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THE RISK OF ADVERSE REPRODUCTIVE AND DEVELOPMENTAL DISORDERS DUE TO OCCUPATIONAL PESTICIDE EXPOSURE: AN OVERVIEW OF CURRENT EPIDEMIOLOGICAL EVIDENCE

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Abstract. The epidemiological studies presented in this paper refer to the association between agricultural occupation of parents and the incidence of infertility, congenital malformations, miscarriage, low birthweight, small-for-gestational-age (SGA) birth, preterm delivery and stillbirth. The results of the analyses showed that employment in agriculture increases the risk of specific morphological abnormalities in sperm, including the decreased sperm count per ejaculate and declined percentage of viable sperm. In general, no effect of exposure to pesticides on sexual hormones was observed. The data on the effect of employment in agriculture on the time to pregnancy are unequivocal, but most of them suggest that there is a relationship between the decreased fecundability ratio and pesticide exposure. Nor does the research on the sex ratio of offspring provide explicit results. The analyses indicate that parental employment in agriculture could increase the risk of congenital malformations in the offsprings, particularly such as orofacial cleft, birthmarks in the form of hemangioma as well as musculoskeletal and nervous system defects. The data on the effect of occupational exposure to pesticides on birthweight are inconsistent. Although most of epidemiological studies do not reveal a significantly increased risk of SGA, a slower pace of fetal development corresponding to SGA in the population of women exposed to pyrethroids has been recently reported. There are also some indications that exposure to pesticides may contribute to stillbirth and female infertility. The literature review suggests a great need to increase awareness of workers who are occupationally exposed to pesticides about their potential negative influence on fertility and pregnancy outcome. In the light of existing although still limited evidence of adverse effects of pesticide exposure on fertility during the preconceptual period, it is necessary to reduce the exposure to pesticides.

Key words:
Endocrine disruptors, Pesticides, Fertility, Pregnancy outcome, Agricultural work, Birth defects, Reproductive disorders, Fecundability ratio

INTRODUCTION

Pesticides are widely used throughout the world especially in agriculture to protect crops. Although pesticides are regularly applied, relatively little is known about their possible adverse health effects, especially reproductive effects, induced by occupational exposure.

The use of pesticides is highest in greenhouses with flower production and lowest in greenhouses with vegetable pro-

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duction and in outdoor gardening [1]. The most often used pesticides are insecticides, fungicides and growth regulators. Herbicides are not used in greenhouses. Dermal exposure to pesticides highly correlates with the manual contact with pesticide-treated plants and it is believed to be the major route of pesticide absorption during occupational use [2]. The working conditions in greenhouses pose significant health hazard, mainly due to high humidity, temperature and poor ventilation.

Pesticide concentrations in the air often exceed the occupational exposure limits in greenhouses when they are sprayed [3]. Most insecticides, such as organophosphates, carbamates and organochlorines easily penetrate the skin [4]. Some pesticides degrade on the leaves into more toxic compounds with even a stronger penetration capacity through the skin than their parent compounds. Workers are exposed to pesticides via inhalation during application activities and to pesticide residues through the skin when they handle the treated plants.

In agriculture, many pesticides like DDT, carbaryl and chlordecone known as having reproductive effects are no longer used, but the problem is likely to persist for a long time in less-developed countries [5]. Currently, chlorotraizine herbicides are the most heavily used agricultural pesticides [6]. The most common of these chemicals is atrazine, to which every day nearly 60% of the population in the United States is currently exposed. Atrazine is used to control annual grasses and broadleaf weeds in the fields of corn, sorghum and sugar cane and in orchards.

Some pesticides are now suspected of being endocrine disrupting chemicals (EDCs). These chemicals might cause an adverse effect by interfering in some way with the body's hormones or chemical messengers. Many of these endocrine disrupters have been linked to adverse effects on either embryonic development or reproductive function in humans and wildlife [7–9].

Endocrine disrupting chemicals may be defined as exogenous agents that change endocrine function and produce adverse effects at the level of the organism, its progeny, and/or sub-populations of the organism [10]. Following the definition proposed by Friends of the Earth England, Wales and Northern Ireland (FOE), endocrine disrupters are exogenous substances that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior [11]. The list of some environmental pesticides reported to have reproductive and/or endocrine disrupting effects are shown in Table 1.

Endocrine disrupting chemicals have been differentiated from classical toxicants such as carcinogens, neurotoxicants and heavy metals because they may interfere with normal blood hormone levels, or the subsequent action of those hormones, but do not have a classical toxic effect. They may disrupt the hormonal regulation of normal cell differentiation, growth, development, metabolism and reproduction throughout life. Endocrine disruption may occur at levels far lower than those of traditional concern to toxicologists. Sometimes high doses suppress the effects that occur at low levels and sometimes low and intermediate doses produce greater effects than those observed at high doses. EDCs affect not only sex hormones but also those produced by the thyroid, adrenal glands and pancreas [11,12].

The effects that may be detected in an organism exposed to an endocrine disrupting chemical depend on which hormone system it is targeted. For example, when the primary

Table 1. The list of some environmental pesticides reported to have reproductive and/or endocrine disrupting effects, the World Wide Found for Nature

Fungicides:
benomyl, etridiazole, fenarimol, fenbuconazole, hexachlorobenzene, mancozeb, maneb, metiram, nabam, penachloronitrobenzene, pentachlorophenol, triadimefon, tributylin, vinclozolin, zineb, ziram

Insecticides:
aldicarb, aldrin, bifenthrin, carbaryl, carbofuran, chlorodane, chlordecone, chlorfentezine, 8-cythalothrin, DDT and metabolites DDE, DDD, deltamethrin, dicofol, dieldrin, dimethoate, dinitrophenol, endosulfan, endrin, ethofenprox, fenitrothion, fenvalerate, fipronil, a-HCH, heptachlor and H-epoxide, lindane (g-HCH), malathion, methomyl, metoxychlor, mirex, oxychlordane, parathion (metyloparathion), photomirex, synthetic pyrethroids, pyrethrins, ronnel (fenchlorfos), toxaphene, transnonachlor

Nematicides:
DBCP

Rodenticides:
n-2-fluorenylacetamide

action of EDC is targeted on the thyroid hormones, the hormones responsible for metabolism and normal brain development, exposure in the womb may affect intelligence and growth. Pesticides like amitrole, inoxynil, dithiocarbamates (maneb, mancozeb or zineb) may act at the thyroid hormone levels. Some endocrine disruptors may exert their action by interfering with the brain's release of hormones. Organophosphates and carbamates are able to reduce acetylocholinoesterase activity and thus block nerve impulses. This effect may be linked to the suppression of the brain's release of such hormones as FSH and LH [13].

A number of male infertility cases were first discovered among workers exposed to 1,2-dibromo-3-chloropropane (DBCP) at a California pesticide factory. Azoospermia, oligospermia and elevated levels of serum FSH and LH were observed during exposure to DBCP [14]. Many other pesticides were studied in subsequent years, some with positive results. Carbaryl, chlordecone and ethylene dibromide have been shown to adversely affect the male reproductive system. The fungicide vinclozolin has been found to exhibit anti-androgenic properties and to increase serum FSH levels among formulators [15].

ADVERSE REPRODUCTIVE AND DEVELOPMENTAL DISORDERS DUE TO OCCUPATIONAL EXPOSURE TO PESTICIDES

Epidemiological studies focused on reproductive outcomes in occupational populations exposed to pesticides, conducted over the last two decades, were identified during search of the MEDLINE and EBSCO literature basis. Key words used were: pregnancy, agricultural work, pesticides, endocrine disruptors, infertility, low birth weight, spontaneous abortion, stillbirth, birth defects and preterm delivery. No criteria for time periods and language of publications were specified.

