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A study of the impact of agricultural pesticide use on the prevalence of birth defects in northeast Italy

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Abstract

Pesticides are probably the most frequently deliberately released toxic chemicals into the environment. However, although the results of experimental studies indicate developmental toxicity hazards for several groups of chemicals used, the studies in humans are contradictory.

There are specific regulations in the European Union (EU) regarding the use of pesticides and there is also considerable awareness about possible related health problems.

In order to investigate whether, in the current EU situation, the use of certain pesticides could be associated with adverse health effects in the outcome of pregnancies, we have performed a 6-year study in an agricultural area in the Veneto Region of, northeastern Italy, where we have been able to define the exact quantity and type of pesticides as well as the exposed population, in order to quantify the risk of congenital malformations related to the use of pesticides.

Data on congenital malformations were obtained from the northeast Italy Congenital malformation Registry, using several sources of ascertainment, while pesticide use were obtained through interviews with users and sellers. The municipalities of three contiguous provinces were divided into those with a high, low or intermediate use of pesticides. In the study period there was a total of 146,239 consecutive pregnancies terminating in birth or induced abortion because of congenital malformation.

No significant differences in the prevalence of congenital malformations were observed between the three different areas (high, low, intermediate risk). Our study confirms that in countries such as Italy, where there is close control of the use of pesticides, there is no epidemiological evidence that pesticides have any effect on the prevalence of congenital malformations.

Keywords: Pesticides; Endocrine disrupting chemicals; EDC; Birth defects; Epidemiology

1. Introduction

Since the introduction of the use of DDT as an insecticide in the late 1930s, billions of kilograms of pesticide active ingredients have been distributed worldwide and pesticides are probably the most frequently released toxic chemicals into the environment.

In the late 20th century, the medical and public media's interest in the environment increased and a number of experimental and epidemiological studies regarding adverse developmental outcomes related to pesticide exposure, including fetal death, intrauterine growth restriction (IUGR), and congenital malformations were published [1–7].

Although the results of experimental (in vitro and in vivo) studies indicate developmental toxicity hazards for several groups of chemicals used, the results in humans are contradictory. Indeed, most epidemiological studies, if not all, have suffered from poor pesticide exposure estimation, relying on job title only and/or the exposure category "any pesticide" as a measure of exposure, and there was limited or inadequate evidence to support causality for all of the associations examined.

In the European Union (EU) there are specific rules regarding the use of pesticides and there is considerable awareness about possible related health problems. The EU has devel-

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oped a very comprehensive regulatory framework, Directive 91/414/EEC, defining strict rules for the authorisation of "plant protection products". The Directive requires extensive risk assessments for effects on health and environment to be carried out before a pesticide can be placed on the market and used, including the definition of maximum residue limits in food and feeding products. Moreover, in 2002, the European Commission adopted a Communication 'Towards a Thematic Strategy on the Sustainable Use of Pesticides' (European Commission, Environment Directorate General). The Communication identifies several objectives, including: encouraging good farming practice, support for epidemiological research on users and consumers, improved controls on the use and distribution of pesticides and reviews of old active ingredients. Lastly, the implementation of the new EU regulatory framework for the Registration, Evaluation and Authorisation of chemicals (European Commission, Environment Directorate General, REACH) is intended to reduce the chance of developmental and reproductive toxicants and/or endocrine disrupting chemicals (EDC) being introduced in the environment and food chains [8–10].

Therefore, it is important that studies with careful exposure assessment investigate whether, in the current EU situation, the use of certain pesticides could be still associated with adverse health effects in the general population or in vulnerable subsets such as pregnant women and fetuses.

We report on an ecological study performed in an agricultural area in the Veneto Region, northeast Italy, where we have been able to define the exact quantity and type of pesticides used, as well as the exposed population in order to quantify the risk of congenital malformations related to the use of pesticides.

2. Materials and methods

The study subjects were all newborns, children or terminations of pregnancy (TOP) for fetal anomaly registered in the northeast Italy (NEI) congenital malformation registry, during the period 1 January 1999–31 December 2004, whose mothers were resident in three contiguous provinces (Verona, Vicenza and Treviso, see Fig. 1) of the Veneto Region, where there is an high proportion of single crop agricultural areas.

