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Association of reproductive disorders and male congenital anomalies with environmental exposure to endocrine active pesticides

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ABSTRACT

There is growing evidence that environmental exposure to pesticides may increase the risk of developing reproductive and developmental disorders. This study determined the prevalence and risk of developing gestational disorders and male congenital genitourinary malformations in areas with distinct exposure to pesticides, many of them with potential endocrine disrupting properties. A population-based case–control study was carried out on pregnant women and male children living in ten health districts of Andalusia classified as areas of high and low environmental exposure to pesticides according to agronomic criteria. The study population included 45,050 cases and 950,620 controls matched for age and health district. Data were collected from computerized hospital records between 1998 and 2005. Prevalence rates and risk of miscarriage, low birth weight, hypospadias, cryptorchidism and micropenis were significantly greater in areas with higher use of pesticides in relation to those with lower use, thus supporting and extending previous information.

1. Introduction

Pesticides are extensively used worldwide because of their benefits for the agricultural sector (they improve the quantity and quality of crop production) and in public health for control of vector-borne diseases. In Spain, the use of pesticides as plant protection products ranged from 98,052 tons in 1999 to 100,373 tons in 2014, with Andalusia representing from 32.7 to 35.2%, respectively, of the total Spanish consumption [1]. This greater use is due to the agricultural practices carried out in this region, which ranges from areas of intensive agriculture within plastic greenhouses (mainly close to the coast) to traditional open-air agriculture (in inland areas).

As pesticides are intrinsically toxic, there is a growing concern on their health impact. The presence of detectable levels of pesticide metabolites in urine [2,3] indicates exposure from indirect sources, including diet (drinking water, food) and the environment (dust, inhaled air). The long-term use of pesticides in areas of intensive agriculture contributes to environmental pollution; therefore, residential proximity to farmland treated with pesticides might pose a risk for human health.

A growing number of studies have shown that many pesticides (or their metabolites) can be considered as endocrine-disrupting chemicals (EDCs) because of their capability to interact with hormone receptors, acting as agonists of the estrogen receptor or antagonists of the androgen receptor [4]. These effects may harm the health, alter the normal function of the organism and impact future generations by interfering the development of the reproductive, nervous, immune and hormonal systems [5,6]. A total of the ninety-eight plant protection products have been categorized for their endocrine disruption potential after conducting detailed human health assessments [7]. From these, those more likely to pose an endocrine active potential include fungicides (mancozeb), herbicides (ioxynil, linuron) and insecticides (abamectin and thiacloprid). For many other pesticides commonly used in agriculture (*e.g.*, chlorpyrifos, clothianidin, β-cyfluthrin, lambda-cyhalothrin, spinosad) further information is need for a proper categorization [7].

In addition, many persistent chlorinated compounds are well known endocrine-active pesticides such as hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), dichlorodiphenyl-trichloroethane (DDT) and its metabolite

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dichlorodiphenyldichloroethylene (DDE). All these pesticides may adversely affect the early stages of human reproduction [8]. Various studies have revealed a link between exposure during pregnancy to EDC, either pesticides or non-pesticides (*e.g.*, polychlorinated biphenyls, bisphenol A), and an increased prevalence and risk of miscarriage [9–12] and low birth rate [13–15].

As the sexual differentiation of male genitals occurs during the first twelve weeks of pregnancy this period has the greatest susceptibility to the effects of endocrine-active pesticides [16]. During sexual differentiation, fetal exposure to these chemicals can lead to congenital genitourinary malformations such as cryptorchidism, hypospadias or micropenis. Because the growth of androgen-dependent structures continues during the second and third trimester of pregnancy, continuous exposure to low levels of endocrine-active pesticides continues interfering genitalia development [17].

Recent epidemiological, toxicological and molecular biology studies have shown an increased prevalence of male reproductive disorders, related to testicular cancer, decrease in the number of sperm and male congenital genitourinary malformations [18]. The origin of the latter abnormalities is likely related to various factors, including exposure to endocrine-active pesticides used in agriculture, which have been linked to cryptorchidism [19–21], hypospadias [22–24] and micropenis [25–27]. All these abnormalities represent an underlying developmental disease, the testicular dysgenesis syndrome [28]. Maternal exposure to the main organochlorine pesticides (DDT, DDE, HCB, HCH, endosulfan, mirex), among other environmental and foodstuff contaminants, has shown a potential effect on the development of these male urogenital defects during pregnancy [18,20,29]. Therefore, the objective of this study was to determine the prevalence and risk of developing gestational disorders (miscarriage and low birth weight), and male congenital genitourinary malformations (cryptorchidism, hypospadias and micropenis) in pregnant woman, and their offspring, who lived in areas with different environmental pesticide exposure, including endocrine-active pesticides.