Time to pregnancy

In the 1980s, Rochootin (1983), Baird (1986), Joffe (1989) [16–18] postulated that the time taken to conceive (time to pregnancy) should be used as an indicator of couple fertility. This idea was soon adopted and became the most frequently used approach in the studies of the occupational and environmental impact on couple fertility. Generally, the information on the time (or the number of menstrual cycles) taken to conceive from the discontinuance of contraception is collected by interview or questionnaire in population samples or samples of pregnant women. Time to pregnancy is measurable only if the starting point is well defined. However, it is estimated that even in western European countries only about 60–70% of all pregnancies are planned [19].

Worldwide epidemiological studies have investigated the relationship between employment in agriculture and decrease in fecundability or increased time to pregnancy (Table 2). Seven studies have examined the relation between exposure to pesticides and the time needed to conceive (time to pregnancy) for farmers and agricultural workers in the Netherlands, Denmark, France, Canada and Italy. Only three of those studies, in the Netherlands [20], Italy [21] and Finland [22] provided statistically significant associations. Others did not find any relationship between pesticide exposure and the time to pregnancy.

An adverse effect of pesticide exposures on the time to pregnancy was observed in the Dutch studies. The application of pesticides solely by the male (usually a farm owner) was associated with a long time to pregnancy, resulting in the fecundability ratio of 0.46 95%CI (0.28–0.77).

Similarly, a low spraying velocity resulted in the fecundability ratio of 0.47 95%CI (0.29–0.76) and was associated with the use of older spraying techniques (i.e., producing higher exposures). During the period from March to November when pesticides were applied, the fecundability ratio was significantly lower – 0.42 95%CI (0.20–0.92) compared to the out of spraying season [20].

The increased risk (OR = 2.4 95%CI (1.2–5.1)) of conception delay beyond 6 months for men was found among Italian greenhouse workers. However, it was noted only for men who worked under conditions of high level of exposure (>100 h of applications per year) [21]. No increased risk was found for men with low-level exposure (1–100 h).

Table 2. Time to pregnancy and occupational exposure to pesticides

Study population	Result	OR	Reference
The Netherlands 91 pregnancies of 43 couples occupationally exposed to pesticides	Exposure to pesticides was associated with a long time to pregnancy, especially when pesticides were applied by farm owners	Fecundability ratio when the pesticides were applied solely by the owners OR = 0.46(95%CI 0.28–0.77) Fecundability ratio when older spraying techniques were used OR = 0.47(95%CI 0.29–0.76) Fecundability ratio during spraying season OR = 0.42(95%CI 0.20–0.92) Fecundability ratio beyond spraying season OR = 0.82(95%CI 0.33–2.02)	20
Denmark 904 traditional farmers (exposed to pesticides) and organic farmers (not exposed to pesticides)	No effect of pesticide exposure on fecundability was found	Fecundability ratio between traditional farmers and organic farmers OR = 1.03(95%CI 0.75–1.40)	23
Denmark 326 Danish farmers exposed to pesticides 123 Danish farmers not exposed to pesticides 121 Danish greenhouse workers exposed to pesticides **France** 142 French rural workers exposed to pesticides 220 French rural workers not exposed to pesticides	No relation between time to pregnancy and male exposure to pesticides	Fecundability ratio for Danish farmers OR = 1.09(95%CI 0.82–1.43) Fecundability ratio for Danish greenhouse workers OR = 0.83(95%CI 0.62–1.18) Fecundability ratio for French rural workers OR = 1.17(95%CI 0.89–1.55)	24
Canada 2012 pregnancies of 1048 couples exposed to pesticides	During exposure intervals in which women (but also their partners) participated in pesticide activities, of the 13 pesticide exposure categories 6 were associated with a 20% decrease in fecundability During exposure in which only men were engaged in pesticide activities, 4 pesticide categories were associated with a 12–15% decrease in fecundability During exposure in which couples did not participate in pesticide activities, but pesticides were used on the farm, 3 pesticide categories were associated with a decrease in fecundability	Of the 13 pesticide categories 6 were associated with decrease in fecundability: – dicamba OR = 0.51(95%CI 0.24–1.05) – glyphosate OR = 0.61(95%CI 0.30–1.26) – phenoxy herbicides OR = 0.80(95%CI 0.57–1.13) – 2,4-dichlorophenoxy acetic acid OR = 0.71(95%CI 0.46–1.10) – organophosphates OR = 0.75(95%CI 0.51–1.10) – thiocarbamate OR = 0.76(95%CI 0.43–1.33) 4 pesticide categories were associated with decrease in fecundability: –2,4-DB OR = 0.85(95%CI 0.63–1.13) – cynazine OR = 0.86(95%CI 0.58–1.26) – fungicides OR = 0.88(95%CI 0.72–1.09) – captan OR = 0.87(95%CI 0.65–1.10) 3 pesticide categories were associated with decrease in fecundability: – dicamba OR = 0.82(95%CI 0.47–1.40) – 2,4-dichlorophenoxy acetic acid OR = 0.75(95%CI 0.49–1.14) – thiocarbamate OR = 0.76(95%CI 0.42–1.40)	25

Study population	Result	OR	Reference
Denmark 253 female greenhouse workers exposed to pesticides 239 non-exposed female greenhouse workers	Female workers in flower greenhouses may have reduced fecundity in terms of increased time to pregnancy	Fecundability ratio for workers in flower greenhouses OR = 1.11(95%CI 0.90–1.36) The handling of cultures for many hours per week OR = 0.69(95%CI 0.47–1.03) The spraying of pesticides OR = 0.78(95%CI 0.59–1.06) The non-use of gloves OR = 0.67(95%CI 0.46–0.98)	1
Italy 127 greenhouse workers exposed to pesticides 173 administrative staff (controls)	The risk of conception was delayed beyond 6 months among the greenhouse workers with high exposure	For low exposure to pesticides (1–100 hours of applications per year) OR = 1.6(95% CI 0.8–3.1) For high exposure to pesticides (>100 hours of applications per year) OR = 2.4(95% CI 1.2–5.1)	21
Finland 578 couples in which men were exposed to pesticides	Fecundability was reduced among the exposed men who did not have effective personal protection	Fecundability for exposed greenhouse workers who were inadequately protected: for high exposure FDR = 0.67(95%CI 0.33–1.35) for medium exposure FDR = 0.92(95%CI 0.45–1.88) for low exposure FDR = 0.77(95%CI 0.46–1.29) Fecundability ratio by the type of pesticide exposure: – organophosphates FDR = 0.70(95%CI 0.42–1.17) – carbamates FDR = 0.55(95%CI 0.27–1.11) – pyrethroids FDR = 0.40(95%CI 0.19–0.85) – benzimidazoles FDR = 0.87(95%CI 0.49–1.53) – miscellaneous fungicides FDR = 0.70(95%CI 0.40–1.20) – herbicides FDR = 0.67(95%CI 0.31–1.48)	22

OR – odds ratio.
FDR – fecundability density ratio.