The methodology of the registration (newborns and fetuses) has been previously described [11] (http://www.eurocat.ulster.ac.uk). The registry is population-based, including all mothers delivering within the covered area irrespective of place of residence. Reports were obtained from 66 participating hospitals, comprising a total of approximately 57,000 annual births. The estimated proportion of non-resident mothers giving birth in the covered hospitals is 1.8% (calculated using controls).

Reporting is voluntary, filling in specific forms in the delivery units of the study area. Briefly, all liveborns, stillborns and fetuses affected with at least one congenital malformation are registered at birth by a pediatrician or an obstetrician. The registration form contains anagraphic and medical information regarding the infant, pregnancy and parents.

Termination of pregnancy is legal under normal circumstances up to 12 weeks of gestation and up to 26 weeks if a fetal anomaly is diagnosed. Most terminations in the latter category, however, are carried out before 22 weeks.

The malformations were classified as isolated when only one malformation was present and associated when one or more additional malformations were diagnosed. Associated anomalies were subdivided into the following groups: chromosomal if associated with a chromosomal anomaly, syndromic if the malformation was part of a non-chromosomal recognized syndrome, or multiple if at least one other major malformation was present in non-chromosomal and non-syndromic cases. Minor anomalies are not included in the registration when isolated according to EUROCAT methodology (http://www.eurocat.ulster.ac.uk/pdf/EUROCAT-Guide-1.3.pdf) (EUROCAT Guide 1.3, Chapter 3.2).

In addition to this prenatal and neonatal registration, an active search for under notification has been in place since the start of the NEI Registry activity. It includes multiple source ascertainment (clinical genetics units, cytogenetics laboratories, pediatricians) in confirming registrations, adding further information and identifying cases not registered at birth.

In order to complete the registration for this study, a further study has been carried out which began in 1999. It includes the analysis of all discharge records (SDO: Schede Dimissione Ospedaliera) of the hospitals of the Veneto Region. Each SDO includes the patient unique identification code (ID), the residence, and the diagnosis code. The NEI registry and SDO system use the same coding system for diagnosis (ICD/BPA 9) [12]. The protection of privacy was assured, therefore preserving confidentiality.

All SDO forms containing a diagnosis coded 74,000–75,899 were transferred to a database (MS Access, Windows 2k/Xp environment) for analysis. As in the case of the first stage of the SDO control, multiple SDOs of the same child were excluded and ID codes were checked using the algorithm created by the Italian National Health Service.

Each SDO referring to a newborn registered in the NEI registry was checked in order to confirm the diagnosis, add information regarding morbidity, survival, and associated anomalies.

The SDOs referring to children born in the study period but not registered in the NEI registry, were verified and all hospital records have been controlled by a pediatrician in the hospital where the child was born and/or was admitted so that the malformation could be carefully described. All of the diagnoses were then verified by a geneticist.

2.1. Exposure assessment

The official statistics regarding the use of pesticides in Italy are provided by the National Institute of Statistics (ISTAT) (www.istat.it). Unfortunately these data are not reliable and detailed enough for the aims of the present work, since they are only related to yearly consumed products grouped in large categories of chemicals, without distinguishing among the active ingredient (AI). To overcome these limitations a survey was planned to collect data, which are as reliable as possible regarding the quantity and the type of AI used in each municipality in the District of Verona, Vicenza and Treviso. These municipalities were chosen on the basis of available data for each of them regarding inhabitants, newborns, product sales and agricultural areas. The methodology to establish the exact use of chemicals in the study was as follows:

(a). Identification of the crop extension. The type of crops and their coverage in hectares were evaluated in all of the 316 municipalities in the three provinces. Based on the data of the fifth agricultural census (ISTAT, 2002) the first database regarding the use of the territory in each municipality was created. Thirty-nine different crop categories were found.

(b). Theoretical use of pesticides. In order to establish the theoretical use of pesticides for each crop listed in the previous database, a second database was created on the basis of the good agricultural practices (GAP), including a list of both the kind and quantities of AI that should be distributed to each crop during the usual practices of their cultivation. The records were in kg/ha.

