies, five were occupational, and seven measured non-occupational exposure. Ten studies examined sperm parameters (mainly sperm concentration, motility and morphology). Only one of these studies reported no association between biomarker levels and the measured effects on sperm quality (Sanchez-Pena et al., 2004). Three studies reported significant associations between PYR biomarkers and sperm parameters (Ji et al., 2011; Meeker et al., 2008; Xia et al., 2008). Four studies measuring OP and one measuring both OP and PYR metabolites, reported weak or borderline significant associations (Meeker et al., 2004a; Padungtod et al., 2000; Perry et al., 2007; Recio-Vega et al., 2008;
### Table 6

Studies investigating effects of pesticides exposure on sperm.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Subjects</th>
<th>Biomarkers</th>
<th>Parameters investigated</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Padungtod et al. (2000)</td>
<td>32 OP manufacture workers/43 non exposed</td>
<td>Urinary PNP</td>
<td>Liquefaction time, seminal volume, pH, sperm concentration, sperm morphology, sperm motility and motility progression score</td>
<td>The exposed groups had significant decreased sperm concentration and sperm motility. Weak evidence of dose–response relationship between p-nitrophenol and sperm parameters are provided. Significant associations between OP metabolites and frequency of sperm aneuploidy were noted.</td>
</tr>
<tr>
<td>Recio et al. (2001)</td>
<td>4 Sprayers/5 agricultural workers</td>
<td>Urinary DAP metabolites</td>
<td>Sperm Aneuploidy</td>
<td>Significant associations between OP metabolites and frequency of sperm aneuploidy.</td>
</tr>
<tr>
<td>Meeker et al. (2004a)</td>
<td>272 Men recruited from an infertility clinic</td>
<td>Urinary TCP</td>
<td>Sperm concentration, motility and morphology</td>
<td>Borderline-significant association for TCP with sperm concentration and motility.</td>
</tr>
<tr>
<td>Meeker et al. (2004b)</td>
<td>260 Men recruited from an infertility clinic</td>
<td>Urinary TCP</td>
<td>Sperm DNA integrity assessed by comet assay</td>
<td>Significant increase in % comet tail DNA in subjects with higher urinary TCP levels</td>
</tr>
<tr>
<td>Sanchez-Pena et al. (2004)</td>
<td>33 Workers with different agricultural activities</td>
<td>Urinary DAP metabolites</td>
<td>Sperm chromatin structure semen volume, sperm concentration, sperm count, sperm motility, sperm viability</td>
<td>Significant association between DETP levels and DNA Fragmentation Index (DFI). No significant associations between semen quality parameters and DAP levels found.</td>
</tr>
<tr>
<td>Perry et al. (2007)</td>
<td>18 men randomly selected from a reproductive cohort</td>
<td>24 Parent compounds and metabolites in urine</td>
<td>Sperm concentration</td>
<td>Some suggestion that higher levels of OP and pyrethroid metabolites are correlated with lower sperm concentration was provided.</td>
</tr>
<tr>
<td>Yuca et al. (2008)</td>
<td>31 Pesticide applicators/31 controls</td>
<td>Urinary DAP metabolites</td>
<td>Liquefaction time, seminal volume, pH, sperm concentration, total sperm number, sperm morphology, sperm motility, sperm viability and concentration of leukocyte</td>
<td>Significant reduction of semen volume and an increase of pH in men with OP exposure. DAP levels had a weaker relation with effects.</td>
</tr>
<tr>
<td>Recio-Vega et al. (2008)</td>
<td>52 Workers with different agricultural activities</td>
<td>Urinary DAP metabolites</td>
<td>Semen volume, sperm concentration, sperm count, sperm motility, rapid progressive motility, Sperm viability, dead sperm</td>
<td>Significant decrease in sperm count and sperm volume among subjects with higher OP exposure. DMGTP levels were associated with sperm viability. No other associations between biomarkers and sperm parameters found.</td>
</tr>
<tr>
<td>Xia et al. (2008)</td>
<td>372 Men recruited from an infertility clinic</td>
<td>Urinary 3-PBA</td>
<td>Semen volume, sperm concentration, sperm count, sperm motility, sperm progression</td>
<td>Significant association between increased 3-PBA levels and sperm concentration and some sperm progression and motion parameters. Non significant or weak associations with other parameters.</td>
</tr>
<tr>
<td>Meeker et al. (2008)</td>
<td>207 Men recruited from an infertility clinic</td>
<td>Urinary 3-PBA, CDCCA-TDCCA</td>
<td>Sperm DNA damage (measured by comet assay) Sperm concentration, sperm motility and sperm motion parameters, sperm morphology</td>
<td>An association was found between % comet tail and 3-PBA and CDCCA values. A significant inverse association between sperm TDCCA and sperm motility and sperm motion parameters was found.</td>
</tr>
<tr>
<td>Ji et al. (2011)</td>
<td>240 Men recruited from an infertility clinic</td>
<td>Urinary 3-PBA</td>
<td>Sperm DNA integrity Seminal volume, sperm concentration, sperm count, sperm motility</td>
<td>Significant association was found between 3PBA levels and sperm DNA fragmentation and sperm concentration. No obvious correlations were found between 3-PBA and seminal volume sperm motility and total sperm count.</td>
</tr>
<tr>
<td>Swan et al. (2003)</td>
<td>36 Cases (males with low sperm parameters)/52 controls</td>
<td>Urinary IMPY, TCP, MDA</td>
<td>Sperm concentration, sperm motility, sperm morphology, sperm count</td>
<td>Men with high levels of IMPY were significantly more likely to be cases than were men with low levels.</td>
</tr>
</tbody>
</table>