In the Netherlands, the fecundability density ratio (FDR) was slightly decreased for men who were inadequately protected from exposure to pesticides. FDR for men with high exposure was 0.67 95%CI (0.33–1.35), medium exposure 0.92 95%CI (0.45–1.88), and low exposure 0.77 95%CI (0.46–1.29). The exposed men who adequately used personal protective equipment were as fertile as the non-exposed greenhouse workers. Exposure to pyrethroids was significantly related to decreased fecundability FDR = 0.40 95%CI (0.19–0.85). Borderline associations were observed for organophosphates FDR = 0.70 95%CI (0.42–1.17) and carbamates FDR = 0.55 95%CI (0.27–1.11) [22].

Epidemiological studies of the effect of pesticide exposure on the time to pregnancy in farm couples (2012 planned pregnancies) in Ontario, Canada [25] revealed that of the 13 pesticide exposure categories 6 were associated with a decreased fecundability during the periods of women participating in pesticide activities. A decrease in fecundability of 20% or more was associated with the use of dicamba OR = 0.51 95%CI (0.24–1.05), glyphosate OR = 0.61 95%CI (0.30–1.26), phenoxy herbicides OR = 0.80 95%CI (0.57–1.13), 2,4-D OR = 0.71 95%CI (0.46–1.10), organophosphates OR = 0.75 95%CI (0.51–1.10) and thiocarbamates OR = 0.76 95%CI (0.43–1.33).

The findings of another study of fertility among female workers exposed to pesticides in flower greenhouses in Denmark also suggest that female workers may have reduced fecundability OR = 1.11 95%CI (0.90–1.36). This effect was related to handling of cultures for many hours per week OR = 0.69 95%CI (0.47–1.03), spraying of pesticides OR = 0.78 95%CI (0.59–1.06) and non-use of gloves OR = 0.67 95%CI (0.46–0.98). However, for all the associations reported above, the OR values were not statistically significant [1].

The studies carried out among farmers and agricultural workers in Denmark and France did not show any relationship between pesticide exposure and time to pregnancy. In Denmark, the fecundability ratio between traditional farmers who used pesticides and organic farmers who did not was 1.03 95%CI (0.75–1.40) [23]. Similarly, the fecundability ratio was 1.17 95%CI (0.89–1.55) for agricultural workers in France and 0.83 95%CI (0.69–1.18) for greenhouse workers in Denmark [24].

Semen quality

Semen analysis is the laboratory assessment of the male reproductive function. Apart from the classical human semen parameters (concentration, motility and morphology), other markers have been used to get a better evaluation of spermatogenesis and the function of accessory sex organs. They include a variety of biochemical parameters and measures of structural and numerical abnormalities of genetic material [26].

There has been a genuine decline in semen quality over the past 50 years. Sperm count decreased from $113 \cdot 10^6$/ml in 1940 to $66 \cdot 10^6$/ml in 1990. As the male fertility is to some extent correlated with the sperm count, the results may reflect an overall reduction in male fertility [27].

The association between exposure to pesticides and semen quality is shown in Table 3. The results of the studies performed in Hawaii, Denmark, China and Mexico indicated that employment in agriculture increased the risk of specific morphological abnormalities of the sperm and decreased sperm count per ejaculate and the percentage of viable sperm. No effects of pesticide exposure on sex hormones could be observed [28–32].

Statistically significant decreases in sperm count per ejaculate, the percentage of viable and motile sperm as well as increases in the proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, abnormal tails) were observed among men exposed to ethylene dibromide (EDB) in the papaya fumigation industry in Hawaii. No effect of exposure to EDB on sperm velocity, the overall proportion of sperm with normal morphology, was observed [28]. Also among Danish greenhouse workers, the median values of sperm concentration and proportion of normal spermatozoa were lower by 60% and 14%, respectively, in the high- and low-level exposure groups. The differences between groups were statistically significant. The median sperm concentration was lower by 40% for men with over 10 years' work experience in a greenhouse than for those with experience below 5 years [29].

Semen quality and reproductive hormones across a spraying season were also examined among Danish farmers (using and not using pesticides) and controls that were asked to give two semen samples [30]. The median sperm concentration declined significantly from the first to the second sample in both groups, but there was no statistical difference in the decline between the two groups. It was concluded that semen quality did not change across the spraying season as a result of pesticide exposure. Sprayers and non-sprayers had an equal decline in sperm concentration from the first to the second semen sample.

Seasonal changes in sperm concentration have been reported in non-equatorial countries in the northern hemisphere, with the lowest values in the three summer months [31]. Experiments with rhesus monkey suggest that such seasonal variation may be induced by an endogenous biological clock that is reset annually by changes in the length of daylight.

In China and Mexico, the studies of the prevalence of sperm aneuploidy [32,33] were conducted. The analysis of chromosomal aneuploidy in sperm is a method for studying the effects of environmental and occupational exposures and other factors of the genetic constitution on spermatozoa [26]. In both countries, the workers were exposed to organophosphorous pesticides like ethyl parathion,

Table 3. Semen quality and occupational exposure to pesticides

Study population	Result	Publication
Hawaii 46 men employed in the papaya fumigation industry, exposed to ethylene dibromide 43 non-exposed men from sugar refinery	Exposure to pesticides statistically decreased sperm count per ejaculate, the percentage of viable and motile sperm. Increase in the proportion of sperm with specific morphological abnormalities (tapered heads, absent heads and abnormal tails) were observed among exposed men compared with controls. No effect of exposure to ethylene dibromide on sperm velocity, the overall proportion of sperm with normal morphology was observed	28
Denmark 161 agricultural workers exposed to pesticides 87 agricultural workers not using pesticides	The median sperm concentration declined significantly from the first to the second sample for the men spraying pesticides and the men not spraying pesticides, but there was no statistical difference in the decline between the two groups. Only minor changes were found in the sperm morphology, vitality, motility, sperm chromatin denaturation (SCSA) and reproductive hormones.	30
China 32 workers from pesticide-manufacturing plant 43 workers from a nearby textile factory free of pesticides	Exposure to organophosphate pesticides (parathion, methamidophos) increased the prevalence of sperm aneuploidy giving the rate ratio of 1.56 (95%CI 1.06–2.31). The specific chromosome abnormalities were disomy for chromosome 18 and the three different types of sex chromosome disomy (XX, XY, YY) Median semen parameters for the exposed (and non-exposed) men were as follows: – proportion of sperm with normal motility – 50.5% (61.3%) – proportion of sperm with normal morphology – 59% (61.5%) – sperm concentration was lower in the exposed group compared with the non-exposed one	32
Denmark 122 greenhouse workers exposed to pesticide: 44 with low exposure 65 with medium exposure 13 with high exposure	The median values of sperm concentration and the concentration and proportion of normal spermatozoa were 60% and 14% in the high- and low-level groups, respectively. No differences were observed for the viability and velocity of sperm and sexual hormones. The median sperm concentration was 40% lower for the men with >10 years' period of work in a greenhouse than for those with <5 years' period	29
Mexico agricultural workers exposed to pesticides	Exposure to organophosphate pesticides has been associated with sperm hyperploidy/polyploidy. There is a significant association between the concentration of organophosphate metabolites and increased frequency of sperm aneuploidies.	33

methamidophos or endosulfan. In China, male workers at a large pesticide-manufacturing plant had an excess risk of aneuploidy, OR = 1.56 95%CI (1.06–2.31) and the risk of specific chromosome abnormalities: disomy for chromosome 18 and the three different types of sex chromosome disomy (XX, XY,YY) [32].

Another study performed in Mexico among agricultural workers exposed to pesticides found aneuploidies in 0.67% of total sperm nuclei. The most frequent aneuploidy was the lack of a sexual chromosome or sex null (0.19%) that further increased during the spraying season. The authors noticed that exposure to organophosphates could interfere with sperm chromosome segregation and increase the risk of Turner's syndrome [33].