2. Material and methods

2.1. Design

A population-based case–control study was conducted in selected areas of Andalusia (Southern Spain) with different environmental exposure to pesticides, because of diverse patterns of pesticide use. The objective was to assess the potential association between exposure to these chemicals and the risk of miscarriage and low birth in pregnant women, and male congenital genitourinary malformations (hypospadias, cryptorchidism and micropenis) in their offspring. Each study area corresponded to an administrative territorial division with a reference hospital (referred to as health district).

2.2. Criteria for the selection of the areas of study and exposure to pesticides

The selected Andalusian health districts were divided into two groups according to agronomic criteria of pesticide use as reported elsewhere [30]. Areas of intensive agriculture with a high number of greenhouse hectares (ha) (>1200 ha), and therefore with high pesticide use, were considered as of high exposure. In contrast, areas with a low number of greenhouse hectares (<1200 ha) or devoted only to extensive agriculture (and thereby with low pesticide use) were classified as of low pesticide exposure. A second criterion to characterize exposure was based on the amount of plant protection products sold normalized by person during the study period (Table 1). Areas of high exposure included the following health districts: West Almeria (Poniente), Centre of Almeria (Centro), South Granada and the Coastline of Huelva. By contrast, areas of low exposure were comprised by: Axarquía (Málaga), Jerez coastline (Cádiz), East Almeria (Levante), Northeast Jaen, North Cordoba and North Seville (Fig. 1).

Table 1
Agronomic criteria for categorizing health districts as areas of high and low pesticide use in Andalusia (Southern Spain).

Agronomic criteria	High pesticide exposure	Low pesticide exposure
Population	800,017	1032.958
Hectares of plastic greenhouses	30,291	2510
Total pesticides (tons used)	8883.74	6145.84
Insecticides (tons)	4777.50	2210.89
Fungicides (tons)	2228.81	1666.28
Herbicides (tons)	599.80	1380.02
Plant growth regulators (tons)	905.21	694.03
Other pesticides (tons)	462.48	235.36
Total pesticides (kg/person)	11.10	5.95
Insecticides	5.97	2.14
Fungicides	2.79	1.61
Herbicides	0.75	1.34
Plant growth regulators	1.13	0.67
Other pesticides	0.58	0.23

2.3. Study population and maternal-infant disorders

The study population consisted of 43,344 women of childbearing age (18–45 year-old) residing in Andalusia and presenting gestational disorders like miscarriage and low birth weight. Besides, 1706 male children (0–14 years) with hypospadias, cryptorchidism and micropenis as major congenital genitourinary malformations were also collected from the study areas between 1998 and 2005 (see distribution in Table 2). The diagnosis was defined according to codes of the International Classification of Diseases (ICD-9): miscarriage (code 634.9), low birth weight (765.1), hypospadias (752.61), cryptorchidism (752.51) and micropenis (752.64). Cases were collected from computerized records of the Andalusian Health Service (referred to as Minimum Dataset) for the 8-year study period. The Andalusian Minimum Dataset (AMD) collects information from the public hospital discharges, including coded clinical data for inpatients. The AMD is recorded when a patient is discharged from a hospital staying for at least one night or more. The main cause for admission (main diagnosis) and other secondary medical diagnoses are routinely recorded in the AMD as are also age, sex, ethnicity and place of residence.

The comparative (control group) consisted of individuals who did not present the illnesses under study and who lived in the same health districts and over the same period of time. The total number of participants was 363,478 women of childbearing age (18–45 years old) with an average age similar to the diseased group, and 587,142 male children 0–14 years old.

2.4. Statistical analysis

Frequencies and percentages were calculated for categorical variables, and mean and standard deviation for quantitative variables. Prevalence rates and risk of developing gestational disorders and male congenital genitourinary malformations in areas of high and low pesticide exposure were calculated by using Chi square test. Odds ratios (OR) and their corresponding 95% confidence interval (CI) were obtained. The Student *t*-test was used to compare age differences in the study populations between the two study areas. The Kolmogorov–Smirnov goodness-of-fit test was used for testing normality. Data were analyzed using SPSS 22.0 and EPIINFO 7 statis-