### Yucra et al., 2008

Also, one case control study reported significant association of IMPY levels with sperm quality (Swan et al., 2003). It should be noted that in three occupational studies the association was strongest when the statistical analysis was based on exposure classification by occupation (Padungtod et al., 2000; Recio-Vega et al., 2008; Yucra et al., 2008).

With regard to effect on sperm DNA, five studies are reviewed. Effects were determined by the Comet assay, sperm chromatin structure analysis, and sperm aneuploidy assessment. All five studies demonstrated significant associations between biomarker levels and effects on sperm DNA.

### 3.3.4. Impact of OP and PYR exposure on the endocrine system

Seven studies examined the hypothesis that OP and PYR exposure causes alterations in hormone levels (Table 7).

Lacasaña et al. attempted to correlate exposure to OP pesticides with serum levels of thyroid hormones in floricultural workers living in the states of Mexico (Lacasaña et al., 2010a, b). Exposure was assessed by questionnaire data regarding occupational history and urinary DAP measurements. Thyroid hormone measurements included the determination of Thyrotropin (TSH), total thyroxine (T4) and triiodothyronine (T3) levels in serum. A significant positive association between total DAP levels and TSH and T4 levels was
demonstrated. An inverse association between DAP and serum T3 was also reported. The same effects were studied in an older study conducted in 322 non-occupationally exposed males (Meeker et al., 2006). Urinary TCP was used to evaluate chlorpyrifos exposure. Meeker et al. also found an association between OP exposure and TSH levels, but in contrast to Lacasana et al., inverse associations were found in relation to T4 serum levels. Serum levels of Follicle stimulating hormone (FSH), Luteinizing hormone (LH), prolactin, testosterone, inhibit B and estradiol were determined in a cross-sectional study among floriculturists in Morelos, Mexico in order to examine the relationship with OP exposure estimated by measurement of DAP metabolites in urine (Blanco-Munoz et al., 2010). We assume that the population sample is the same as the Lacasana et al. study because it has identical characteristics although it is not clearly stated in the article. A negative association was found between inhibit B and four DAP metabolites. Also, DEP was correlated with decreased FSH concentrations and DETP was marginally associated with lower LH levels. No other correlations were found. A similar study was conducted by Recio et al. (Recio et al., 2005). The authors also found a negative association between FSH levels and two DAP metabolites (DMTP and DMDTP), and a negative association between LH levels and DMTP. Estradiol and testosterone levels were not related to urinary DAP metabolites. Non-occupational exposure to pyrethroid pesticides and its relation with the hormone profile was investigated by Meeker at al. in a study that examined 161 men from an infertility clinic in the USA (Meeker et al., 2009). A variety of hormone levels were determined in serum. Significant positive associations between FSH and 3-PBA and cis- and trans-DCCA levels in urine were reported. In addition, testosterone levels were inversely associated with total PYR metabolites. 3-PBA levels were also associated with increased LH and reduced Estradiol levels in a cross-sectional study involving 212 men diagnosed as infertile (Han et al., 2008). Yucra et al. reported no significant associations between urinary DAP’s and hormone levels (Yucra et al., 2008).

Almost all (6 out of 7) studies demonstrated associations between biomarker levels and effects on hormone function, providing some evidence to support the endocrine disrupting action of OP and PYR insecticides.

3.3.5. Studies that examined DNA damage

DNA damage and oxidative stress have been proposed as mechanisms linking pesticide exposure to long-term health effects (Bagchi et al., 1995; Banerjee et al., 2001).