(c). Empirical use of pesticides. This step was carried out through personal interviews and data requests to the technicians of 15 public or private organizations such as the IRA (Ispettorato Regionale Agricolo) bureaus of Verona, Vicenza and Treviso; Agricultural Consortiums; Cooperatives; companies selling agricultural products. At the end of this process, the coverage of each crop was revised. Some crops with similar pesticide needs were grouped together reducing the previous 39 categories to 17, which are listed in Table 1.

(d). Correction of the second databases by means of "field investigations" in 15 model-farms (5 per District) and through consultation of the climatic reports of the Regional Environment Agency (ARPAV, Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto: http://www.arpa.veneto.it/indice.asp?l=cmt/meteo/meteo.htm). The farm

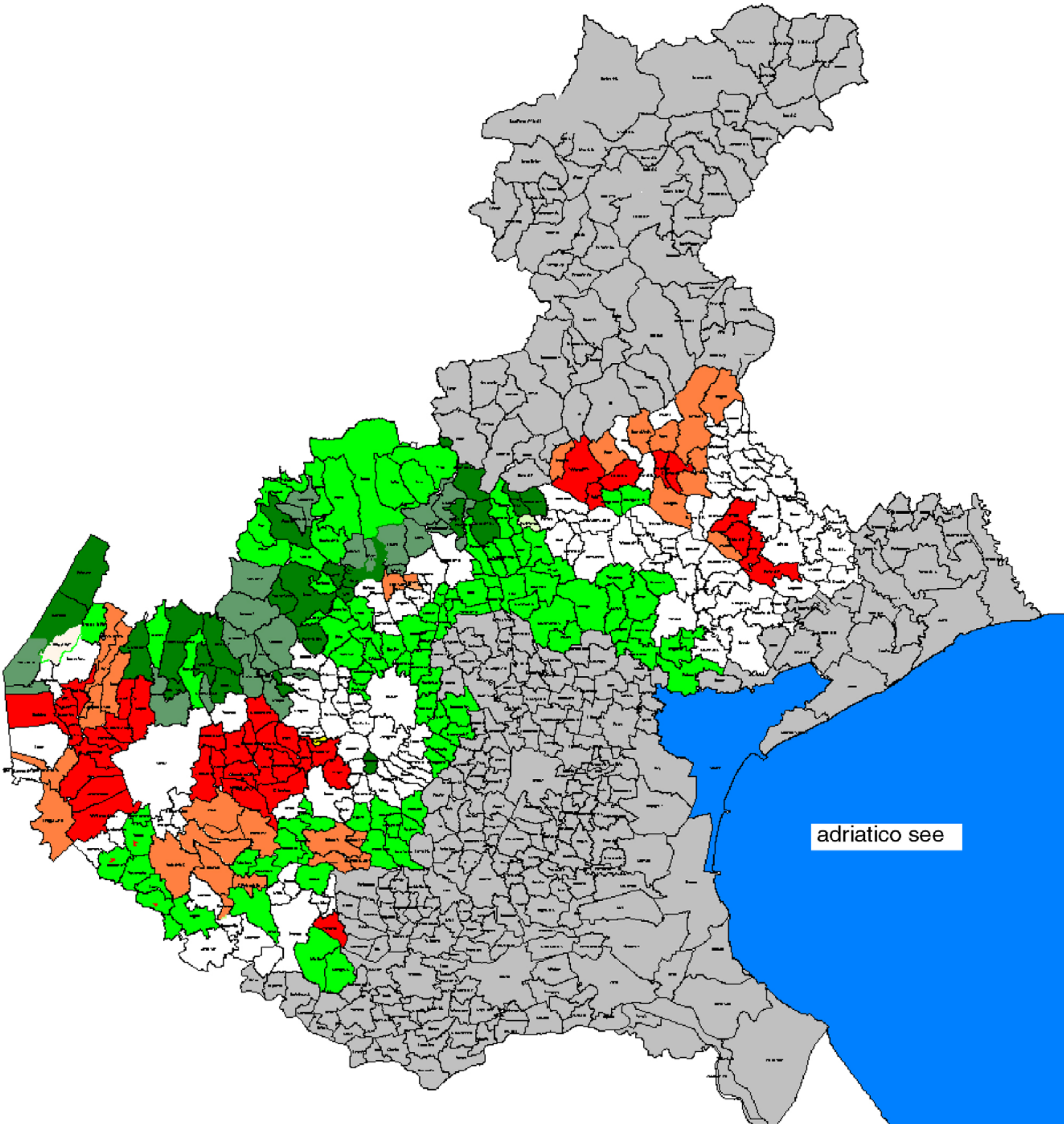

Fig. 1. Veneto Region Map. Study are: high (red), intermediate (white), and low (green) use of pesticides. Gray areas are not included in the study.

investigations were necessary to adapt the theoretical data to the particular demands and actual uses of pesticides in each different agricultural area. Climatic information (temperatures and the frequency and quantity of rainfall in each of the 6 years considered) served to determine the intensity of the treatments. In fact, less control of weeds, insects and plant diseases is needed during warm dry years.

(e). Assessment of the type and quantity of pesticides used per year in each municipality. A third database was produced by multiplying the values of the second database (quantities, in kg/ha, of each AI used for each crop) by those of the first database (coverage, in ha, of these crops) and adding the results obtained for the same kind of AI. These data were organized by municipality and year.

An average of more than 160 different types of AI were used per year and 59 of them have been considered to be, or suspected to be, endocrine disrupting chemicals (EDC) or to have reproductive and developmental toxicity.

2.2. Statistical analysis

Analyses were made using the Mantel-Haenszel procedure with adjustment for selected confounders (maternal and paternal age, parity, parental job). The 95% confidence intervals (95% CI) of odds ratios (OR) were calculated. The observed/expected ratio then represented a risk ratio, and its 95% confidence interval was calculated using exact Poisson distributions. Odds ratios were compared as two-tailed z-tests.