Infertility

Several epidemiological studies of the effects of pesticide exposure on infertility were based on couples attending infertility clinics [34–36]. Infertility was defined based on medically confirmed diagnosis. The studies demonstrating an association between agricultural work and infertility are summarized in Table 4. The findings indicate that agricultural work related to pesticide exposure might be a significant risk for infertility.

The Iowa State study performed on women working in agriculture-related industries and in those residing on a farm, showed a significantly increased risk of infertility. The risk estimates were OR = 7.0 95%CI (2.3–20.8) and OR = 1.8 95%CI (1.2–2.7), respectively [34]. Also in the Netherlands the effect of occupational exposure on fertility was investigated in 836 couples who sought *in vitro* fertilization treatment. Fertilization rates were significantly decreased for 20 couples with paternal pesticide exposure [35]. Kenkel et al. [36] noticed that among 2054 infertile men receiving treatment at infertility clinics, the farmers had a significantly higher proportion of reduced sperm

Table 4. Infertility and agricultural work

Study population	Result	OR	Reference
The United States 281 female agricultural workers with medically-confirmed diagnosis of infertility 216 women at risk for infertility	Women were at an increased risk of infertility if they had worked in industries associated with agriculture	Women in industries associated with agriculture OR = 7.0(95%CI 2.3–20.8) Women who worked in agriculture prior to conception OR = 11.3(95%CI 2.6–48.8) Women who resided on a farm OR = 1.8(95%CI 1.2–2.7) Yearly duration of farm residence OR = 0.99(95%CI 0.95–1.03)	34
The Netherlands 836 couples who sought *in vitro* fertilization treatment (in 16 couples the male was exposed to pesticides: 9 with moderate exposure, 7 with high exposure)	Fertilization rates were significantly decreased for couples with paternal pesticide exposure	Moderately exposed men OR = 0.52(95%CI 0.22–1.24) Highly exposed men OR = 0.22(95%CI 0.06–0.80)	35
Germany 46 infertile farmers 20 infertile gardeners and foresters under treatment at infertility clinic	Agricultural work is associated with male infertility	Farmers had a significantly higher proportion of reduced sperm count OR = 2.13(95%CI 1.18–3.88) and severely reduced sperm concentration	36

OR – odds ratio.

count OR = 2.13 95%CI (1.18–3.88) and severely reduced sperm concentration.

Sex ratio

A recent report concludes that male/female birth ratio in several industrial countries had decreased [37]. In Finland, where birth sex ratio was analyzed for the period of 1751–1997, such a trend was noted after 1920 and was related to the introduction of a heavy use of industrial chemicals. Similar trends have been observed in other countries [38]. The studies of offspring sex ratio in families with occupational exposure to pesticides revelated the increased likelihood of conceiving girls than boys [39]. The reduction in the number of male infants was noticed also for children born to workers using dibromochloropropane (DBCP) and organochlorine pesticides [40]. Also Garry et al. [41] in a study of male pesticide applicators in Minnesota noticed a deficit in the number of male children born to the spouses of fungicide applicators ($p = 0.04$). However, more male children were born with birth defects (M/F sex ratio = 1.8) when other than fungicide pesticides were applied. When fungicides were applied by the male partner, far fewer male children with births defects were born (M/F sex ratio = 0.57, $p = 0.02$). This study pointed out that fungicides could be a significant factor in the determination of sex of the children of farmers' families from Minnesota. On the other hand, another study performed only on female workers revealed that pesticide exposure seemed to increase the chance to conceive a boy [42]. Savitz at al. [43] did not find an association between sex ratio and chemical activities on the farm (OR = 1.0 95%CI (0.8–1.2)).

Spontaneous abortion

An increased risk for spontaneous abortion has been found among women in agricultural occupations and among gardeners who sprayed pesticides in Canada [43–46], Columbia [47], Norway [48,49], Italy [50], the USA [51] and the Philippines [52]. Only in the study carried out in West Sumatra, Indonesia, the number of spontaneous abortions among female spraying operators was not significantly increased compared to female rice farmers [53]. In one study, parental preconception exposure to phenoxy herbicides was associated with the risk of early (< 12 weeks) spontaneous abortion [45] (Table 5).

In Quebec, Canada, substantially increased O/E ratios were found for late but not for early abortion in women working in agriculture and horticulture (2.4; $p < 0.05$) [44]. In the Ontario study, the authors analyzed 3984 pregnancies in 1898 farm couples. An increased risk was found in the couples who reported using thiocarbamates

Table 5. Reproductive effects of exposure to pesticides: the risk of spontaneous abortion

Country and definition of exposure	Reproductive outcome	OR, RR, O/E	Reference
Montreal, Canada Work in agriculture and horticulture	Late spontaneous abortion (gestational weeks 16–28)	O/E = 2.4 ($p < 0.05$)	44
Bogota, Colombia Pesticide exposure in floriculture	Spontaneous abortion	OR = 2.20 (95%CI 1.82–2.66) – for female workers exposed to pesticides OR = 1.79 (95%CI 1.16–2.77) – for the wives of male workers exposed to pesticides Female workers with: OR = 2.01 (95%CI 0.96–4.21) – low exposure OR = 2.31 (95%CI 1.70–3.14) – medium exposure OR = 2.21 (95%CI 1.62–3.02) – high exposure Wives of male workers with: OR = 2.09 (95%CI 1.03–4.27) – medium exposure OR = 2.05 (95%CI 1.03–4.08) – high exposure	47
Norway Exposure to pesticides in grain farming	Late spontaneous abortion (gestational weeks 16–27)	OR = 1.9 (95%CI 1.6–2.3) – exposure to pesticides in grain farming OR = 2.4 (95%CI 1.5–3.8) – seasons with poor quality harvest	49
Ontario, Canada Parental work in agriculture	Spontaneous abortion	OR = 1.1 (95%CI 0.8–1.3) – all pesticides OR = 1.9 (95%CI 1.1–3.1) – crop herbicides + carbaryl, crop herbicides + thiocarbamates OR = 2.1 (95%CI 1.1–4.1) – crop insecticides or fungicides + carbaryl	43
Ontario, Canada Parental work in agriculture	Spontaneous abortion	OR = 2.5 (95%CI 1.0–6.4) – early spontaneous abortions of < 12 weeks, preconception exposure to phenoxy herbicides OR = 5.0 (95%CI 0.7–36.2) – not wearing protective equipment during application OR = 3.8 (95%CI 1.2–12.7) – early spontaneous abortions, postconception exposure to phenoxy herbicides	45
West Sumatra, Indonesia Female spray operators and female rice farmers	Insignificant number of spontaneous abortion	RR = 1.1(95%CI 0.7–1.6)	53
Italy Pesticide applicators occupationally exposed to pesticide (parental exposure) and food retailers (control population)	Spontaneous abortion	OR = 0.27 ($p< 0.01$) – for applicators OR = 0.07 ($p< 0.01$) – for retailers OR = 3.8 (95%CI 1.2–12.0) – multiple logistic regression OR = 7.6 (95%CI 2.0–28.9) – with interaction effects	50
Canada Parental exposure to pesticides (parents living on the farm)	Early spontaneous abortion (< 12 weeks) Late spontaneous abortion (12–19 weeks)	Early spontaneous abortion and preconception exposure to pesticides: – phenoxy acetic acid OR = 1.5 (95%CI 1.1–2.1) – 2,4-D OR = 1.3 (95%CI 0.9–2.0) – 2,4-DB OR = 1.4 (95%CI 0.7–2.8) – triazine OR = 1.4 (95%CI 1.0–2.0) – herbicide OR = 1.4 (95%CI 1.1–1.9) Late spontaneous abortion and preconception exposure to pesticides: – glyposate OR = 1.7 (95%CI 1.0–2.9) – thiocarbamate OR = 1.8 (95%CI 1.1–3.0) – fungicide OR = 1.4 (95%CI 0.9–2.1) – miscellaneous class of pesticide OR = 1.5 (95%CI 1.0–2.4) Late spontaneous abortion and postconception exposure to pesticides: – miscellaneous class of pesticide OR = 1.9 (95%CI 1.2–3.0)	46