Four biomonitoring studies on agricultural workers are reviewed. These studies mainly measured 8-hydroxydeoxyguanosine (8-OHdG) levels in urine, as a marker of oxidative stress and DNA damage determined by the Comet assay. A study on 17 horticultural workers recruited in Almeria, Spain and 7 non-occupationally exposed volunteers showed significant positive associations between DAP levels and DNA damage and 8-OHdG levels (Atherton et al., 2009). In a similar pilot study, DAP metabolites, DNA damage and oxidative stress were determined in pesticide applicators (n = 13), farm workers (n = 10) and controls (n = 12) (Muniz et al., 2008). Significant differences in oxidative stress and DNA damage between the three groups were reported, indicating adverse effects due to OP exposure. However, these differences were significantly weaker when correlations between OP metabolites and markers of oxidative stress and DNA damage were investigated. In a larger study involving 132 agricultural workers and 54 controls, Comet parameters, and urinary DAPs were determined. No associations were found between DNA

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Subjects</th>
<th>Biomarkers</th>
<th>Hormones measured</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacasana et al. (2010)</td>
<td>136 Floriculturists</td>
<td>Urinary DAP metabolites</td>
<td>TSH, T3, T4</td>
<td>A significant positive association between total DAP levels and TSH and T4 levels was demonstrated. An inverse association between DAP and serum T3 was also reported.</td>
</tr>
<tr>
<td>Meeker et al. (2006)</td>
<td>322 Non-occupationally exposed recruited through an infertility clinic</td>
<td>Urinary TCP</td>
<td>TSH, T3, T4</td>
<td>Positive association between TCP levels and TSH levels, inverse associations were found in relation to T4 serum levels</td>
</tr>
<tr>
<td>Blanco-Munoz et al. (2010)</td>
<td>104 Floriculturists</td>
<td>Urinary DAP metabolites</td>
<td>LH, prolactin, testosterone, inhibit B and estradiol</td>
<td>A negative association was found between inhibit B and four DAP metabolites. Also DEP was correlated with decreased FSH concentrations and DETP was marginally associated with lower LH levels. No other correlations were found.</td>
</tr>
<tr>
<td>Recio et al. (2005)</td>
<td>64 Agricultural workers</td>
<td>Urinary DAP metabolites</td>
<td>FSH, LH and prolactin</td>
<td>Negative association between FSH levels and two DAP metabolites (DMTP and DMDTP), and a negative association of LH levels with DMTP. Estradiol and testosterone levels were not related to urinary DAP metabolites. Significant positive association between FSH and 3-PBA and cis and trans DCCA levels in urine, testosterone levels were inversely associated with total PYR metabolites</td>
</tr>
<tr>
<td>Meeker et al. (2009)</td>
<td>161 Men recruited from an infertility clinic</td>
<td>Urinary 3-PBA, cis-DCCA and trans-DCCA</td>
<td>FSH, LH, Inhibit B, testosterone, estradiol, prolactin, TSH, T3, T4</td>
<td>Significant positive association between FSH and 3-PBA.</td>
</tr>
<tr>
<td>Yucra et al. (2008)</td>
<td>31 Pesticide applicators/31 controls</td>
<td>Urinary DAP metabolites</td>
<td>FSH, LH, estradiol, testosterone</td>
<td>No significant associations reported</td>
</tr>
<tr>
<td>Han et al. (2008)</td>
<td>212 Non occupational exposed males diagnosed unexplained male factor infertility</td>
<td>Urinary DAP metabolites</td>
<td>FSH, LH, estradiol, testosterone</td>
<td>Positive association between LH and 3-PBA. Negative association between estradiol and 3-PBA</td>
</tr>
</tbody>
</table>
damage and DAP metabolites. However, tail intensity and tail moment were significantly greater for agricultural workers compared to controls (McCauley et al., 2008). 18 male sprayers and 18 controls were assessed for oxidative DNA damage (8-OH-DG), and pesticide exposure (DAP metabolites) in a cross-sectional study conducted in central Japan (Lee et al., 2007). A significant association was found between urinary DAP and 8-OH-DG levels.

Only a few studies measured DNA damage, and with relatively small population samples, thus it is not safe to make valid conclusions. It should be noted that in two out of four studies, exposure classification by occupation was more efficient in demonstrating exposure–effect relationships than biomonitoring data.

3.3.6. Other effects
We found two studies assessing OP exposure and self-reported acute health symptoms (Rees, 1996; Strong et al., 2004). None of these studies reported associations between health symptoms and OP metabolite levels in urine.

The only study we found that examined biomarker levels in cancer was a recent study that attempted to correlate OP exposure to acute lymphoblastic leukemia (Soldin et al., 2009). Soldin reported that children with ALL had significantly increased DAP levels.

4. Discussion

Biomarkers of exposure to OP and PYR insecticides, used as direct exposure measurements, were used in a variety of epidemiological studies. In this review we summarized studies measuring a variety of different dosimeters and attempted to correlate the measurements with specific health effects.

In the studies included, urine was the most commonly used matrix. Urine sampling is non-invasive and relatively easy to perform. In addition, many studies have chosen to measure DAP metabolites which are measured mainly in urine. DAPs were used to estimate exposure to a variety of OPs and not a single compound. DAP metabolites were measured in more than half the studies.