Table 1
Categories of crops in the study area

Actinidia (kiwi fruit)
Apple tree
Grapevine
Herbaceous leguminosae
Maize
Olive tree
Pear tree
Potato
Rice
Small cereals
Stonefruit tree
Sugar beet
Tobacco
Tomato
Other vegetables
Other woody plants of agricultural interest
Nursery

3. Results

The 316 municipalities were divided into three groups on the basis of the extension of crops (e.g., vineyards and orchards, which require an intense use of pesticides); the method of pesticide distribution (e.g., via aerial spraying or by soil wetting); and the quantity of AI used per hectare.

Group 1 (shown in red in Fig. 1): areas with high use of pesticides, mainly distributed by aerial spraying. This group was formed of the municipalities where the crop surface (vineyards + orchards) was >50% of the arable land or where the difference between the surface of arable land sown with cereals and that covered with vineyards + orchards was <10. Group 1 also includes two other categories of municipalities: those where tobacco + sugar beet + horticultural crops was ≥30% of arable land, and those surrounded by areas with an intense use of pesticides distributed by aerial spray or by helicopter. This group included 65 municipalities in total.

Group 2 (shown in green in Fig. 1): areas with a low use of pesticides, mainly distributed by soil wetting. This group was formed of the municipalities where cereals occupied >70% of the arable land or where the arable land was <10% of the whole area (mountains or hills prevalent). This group included 93 municipalities in total.

Group 3 (shown in white in Fig. 1) was formed by the municipality not included in groups 1 and 2, i.e., those with an intermediate use of pesticides. In this group were included 158 municipalities in total.

The average of quantities of both ECDs and total pesticides, used in the municipalities of each of the three groups are shown in Table 2.

3.1. Population

In the three provinces there was a total of 146,239 consecutive births, 74,940 males and 71,299 females, with a sex ratio 0.51.

In the municipality areas with a high pesticide use (Group 1) there were 29,046 births (14,939 males, sex ratio 0.51), in those with a low pesticide impact (Group 2) 33,184 (17,101 males, sex ratio 0.51), and in the intermediate zone (Group 3) 84,009 (42,900 males, sex ratio 0.51).