Country and definition of exposure	Reproductive outcome	OR, RR, O/E	Reference
Minnesota, USA Parental exposure to pesticides	Miscarriage	Miscarriage when pesticides were applied by man: OR = 2.11 (95%CI 1.09–4.09) – for sulfonylurea OR = 2.56 (95%CI 1.11–5.87) – for imidizolinone OR = 2.94 (95%CI 1.40–6.16) – for chlorophenoxy + sulfonylurea + benzothiodiazole Miscarriage when pesticides were applied by woman: OR = 1.81 (95%CI 1.04–3.12) Pregnancy loss and specific fungicide use: – organotin (OR = 1.55 95%CI 1.01–2.37) – EDBC = ethylene bisdithiocarbamate-containing fungicides (maneb and mancozeb) OR = 1.77 95%CI 1.11–2.83	51
Nueva Ecija, The Philippines Parental exposure to pesticides (farming householders who used pesticides regularly and people who practised integrated pest management and used pesticides as necessary on injured crop areas)	Spontaneous abortion	Spontaneous abortion were more common in the farming householders group OR = 6.17 (95%CI 1.37–27.86)	52

OR – odds ratio.
RR – risk ratio.
O/E – observed to expected ratio.

(OR = 1.9 95%CI (1.1-3.3)) and carbaryl (OR = 2.1 95%CI (1.1–4.1)) [43].

In another Canadian study [46], couples living on the farms also showed a moderately increased risk of early spontaneous abortion and late abortion (12–19 weeks) due to preconception exposure. The risk of early spontaneous abortion was observed in the exposure to phenoxy acetic acid, triazine herbicide, 2,4-D, 2,4-DB, and of late spontaneous abortion in the exposure to glyposate, thiocarbamate, fungicide and miscellaneous class of pesticides. The risk of late abortion for postconception exposures applied to the miscellaneous class of pesticides.

The excessive risk of spontaneous abortion was also noted in a population of female workers occupationally exposed to pesticides in Columbia and among the wives of male workers OR = 2.20 95%CI (1.82–2.66). The risk of spontaneous abortion for female workers was observed for low OR = 2.01 95% CI (0.96–4.21), medium OR = 2.31 95% CI (1.70–3.14), and high OR = 2.21 95% CI (1.62–3.02) exposures [47]. Another study performed in Italy also showed that the odds ratio of spontaneous abortion for pesticide applicators was 3.8 95%CI (1.2–12.0) [50]. An excess risk of spontaneous abortion was observed in the study in Nueva Ecija, Philippines, where conventional pesticide applicators experienced a higher risk of spontaneous abortion, RR = 6.17 95%CI (1.37–27.86) as compared to farmers practising integrated pest management [52].

The risk of late-term abortions (gestational weeks 16–27) was higher among Norwegian farmers than among non-farmers (OR = 1.9 95%CI (1.6–2.3)). For women involved in grain farming, the risk was higher for birth after the harvest (OR =1.8 95% CI (1.1–2.8)) and in seasons with poor quality harvest (OR = 2.4 95% CI (1.5–3.8)). The effect of grain farming on the risk of late-term abortions was even more pronounced in the case of multiple pregnancies (OR = 3.8 95% CI (1.7–8.2)) [48,49].

In the Minnesota study reported above, Garry et al. [51] noticed that the first-trimester miscarriages had occurred most frequently in the spring, in the time the herbicides are applied. The use of sulfonylurea products OR = 2.11 95%CI (1.09–4.09), imidizolinone OR = 2.56 95%CI

(1.11–5.87), 9100 mixture (chlorophenoxy + sulfonylurea + benzothiodizole) by male applicators was significantly (OR = 2.94 95%CI (1.40–6.16)) associated with an increased risk for miscarriage during the spring season. Women who were engaged in the application of these products were also at a demonstrable (OR = 1.81 95%CI (1.04–3.12)) risk of miscarriage. Pregnancy loss was also associated with specific fungicide use: organation OR = 1.55 95%CI (1.01–2.37) and etylene bisdithiocarbamate-containing fungicides (EDCB) (maneb and mancozeb) OR = 1.77 95%CI (1.11–2.83).

Birth defects

An increased rate of birth defects has been associated with some individual pesticides or pesticide mixtures. In several studies, the authors reported an increased risk of limb anomalies, orofacial cleft or birthmarks, while several others did not support such evidence (Table 6). A comprehensive review of the methods applied and of the results of epidemiological studies has recently been performed by Garcia [54].

An excess risk of musculoskeletal malformations was noted for infants of gardeners in Finland (OR = 5.0; $p < 0.05$) [55]. Similar results with respect to developmental defects were found in offsprings of parents working in agriculture and horticulture in Quebec (RR = 4.5; $p < 0.05$) [56]. In a study carried out among 8867 floriculture workers in Bogota, an increased risk for birthmarks, particularly hemangiomas was found (OR = 6.6; $p < 0.05$) [57].

Several studies indicated an increased risk of limb defects in infants born to parents exposed to pesticides. In California, the cases with limb reduction defects (n = 237) and randomly selected controls (n = 475) were compared with respect to parental occupation and maternal county of residence. Among the parents involved in agricultural work, an estimated relative risk of parenting a child with a limb reduction defect approximated 0.9 [58].

Also in the retrospective study, using Washington State birth records (1980–1993), maternal employment in agriculture was associated with an elevated risk of limb defects in the offspring. The risk of limb defects observed in the exposed group was compared with non-agricultural group OR = 2.6 95%CI (1.1–5.8) and paternal agriculture group OR = 2.6 95%CI (0.7–9.5) [59].

In a case-control study conducted in Spain, 261 cases and 261 controls were selected from the infants born in eight public hospitals during 1993–1994. Adjusted odds ratio for congenital malformations was 3.1 95%CI (1.1–9.0), primarily due to an increased risk for nervous system defects, oral cleft and multiple anomalies. A statistically significant relationship was found for pyridil derivatives OR = 2.8 95%CI (1.2–6.4). No increased risk was noted in the case of paternal exposure to pesticides in the classes of organophosphates, carbamates, organochlorines**,** chloroalkylthio fungicides and organosulfurs [54,60].

In a Finnish study, an elevated risk of orofacial clefts (1.9 95% CI 1.1–3.5) was documented [61]. In Norway, an increased risk of spina bifida and hydrocephaly was found, the associations being strongest for pesticide exposure in orchards and greenhouses [49].

