DEPARTAMENTO DE MEDICINA PREVENTIVA Y SALUD PÚBLICA

FACULTAD DE MEDICINA

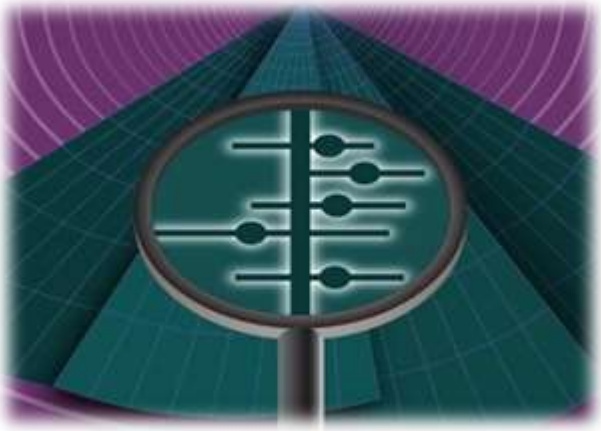
UNIVERSIDAD DE GRANADA

PESTICIDE EXPOSURE & PROSTATE CANCER:

A SYSTEMEATIC REVIEW

&

META-ANALYSIS



ANNE-MARY LEWIS MIKHAEL SAAD

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Departamento de Medicina Preventiva y salud Pública

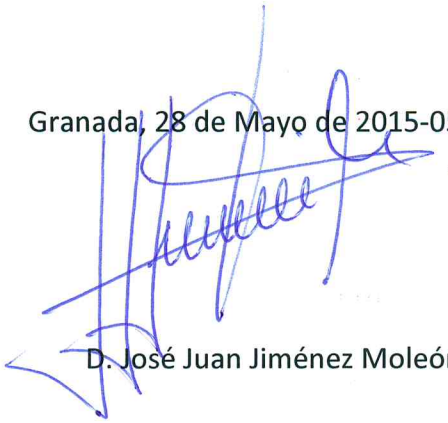
Universidad de Granada

D. José Juan Jiménez Moleón, profesor Titular del Departamento de Medicina preventiva y Salud Publica de la Universidad de Granada

CERTIFICA

Que la memoria de la Tesis Doctoral que presenta al superior juicio del Tribunal que designe la Comisión de Doctorado doña Anne-Mary Lewis Mikhael Saad, titulada "Pesticide expsoure and prostate cancer: A systematic review and meta-analysis", es expresión de la capacidad investigadora e interpretativa de su autora, en condiciones que la hace acreedora al título de Doctor, siempre que así lo considere el citado Tribunal.

Granada, 28 de Mayo de 2015-05-28



D. José Juan Jiménez Moleón

To

Basil & Angelina

The formulation of a problem is often more essential than its solution

Albert Einstein

Research is to see what everybody else has seen,

& to think what nobody else has thought

Albert Szent-Györgyi

The measure of greatness in a scientific idea is the extent to which

it stimulates thought and opens up new lines of research

Paul A.M. Dirac

To all the ***members of the Department of Preventive Medicine and Public Health***

.

To my very dear professors

To ***Dr. Aurora,*** *who has always helped and encouraged me.*

You are a real icon of hard work and character,

an example for success that shines on all those around you.

I will always put you as an example to follow.

To ***Dr. José-Juan****, who has taught me perseverance, patience and positivity.*

For three years, you have helped, advised, supported and encouraged me on a daily basis. I will never forget your solving each and every problem that came into my way.

Thank you for everything

You are a great professor that I will always remember.

To my best friends forever

To ***Mamen****, my beautiful & dear friend. Your courage & strong spirit is inspirational.*

Thanks for always being there for me.

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To ***Carmen****, a lovely person. Thanks for advising me.*

To ***Eladio****, thanks for making me laugh at times of trouble.*

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I am sure that wherever life will take me, I will leave part of me here, in Granada, in this department….