3.2. Malformation

In the study period there were a total of 75,594 SDOs. After excluding the duplicates and the SDOs with discharge codes not referring to a malformation, a total of 15,611 SDOs were analyzed. A total of 12,895 SDO were excluded from the study for at least one of the following reasons: mother resident in an area not considered in this study, data of birth before 1 January 1999, presence of a minor malformation, or miscoding. A total of 2716 SDOs were finally compared with the data of the NEI registry. There were 1061 children and 334 fetuses affected with at least one major malformation registered in the NEI registry from the study area during the study period.

After comparing the ID in the SDO and NEI Registry a total of 3473 cases were included: 334 fetuses, 427 children registered only in the NEI registry, 634 children registered in both SDO and NEI registry and 2082 children identified through the SDO.

The birth prevalence of children with congenital malformation(s) and of groups of congenital malformations for each category of pesticide exposure is given in Table 3. The prevalence of groups of congenital malformations is given in Table 4. No significant differences either in the prevalence of malformed children and/or in the incidence of malformations were observed between the three areas.

Maternal and paternal age at birth, parity, and the type of job were not significantly different between the three groups (data not shown).

Table 2
Use of pesticides in the three groups of municipalities

	Total of pesticides (as active ingredient)						ECD pesticides or with reproductive and developmental toxicity (as active ingredient)					
	Group 3		Group 2		Group 1		Group 3		Group 2		Group 1	
	kg/ha Mun	kg/ha Colt	kg/ha Mun	kg/ha Colt	kg/ha Mun	kg/ha Colt	kg/ha Mun	kg/ha Colt	kg/ha Mun	kg/ha Colt	kg/ha Mun	kg/ha Colt
Average	10.73	19.33	1.90	3.65	17.32	30.69	1.93	3.78	0.48	0.91	4.03	7.28
S.D.	8.03	8.68	1.99	2.42	8.67	9.20	1.13	1.29	0.38	0.25	1.81	1.95

kg/ha Mun: kg of active ingredient per hectare of total municipality area; kg/ha Colt: kg of active ingredient per hectare of the cultivated land.

Table 3
Birth prevalence per 10,000 of children with malformations

	No. of cases		
	Low risk area	High risk area	Intermediate risk area
Skeletal dysplasia	0.60	1.72	1.43
NTD	3.62	2.07	3.21
Hydrocephalus	3.62	2.75	2.62
Eye	2.41	2.41	2.86
External ear	3.31	2.07	3.09
Cleft lip	4.82	3.79	6.19
Cleft palate	3.31	4.82	3.21
Heart malformation	87.99	91.23	78.92
Oesophageal atresia	2.11	2.41	1.31
Intestinal atresia	1.21	0.69	1.79
Anorectal atresia	3.01	1.03	1.31
Omphalocele	0.30	0.0	0.36
Gastroschisis	0.0	0.34	0.24
Cloaca	0.30	0.0	0.12
Bladder extrophy	0.0	0.34	0.0
Epispadias	0.30	0.0	0.0
Hypospadia	12.36	9.64	12.14
Bilateral renal agenesia	0.0	0.0	0.24
Limb reduction anomalies	2.41	2.75	1.79
Polydactyly	6.93	6.89	7.74
Syndactyly	4.52	7.23	5.48
Trisomy 21	16.88	11.36	17.97
Others	46.11	50.27	47.97
Associations	22.60	24.79	21.90
Syndromes	10.25	6.89	13.57
Total	238.97	241.69	235.45

Table 4
Prevalence per 10,000 of groups of congenital malformations

Malformation	Exposure		
	Low	High	Intermediate
NTD	4.82	2.75	4.40
Hydrocephalus	4.22	4.13	4.64
Eye and ear	8.44	6.88	8.09
Cleft lip	5.12	4.13	8.33
Cleft palate	3.92	6.54	5.59
Heart	103.06	105.00	91.06
Hypospadia	15.37	11.70	14.99
Limb reduction	3.01	4.13	4.17
Digestive system	8.14	7.57	5.12