Garry et al. [59] identified 4935 births in the population of 34 772 state-licensed private pesticide applicators in Minnesota (1989–1992) and compared them to 210 723 live births in this State. Information on pesticide use was obtained from a survey of the Minnesota Department of Agriculture. Pesticide applicators had relatively more children with circulatory/respiratory, musculoskeletal/integumental and urogenital anomalies (OR = 1.57 95%CI (1.22–2.01)). Families residing mostly in the agricultural regions of Minnesota with a high use of chlorphenoxy herbicides/fungicides were more likely to have children with birth defects. The following pesticides were associated with excess risk of birth defects: trifluralin, atrazine, 2,4 D and MCPA. In the regions where chlorphenoxy herbicides/fungicides were frequently used, infants conceived in the spring showed a significant increase in birth defects compared to infants conceived in the other seasons OR = 1.15 95%CI (1.03–1.28) [59]. Birth defect rates per 1000 births in the first year and in the first three years of life were 31.3 and 47, respectively. Offspring of applicators who applied phosphine fumigants had an increased risk of birth defects in general (OR = 2.3 95%CI (0.9–6.1)) as well as of defects of the central nervous system or neurobehavioral problems (OR = 2.5 95%CI (1.2–5.1)). The use of herbi-

Table 6. Reproductive effects of exposure to pesticides: the risk of birth defects

Country and definition of exposure	Reproductive outcome	OR, RR, O/E	Reference
Finland Agricultural work	Musculoskeletal defect	OR = 5.0 ($p < 0.05$)	55
Montreal, Canada Work in agriculture and horticulture	Congenital defects Developmental defects	O/E = 2.6 ($p < 0.05$) O/E = 4.5 ($p < 0.01$)	56
California, USA Parental occupation in agriculture	Limb reduction defects Limb reduction defects and additional defects	RR = 0.9 (95%CI 0.4–.7) OR = 1.6 (95%CI 0.7–3.6)	58
Comunidad Valeriana, Spain Parental work in agriculture	Congenital defect	OR = 3.2 (95%CI 1.1–90) OR = 2.8 (95%CI 1.2–6.4) – pyridil derivatives	60
Bogota, Colombia Pesticide exposure in floriculture	Congenital defects Hemangiomas	RR = 1.8 (95%CI 1.2–2.7) RR = 6.6 ($p < 0.05$)	57
Finland Maternal agricultural work	Congenital defects Orofacial cleft	OR = 1.4 (95%CI 0.9–2.0) OR = 1.9 (95%CI 1.1–3.5)	61
Minnesota, USA Parental exposure to pesticide	Circulatory/respiratory, musculoskeletal/integumental and urogenital anomalies	OR = 1.57 (95%CI 1.22–2.01) – pesticide appliers OR = 1.15 (95%CI 1.03–1.28) regions with high exposure to chlorphenoxy herbicides/fungicides	59
Washington, USA Maternal exposure to agriculture chemicals	Limb defects	PR = 2.6 (95%CI 1.1–5.8) relative to the non-agricultural group PR = 3.0 (95%CI 0.9–9.6)	62
Nueva Ecija, Philippines Parental exposure to pesticides (farming householders who used pesticides regularly and people who practised integrated pest management and used pesticides as necessary on injured crop areas)	Birth defects	Birth defects were more common in the farming householders OR = 4.56 (95%CI 1.21–17.09) $p = 0.05$ – fathers mixed or applied pesticides during the first trimester of their wives pregnancies	52
Minnesota, USA Parental exposure to pesticides	Birth defects Neurobehavioral developmental effects	In the first year of life the birth defects rate was 31.3 per 1000 births In the first three years of life and later the rate was 47.0 per 1000 births – application of fumigant posphine (OR = 2.3 95%CI 0.9–6.1) – central nervous system or neurobehavioral problems (OR = 2.5 95%CI 1.2–5.1) – application of phosphonamino herbicides by fathers (OR = 3.6 95%CI 1.4–9.7)	41
Minnesota, Montana, North and South Dakota Parental exposure to pesticides	Birth defects (male and female births together): – circulatory/respiratory – musculoskeletal/ integumental Musculoskeletal/ integumental defects in female infants	 OR = 1.65 (95%CI 1.07–2.55) OR = 1.50 (95%CI 1.06–2.12) OR = 1.62 (95%CI 1.01–2.60)	63

OR – odds ratio.
RR – risk ratio.
O/E – observed to expected ratio.

cide glyposate was associated with an increased risk in the neurobehavioral category OR = 3.6 95%CI (1.3–9.6) [62]. Schreinemachers et al. [63] also found an increase in the odds of having a child with birth defects among farmers exposed to chlorphenoxy herbicides. When examining male and female births jointly, the odds ratio for circulatory/respiratory defects was 1.65 95%CI (1.07–2.55) and for musculoskeletal/integumental defects OR = 1.50

95%CI (1.06–2.12). Female infants had an elevated risk of musculoskeletal/integumental defects (OR = 1.62 95%CI (1.01–2.60)) [63].

An increase in birth defects was also observed in a retrospective study in Nueva Ecija, the Philippines (adjusted RR = 4.56 95%CI (1.21–17.09)). Birth defects were found to be more common in the group of farming households which used pesticides regularly than in the group practising integrated pest management where pesticides are used as necessary on heavily damaged areas. An excess of birth defects was observed in families in which husbands mixed or applied pesticides during the first trimester of their wife's pregnancy [52].

Preterm delivery

Evidence obtained thus far, however limited, indicates in general that working in agriculture is not related to an increased risk of preterm delivery [47,64,65] (Table 7). However, in one study, preterm delivery was associated with mixing or applying atrazine (OR = 4.9 95% CI (1.6–1.5)) and 4-[2,4-dichlorophenoxy] butric acid (OR = 3.5 95% CI (1.2–9.9)) within a three-month period prior to conception [43]. No significant effect of grain farming was documented in the Norwegian study. The mean pregnancy duration was even by one day longer in farmers than in non-farmers [49].

Birthweight and small-for-gestational-age

No increased risk for low birth weight (LBW) and small-for-gestational-age (SGA) was found among women working in agricultural occupations in Scotland and Canada [43,65,66] (Table 8). In Norway, the overall mean birthweight was 3541 g for farmers' offspring and 3509 g for non-farmers' offspring [49].

Despite the most negative results on the risk of LBW, there are some indications for further research in this area. The implication derives from the studies carried out in Northeast Brazil, West Sumatra, Indonesia, and Central Poland. In Northeast Brazil, the mean birth weight of infants born to women who worked in agriculture was by 190 g lower than that in the non-exposed group. After controlling for confounding factors, the adjusted effect was 117 g (p = 0.05) [67].

The Indonesian study performed among 161 female spraying operators and 352 female rice farmers as controls showed that more women delivered infants with low birthweight among the rice farmers compared to the sprayer cohort (RR = 3.6 95%CI (2.4–5.4)) [53].

Maternal exposure to pesticides in the 1st and 2nd trimesters of pregnancy affected infant birthweight in a population of Polish female farmers who had infants with birtweight lower by 100 g than that of infants born to non-exposed women. The results were adjusted for pregnancy duration and other factors, including smoking, which might influence birthweight [68].