PYR metabolite levels were determined in only six studies. However, all of these studies were published recently. Since OPs are gradually being eliminated from the field of residential and agricultural insecticides, research seems to be turning to pyrethroids.

With regard to the analytical approaches used, it should be noted that in some studies it was difficult to trace the analytical methods used for biomarker quantification, and importantly, the validity of the methods used was not demonstrated. The efficiency of analytical methods is crucial for the accurate quantification of biomarkers and can affect the eventual conclusions of the study. Thus, the validity of the analytical methods used should be demonstrated. Additionally there should be find consensus of concentrations presentation so that the data from different research groups could be directly compared as well as conclusions made on the basis of these data.

In some occupational studies, biomarker measurement failed to demonstrate the exposure level–effect relationship compared to exposure classification by occupation only. In seven studies, exposure classification by occupation or based on questionnaire data, demonstrated stronger relationships with the examined effect than biomarkers. Yuca et al. states clearly that “current presence of OP metabolites in urine is not an adequate marker to demonstrate the effect of long-term exposure to OP pesticides” (Yuca et al., 2008). Biomonitoring is a labor intensive and expensive technique. The concern that arises almost automatically is why apply biomonitoring, since exposure classification by occupation or single exposure history questionnaires can better describe the exposure–effect relationship. The major issue when assessing exposure using biological measurements is that biological half lives of toxins or metabolites are short, reflecting only recent exposure. In epidemiological studies, especially in those investigating the long-term effects of chronic repeated pesticide exposure, single measurements may not be reliable. Therefore, exposure assessment must not rely only on biomarkers. This is especially true in occupational studies where the variability of pesticide biomarker levels in one single day can be huge. In this case, the time between the exposure incident and the sample collection must be recorded and used to better interpret the results. Another approach adequate to reduce the uncertainties related to the toxicokinetics of the compound, the intermittence of exposure (and the variations in the concentration of the sample) is the collection of 24-h samples. Also, data regarding the frequency and duration of exposure must be collected in order to estimate chronic exposure. The studies by Pilkington et al. and Albers et al. are good examples of how biomarker measurements can be combined with other techniques, in order to estimate cumulative exposure. Biomarkers can sometimes confuse researchers when they are used as a stand-alone exposure assessment tool.

Biomarkers of chronic exposure can be an ideal tool in exposure assessment science. Pesticide residues in hair can reflect exposure that occurred months or even years before the sampling date, and can estimate chronic, aggregate exposure. Analytical methods are being developed in this direction (Margariti and Tsatsakis, 2009a,b; Tsatsakis et al., 2010). Hair analysis can contribute to the substantial progress in the use of non-persistent pesticide exposure biomarkers in epidemiological studies (Tsatsakis et al., 2009b, 2011). Also, meconium analysis can provide a longer historical record for prenatal exposure to pollutant residues (Tsatsakis et al., 2009a).

Studies focused on specific health effects include birth outcomes, neurodevelopment, neurologic effects, male infertility and DNA damage. For each health effect studied, many different methods were used to measure the extent of the health impact, especially in studies that focused on neurologic health effects.

With regard to the findings of these studies, significant evidence supported the suggestion that prenatal levels of biomarkers were associated with effects on children’s neurodevelopment. This conclusion is based on the fact that the studies were well designed cohorts, with adequate sample size and the results did not suffer from contradiction or major inconsistencies. The hypothesis that OP and PYR exposure affects male fertility was also supported by the findings of the studies. Regarding the association between biomarker levels and neurologic effects in occupationally exposed males, DNA damage, birth outcomes and neurobehavioral deficits as a consequence of children’s exposure, some suggestions are provided, but the results are not conclusive.

5. Conclusions

Biomarkers were used in numerous epidemiological studies with different approaches and exposure assessment methodologies. The epidemiological studies supported by quantification of biomarkers in urine or plasma samples lead to more (highly) validated conclusions on the associations between human exposure to OP or PYR insecticides and health outcomes. These studies suggested negative health effects of OP exposure on children’s neurodevelopment and on male reproduction. With regard to exposure to PYR, it should be noted that to date there are few studies investigating the adverse health outcomes of PYR insecticides. The effects studied in relation to PYR exposure were mainly the effects on male reproduction (sperm quality, sperm DNA damage and reproductive hormone disorders). Significant evidence supported the hypothesis that PYR exposure is adversely associated with effects on the male reproductive system.

However, for valid and reliable results and conclusions, attention should be paid to the validity and sensitivity of the analytical methods used, study designs and the measured toxicant characteristics. Additionally, information on exposure duration and
frequency should be combined with biomarker measurements in order to accurately estimate exposure.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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