4. Discussion

4.1. Previous knowledge

Pesticides or, according to the European definition of Directive 91/414/EEC, plant protection products, are a broad and rather diverse group of compounds. Pesticides have various chemical structures, mechanisms of action and toxicity profiles. Also, different patterns of use lead to variable potentials for exposure. Indeed, from the exposure standpoint, pesticides are rather unique among pollutants in that they are deliberately inserted into the environment in order to support crop production, and also animal farming, rather than being accidentally released. Thus, three different exposure scenarios may occur: (i) occupational exposure, (ii) environmental exposure (especially for communities living in areas with intensive agricultural activity [13]) and (iii) dietary exposure of the general population through residues in foods of both vegetable and animal origin.

Several major groups of pesticides, such as most cholinesterase-inhibiting organophosphorus insecticides, are not considered to pose significant teratogenic hazards since adverse fetal effects in laboratory animals appear to be secondary to maternal toxicity [14]. On the contrary, some groups of pesticides are considered developmental toxicants as they are able to directly affect the intrauterine development of one or more laboratory animal species such as benzimidazoles (benomyl and carbendazim) which induces brain malformations in rats [5].

Whereas experimental studies are essential for pesticide regulation (labelling, limitations of use, etc.), they do not directly indicate an actual risk for human development. Indeed, in most cases malformations are induced following high peak plasma concentrations, which require intense exposures in humans. Such exposure is more likely to be encountered in areas with highly intensive use and/or less strict regulation of agrochemicals. In Mexico, Lacasana et al. [15] found a significantly increased risk of anencephaly in the offspring of pesticide applicators. In Spain, an ecological study observed that paternal agricultural work in the areas where pesticides are massively used increased the risk of fetal death from congenital anomalies [16]. On the other hand, in Denmark a large study on the offspring of gardeners and farmers found no association with occupation and birth defects or other adverse pregnancy outcomes, except for an increased risk of extreme preterm birth for gardeners [17]. Further attention could be devoted to other developmental impairments such as the risk of neurobehavioural deficits [1].

A specific public health issue concerns endocrine disrupting chemicals (EDC), a heterogeneous group of compounds sharing the ability to interfere with endocrine homeostasis [18,19]. A significant portion of EDC is represented by pesticides that are still in use, or have recently been just phased out shortly, in industrialized countries [19–20]: major examples include the chlorinated insecticide methoxychlor [21], androgen receptor antagonists (dicarboximide fungicides such as vinclozolin and procymidone; herbicides such as linuron) [22], aromatase inhibitors such as triazoles, thyrostatic agents such as ethylene bisdithiocarbammates and their metabolite ethylenethiourea [2] and compounds interfering with the hypothalamus–pituitary–gonad axis such as triazine herbicides [23]. A number of toxicological studies exist on the developmental effects of EDC, especially on the induction of malformations of the reproductive tract, e.g., hypospadia induced by vinclozolin and linuron [23]. In other cases, EDC elicit more subtle effects on reproductive or endocrine maturation, such as in rats prenatally exposed to atrazine [24] or to the potentially thyrostatic fungicide methyl-thiophanate [25]. There is also evidence that EDC pesticides may increase the risk of early pregnancy loss in women under certain occupational exposure situations [20]. On the contrary, only limited evidence exists to indicate that human exposure to EDC pesticides is associated with relevant birth defects, such as hypospadia. A recent Arkansas study found no or even negative association between hypospadia and the agricultural use of pesticides identified as potential EDC and/or developmental toxicants; only a weak, but significant positive association with exposure to the chloro-phenoxy herbicide diclofop-methyl was found [4]. A Dutch case–control study found a significant association between chryptorchidism risk and paternal pesticide exposure as well as between hypospadia and paternal smoking [26]; it remains to be ascertained whether paternal exposure is directly relevant or is just an indirect indicator of maternal exposure. On the other hand, it cannot be excluded that exposure to pesticides with endocrine activities might be associated with long-term effects on development, such as impaired reproductive capacity. More research is needed to clarify this topic, especially regarding the development of predictive biomarkers to be used in epidemiological studies [27].