Another study, carried out in Poland, in a group of 104 women who delivered a single live infant, showed that mothers who reported involvement in field work delivered infants with a significantly higher birthweight than mothers not reporting such activities in the 1st or 2nd trimester

Table 7. Reproductive effects of exposure to pesticides: the risk of preterm delivery

Country and definition of exposure	Reproductive outcome	OR, RR, O/E	Reference
Montreal, Canada Work in agriculture and horticulture	Preterm delivery	OR = 1.86 (95%CI 1.59–2.17) – for female workers exposed to pesticides	64
Bogota, Colombia Pesticide exposure in floriculture	Preterm delivery	OR = 2.75 (95%CI 2.01–3.76) – for the wives of male workers exposed to pesticides	47
Scotland, UK Parental work in agriculture	Preterm delivery	RR = 1.4 (95%CI 0.9–2.1) – maternal exposure RR = 1.1 (95%CI 1.0–1.3) – paternal exposure	65
Ontario, Canada Parental work in agriculture	Preterm delivery	OR = 1.2 (95%CI 0.7–1.9) – any chemical OR = 4.9 (95%CI 1.6–1.5) – crop herbicides + atrazine OR = 3.5 (95%CI 1.2–9.9) – crop herbicides + 2.4-DB	43

OR – odds ratio.
RR – risk ratio.
O/E – observed to expected ratio.

Table 8. Reproductive effects of exposure to pesticides and the risk of low birthweight

Country and definition of exposure	Reproductive outcome	OR, RR, O/E	Reference
Montreal, Canada Work in agriculture and horticulture	Low birthweight	O/E = 1.2	[64]
Scotland Parental work in agriculture	Low birthweight Small-for-gestational-age infant	RR = 1.4 (95%CI 0.9–2.2) – maternal exposure RR = 0.9 (95%CI 0.8–1.0) – paternal exposure RR = 0.8 (95%CI 0.6–1.1) – maternal exposure RR = 0.7 (95%CI 0.2–2.5) – paternal exposure	[65]
Ontario, Canada Parental work in agriculture	Small-for-gestational-age infant	OR = 1.0 (95%CI 0.6–1.3) – any chemical	[43]
West Sumatra, Indonesia Female spray operators and female rice farmers	Low birthweight	RR =3.6 (95%CI 2.4–5.4) – among the rice farmers	[53]
Norway Exposure to pesticides in grain farming	Birthweight	by 32 g higher	[49]
Northeast Brazil Maternal work in agriculture	Birthweight	by 117 g lower (p = 0.05)	[67]
Poland Maternal exposure to pesticides	Birthweight	lower by 100 g among infants born to women exposed to pesticides in the first or second trimester (p = 0.67)	[68]
Poland Maternal exposure to pesticides	Birthweight	Maternal exposure to synthetic pyrethroids in the first or second trimester was associated with a decreased birth weight (p = 0.02)	[69]

OR – odds ratio.
RR – risk ratio.
O/E – observed to expected ratio.

of pregnancy (p = 0.04). Also maternal exposure to synthetic pyrethroids in the first or the second trimester was associated with a decreased birthweight (p = 0.02) [69].

Stillbirth

The rate of stillbirth without a birth defect was increased among women who worked in agriculture or horticulture more than 30h/week [66] (Table 9). On the other hand, women with even low-level exposure to pesticides or germicides demonstrated an increased risk of stillbirth [70]. The study of the Norwegian grain farmers did not reveal a higher risk of stillbirth in farmers than in non-farmers. (OR = 0.88 95%CI (0.79–0.98)) [48,49].

Among the farmers in California [71], occupational exposure to pesticides during the first two months of gestation was positively associated with stillbirth due to congenital anomalies (OR = 2.4 95%CI (1.0–5.4)), while during the first and second trimesters with stillbirth due to all causes of death (RR = 1.4 95%CI (1.0–1.7)).

The potential association between fetal death and residential proximity to agricultural pesticide applicators was examined in another study in California**.** The risk of fetal death was observed for women who during the second trimester of pregnancy resided near the application of: carbamates (OR = 1.3 95%CI (1.0–1.8)), estrogenic pesticides (endocrine disruptors) (OR = 1.4 95%CI(0.8–2.5)), and carbamate acetylcholinoesterase inhibitors (OR = 1.3 95%CI (1.0–1.8)). Women who when in the third trimester resided near the application of pyrethroids were found to be at an increased risk of fetal death (OR = 1.4 95%CI (0.7–2.8)). In the month-by-month analysis, elevated risks were observed when exposure to carbamates and carbamate inhibitors occurred during the third and fourth gestational months [72].

No associations between pesticide exposure and the risk for spontaneous abortion were found among female spraying operators and rice farmers in West Sumatra, Indonesia.

Table 9. Reproductive effects of exposure to pesticides: the risk of stillbirth and fetal death

Country and definition of exposure	Reproductive outcome	OR, RR, O/E	Reference
Montreal, Canada Exposure to pesticides and bactericides	Stillbirth without major malformations (gestational weeks 20)	OR = 3.1 (95%CI 1.1–8.6)	[70]
Norway Exposure to pesticides in grain farming	Perinatal mortality Stillbirth due to congenital anomalies	15% higher in farmers OR = 2.4 (95%CI 1.0–5.9) – pesticide exposure during the first two months of gestation	[49] [71]
California, USA Prenatal exposure to pesticides	Stillbirth due to all causes of death	RR = 1.4 (95%CI 1.0–1.7) – exposure during the first and second trimester	
West Sumatra, Indonesia Female spray operators and female rice farmers	No significant number of stillbirths	RR = 1.2 (95%CI 0.6–2.2)	[53]
California, USA Parental exposure to pesticides	Fetal death	For women who during the second trimester of pregnancy resided near application of: – carbamates OR = 1.3 (95%CI 1.0–1.8) – estrogenic pesticides OR = 1.4 (95%CI 0.8–2.5) – carbamate acetylocholinestrase inhibitors OR = 1.3 (95%CI 1.0–1.8) For women who during the third trimester of pregnancy resided near application of: – pyrethroids OR = 1.4 (95%CI 0.7–2.8)	[72]

OR – odds ratio.
RR – risk ratio.
O/E – observed to expected ratio.

The number of stillbirths was slightly higher among the rice farmers but the difference was not significant [53].

IMPLICATION OF REPRODUCTIVE EPIDEMIOLOGY FOR FARMERS' HEALTH PROTECTION

Much emphasis has been laid on epidemiological investigations, since they concern humans and the exposure scenarios are realistic. However, despite giving high priority and performing a considerable number of studies, the epidemiological methods employed thus far have not significantly contributed to the assessment of adverse reproductive and developmental toxicity of pesticides. The results of epidemiological studies are not consistent even for the most frequently approached reproductive outcomes such as early fetal loss and congenital malformations. Nevertheless, they indicate the need to increase awareness among workers occupationaly exposed to pesticides about the association between agricultural work, fertility and pregnancy outcome.

We try to analyze the exposure to specific pesticides which may cause adverse reproductive and developmental outcomes. In the majority of studies, the authors do not show which pesticides are responsible for which reproductive disorders. They have just claimed the exposure to pesticides without giving their chemical names, and limiting their analyses only to the duration of exposure to pesticides. Only in some cases we were able to show that exposure to certain kinds of pesticides may be dangerous to pregnant woman. There are several pesticides that induce reproduction disorders. Dicamba is related to the risk of spontaneous abortion and prolonged time to pregnancy, and glyposate to spontaneous abortion, prolonged time to pregnancy, birth defects and preterm delivery. Exposure to phenoxy herbicides may cause spontaneous abortion and have impact on time to pregnancy; triazines increase risk of spontaneous abortion and preterm delivery; organophosphate pesticides are associated with an increased risk of infertility, miscarriage and preterm delivery; perythroids influence time to pregnancy, birth weight and fetal death, and mankozeb and maneb might cause birth defects (Table 10).

Table 10. Risk of adverse reproductive and developmental outcomes and exposure to specific pesticides

Pesticide category	Time to pregnancy	Semen quality	Birth defects	Spontaneous abortion	Fetal death	Preterm delivery	Birth weight	Sex ratio
Dicamba	+ [25]			+ [46] + [43]			– [43]	
Glyposate	+ [25]		+ [41]	+ [46] + [43]		+ [43]	– [43]	
Phenoxy herbicides	+ [25]		+ [63] + [41]	+ [45,46] + [43]		+ [43]	– [43]	
2,4-DB	+ [25]			+ [43,45,46]		+ [43]	– [43]	
2,4-D	+ [25]			+ [45,46] + [43]				
MCPA	– [25]			+ [43] + [45]			– [43]	
Trazines	– [25]			+ [46] + [43]		+ [43]	– [43]	
Atrazine	– [25]			+ [43]		+ [43]	– [43]	
Cynazine	+ [25]						– [43]	
Carbamates	+ [22]				+ [72]			
Thiocarbamate	+ [25] + [22]			+ [46] + [43]			– [43]	
Organochlorines	– [22]							
Pyretroids	+ [22]				+ [72]		+ [69]	
Organophosphates	+ [25] + [22]	+ [32] + [33]		+ [43]		+ [43]		
Benzimidazoles	+ [22]							
Carbaryl	– [25]						– [43]	
Captan	+ [25]							
Maneb			+ [41]					
Mancozeb			+ [41]					
Phosphine fumigants			+ [41]					
Ethylene dibromide		+ [28]						
Pyridil derivates			+ [54]					
Sulfonylurea				+ [41]				
Imidizolinone				+ [41]				
Organotion			+ [41]					
Estrogenic pesticides					+ [72]			
Carbamate acetylo-cholinoesterase inhibitors					+ [72]			
Herbicides	– [25] + [22]			+ [46] + [43]		+ [43]	– [43]	
Insecticides	– [25]			+ [43]		+ [43]		
Fungicides	+ [25] + [22]			+ [46] + [43]				+ [41]

(+) Observed effect.
(–) Not observed effect.

It will be very difficult to eliminate pesticides from agriculture in the near future. However, being aware of the risks they pose to human health and the environment we should undertake some steps to minimize their use. There are over 400 different pesticide chemicals approved for use in agriculture, which might be formulated into thousands of different products available for users. Farmers may use dozens of different chemicals over a season and they may vary from season to season. They cannot be expected to know in detail the profile of each active ingredient, but at the same time they should be responsible for using the least toxic product. Good practice also dictates the use of products with the least harmful environmental profile

FAVORING THE HYPOTHESIS BASED ON MECHANISTIC MODELS AND BIOLOGICAL KNOWLEDGE

In the postgenomic age, epidemiology will have to shift the emphasis from the studies based on the risk factor analysis towards the verification of hypotheses rooted in biological knowledge, and launched to investigations into the effect of environmental exposures on the immune and endocrine regulation and placental function during pregnancy. Amongst others, more studies are needed to investigate gene-environment interaction and male-mediated effects.

The natural context for pregnancy outcomes evaluation is a prospective observation. It enables better evaluation of the risk of selective loss of the pregnancies as well as valid risk estimates in the studies of chromosomal aberrations, birth defects and spontaneous abortions. The recent years have witnessed a rapid progress in the study design and performance. National-based cohorts provide a valuable basis for parental exposure studies. The establishment of sibship-based cohorts is a particular asset making it possible to consider the reproductive history and selective fertility [73–75].

The use of the national registers of birth defects is common in the Scandinavian countries, and probably remains the most instrumental in the area of developmental outcomes risk assessment, in particular when supplemented by more precise and relevant information in nested case-control studies [76,77]. The possibility of establishing large prospective cohorts of farming couples who plan procreation is definitely worth considering. An important virtue of this strategy is the possibility to investigate several interrelated outcomes for which the combined results may add inferential value and credibility. Cohorts open the potential for performing nested case-referent studies with better exposure parameters.

Exposure assessment in farming activities, especially those performed by pregnant women is one of the crucial aspects of validity of epidemiological studies. The semiquantitative methods of exposure assessment and extrapolation of current exposure data to past scenarios were intensively used in the past. However, epidemiologists were aware of numerous limitations of this approach. A comprehensive assessment includes evaluation of exposure to pesticides, biological factors, commercial fertilizers and the level of physical effort.

The information on pesticide use obtained via questionnaires is usually not sufficient for valid dose assessment. The prospective approach is probably the only way to improve the quality of pesticide exposure data. It also allows for validation of farm holders' reports by using the methods of biological monitoring. These methods have not as yet been introduced to developmental epidemiology but their applicability is strongly postulated. Standard protocols for the biological monitoring of the most prevalent pesticides have to be developed.

The exposure to fungi and mycotoxins is inherent in farming activities. A valid recognition of the problem requires a biological characterization of the crops [78]. Although the use of systematic data on late blight was successfully applied in the Norwegian studies on reproductive health, the prospective approach may contribute to a more comprehensive recognition of the problem.

The introduction of molecular biology to epidemiology offers a possibility to evaluate individual susceptibility to adverse reproductive and developmental effects, including those resulting from occupational exposure in agriculture. It has been observed that there are pronounced inter-individual differences in the capacity for metabolising different xenobiotics. This variation is due not only to

physiological, environmental or pathological factors but also to genetic polymorphism of the genes encoding specific enzymes. An unfavorable combination of alleles of those genes may be the cause of enhanced or decreased susceptibility to adverse reproductive and developmental outcomes in farmers. The markers of susceptibility could prove to be most valuable.

It is well known that in the absence of chronic maternal and fetal diseases, the birth weight is affected by genetic factors (e.g., measured as birthweight of sibs or the mother), the duration of pregnancy, sex of infant, some socio-economic variables (education, marital status) and intrauterine exposure to nicotine and carbon monoxide as a result of maternal smoking. While the biological nature of the effect of smoking is well examined, the influence of the socio-economic factors is still not adequately explained. For most developmental outcomes, the mother's reproductive history has great impact on the outcome of pregnancy. Accordingly, the number of covariates, which should be taken into account, is fairly large [79].

The reproductive epidemiologists should discuss several options of analyzing data on pregnancy outcomes. The handling of the subjects' reproductive history may serve as one of examples. Although the necessity for identification and control of confounders is commonly acknowledged, this part of analysis is often neglected in retrospective studies.

It is also important to remember that analytical strategies are closely linked with biology. The use of causal diagrams may serve as a useful tool in analytical modeling [80].

CONCLUSIONS

In epidemiological studies, the analyses have been focused on the relationship between agricultural occupation of parents and the incidence of infertility, congenital malformations, miscarriage, low birthweight, small-for-gestational-age birth, preterm delivery and stillbirth. Their results imply that employment in agriculture might increase the risk of specific morphological abnormalities the sperm and decrease sperm count per ejaculate and the percentage of viable sperm. In general, no effect of pesticide exposure on sexual hormones could be observed. The data on the effect of employment in agriculture on the time to pregnancy are unequivocal, but most of them suggest that there is a relationship between the decreased fecundability ratio and exposure to some pesticides.

The findings indicate that parental employment in agriculture could increase the risk of congenital malformations in the offspring, particularly orofacial cleft, birthmarks in the form of hemangioma as well as defects of musculoskeletal and nervous systems. The data on the effect of occupational exposure to pesticides on low birthweight are inconsistent**.** However, the recent epidemiological study did reveal a significantly lower pace of fetal development corresponding to SGA in the population of women exposed to pyrethroids**.** There are also some indications that exposure to pesticides may contribute to stillbirth and female infertility.

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