Besides the issue of occupational exposure, exposure of the general population must receive more attention from well-targeted epidemiological studies. Residues in foods are likely to be a major source of exposure to pesticides. In the EU (European Commission, 2004), the exposure to residues through vegetable foods is generally under control, although some issues of potential concern remain. More attention could be given to the additive intake of residues of compounds with similar mechanisms of toxicity and targets. Jensen et al. [3] evaluated the overall intake of neurotoxic, cholinesterase-inhibiting insecticides (35 organophosphates and carbammates, altogether) in Denmark; no potential risk for consumers was identified as compared with regulatory limits.

Besides diet, living near sites where pesticides are used, manufactured or disposed may also significantly increase the environmental exposure through air, water and soil. Villanueva et al. [28] investigated the association between levels of atrazine (a triazine herbicide and EDC) in drinking water and adverse pregnancy outcomes, finding only a limited increase of risk for small-for-gestational-age status. A study performed in the U.S.A. found a significant presence of cholinesterase-inhibiting insecticides (chlorpyrifos, diazinon, propoxur) in maternal and cord blood of a mother–child cohort from a low-income New York area; the exposure was consistently associated with pest control [29]. These data deserve further attention, since experimental data indicate that such compounds may affect neurobehavioural development, even without eliciting morphological abnormalities [7]. However limited, the data on exposure of the general population to pesticides do not point to a major developmental risk for humans, at least in developed countries. On the other hand, it cannot be excluded that the upper range of exposure to certain pesticides may interact with genotype and/or diet in the pathogenesis of multifactorial birth defects. An ecological study in the U.S.A. found an association between the use of chlorophenoxy herbicides (in grain farming and park maintenance) and vascular/respiratory malformations [6]; in California a significant association between the incidence of neural tube defects and maternal residential proximity to agricultural pesticide applications was found; amide, benzimidazole, methyl carbamate, and organophosphorus pesticides were specifically associated with higher risks [30].

4.2. Strengths and weaknesses of this study

The present study is the first part of a study concerning the relationship between exposure to pesticides and reproduction in a well defined (three contiguous provinces) and large population (146,239 consecutive births) over a specific period of time (1999–2004) with a complete estimation of pesticide exposure. The ascertainment of birth defects included the registration at birth of newborns/abortions and registration by pediatricians using multiple sources of data. The methodology used allowed the residence of the mothers during the pregnancy to be known.

The exposure in each municipality has been determined in a 6-year period and only those municipalities with a high proportion of specific crops and high pesticide exposure have been included in the high risk category.

A comparison was made between populations of the same ethnic group, in the same geographical area, but with different exposure to pesticides. Unfortunately, no “control population” could be defined, since there is no corresponding non-exposed population. In fact, pesticides are present in drinking water, fruit and vegetables. However, although the difference in exposure is relevant, we did not find any difference in malformation prevalence. Pesticide exposure has been estimated on the basis of their open-air cultivation of grapes, fruit and vegetables, hypothesizing a higher exposure of populations living in the affected municipalities.

Finally, no investigation on biomarkers of exposure to relevant pesticides or on other adverse pregnancy outcomes (e.g., spontaneous abortion, growth retardation) was carried out. Also, our study has not taken possible late functional effects, such as those connected to behaviour, into consideration, since they would require a different type of study, namely cohort studies.

4.3. Bias and confounding factors

Underreporting cannot be excluded (for example malformations in stillbirths or in induced abortions). However, there is no reason to suspect any underreporting bias between the three considered geographic areas. Various confounding factors such as parental age, parity and parental job were taken into account.

5. Conclusions

Our study confirms that in countries such as Italy, where there is close control of the use of pesticides, there is no evidence that pesticides have any effect on the prevalence of congenital malformations. This is true for all types of malformations encountered and for each pesticide used.

Although our study presents some limitations due to the fact that it is based on data of a congenital malformation registry, the results have been obtained from a large population.

We can conclude that living in an agricultural area where pesticides are widely used, according to current EU regulations, does not appear to increase the additional risk of congenital malformations in offspring.

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