To all my family ….especially

*To my **father, Lewis Mikhael**, Thanks for teaching me that nothing is impossible.*

Your believing in me since I was a little girl has always given me strength.

Your hard working and successful nature has always inspired me.

*To **Basil** and **Angelina**, the light of my life and God's greatest blessing.*

You both have given me unconditional love and taught me happiness and contentment on a daily basis. You are my heroes, Thanks for supporting me in every step, enduring with me hard times and giving me a reason for going on.

I am proud of you.

*To my friend, soul mate, and my life's companion **Hani**, thank you for tolerating being miles away from me and the kids to give me the chance to follow my dreams.*

I will always appreciate this.

SUMMARY

Prostate Cancer is the second most common type of cancer and the fifth cause of cancer mortality in men worldwide. However, its etiology is still very unclear and established risk factors include only advancing age, having a positive family history of prostate cancer and ethnicity. In search for possible modifiable risk factors, many studies have examined the role of various environmental and occupational exposures.

Pesticide exposure has received much attention, as experimental studies had suggested carcinogenic potential of many pesticides types. On the other hand, a large number of pesticides have been postulated to possess endocrine disrupting properties, which make them of specific relevance to prostate cancer, being a hormone dependant malignancy. However, in spite of the availability of a large body of epidemiological literature relating farming and pesticide exposure to prostate cancer, results are controversial and inconsistent.

Accordingly, we have systematically reviewed available epidemiological literature relating pesticide exposure to prostate cancer in order to examine the hypothesis that farmers are at an increased risk of developing prostate cancer. We have also aimed to evaluate the potential association between different levels of pesticide exposure and prostate cancer, and to explore potential sources of heterogeneity between studies.

We searched PubMed, Web of Science and Scopus databases for case-control and cohort studies published from 1986 till 2015. We assessed the quality of the included articles using the Newcastle-Ottawa scale (NOS). Pooled estimates were calculated using

the random-effects model. Heterogeneity was explored using sub-set analyses, sensitivity analyses and meta-regression.

We have conducted three consecutive meta-analyses, the first included 14 case-control and 11 cohort studies relating farming to prostate cancer. For the cohort studies, pooled estimates showed high heterogeneity. Homogeneity was revealed by sensitivity analysis and the pooled estimate showed no association; 0.99, 95% CI 0.96-1.02, I^2 =22.3%, p=0.252. For the case-control studies, there was moderate heterogeneity which was explained by location of the studies, decades of prostate cancer diagnosis, and type of control population. A higher association was observed for studies conducted in the USA, for older studies where data was collected between late 70s and late 80s, as well as for studies that used cancer patients as controls. The repeatedly obtained pooled estimate showed consistently a weak yet statistically significant association. The pooled estimate from the sensitivity analysis was 1.26, 95% CI 1.19-1.33, I^2 = 0.00%, p = 0.570.

For the second meta-analysis, which included four cohort studies and 21 case-control studies that quantified pesticide exposure, there was no association between low exposure to pesticides and prostate cancer. However, association was weak but significant for high exposure, pooled OR 1.33, 95% CI 1.02-1.63, I^2 = 44.8%, p = 0.024. Heterogeneity was explained by a number of variables including the method used to assess pesticide exposure. Pooled OR was weak and non-significant for those having high serum levels of pesticides, 1.12(0.74-1.50), I^2 = 0.00%, p=0.966, while a high and significant association was detected for studies that applied grouped non-individualized

assessment of exposure, pooled OR was 2.24(1.36-3.11), I^2 = 0.00%, p=0.955. On the other hand, for studies that used self-reporting of pesticide exposure, pooled OR was 1.34(0.91-1.77), I^2=0.00%, p=0.493. Also, higher pooled estimates were observed for studies conducted in USA/Canada, those where the controls were cancer patients, and those having lower quality according to NOS criteria. Also, studies addressing pesticide exposed farmers and organochlorine pesticides showed higher pooled ORs. Nevertheless, an impact of the exposure assessment methodology and study quality was consistently observed. We have also noted an increased risk of prostate cancer for high exposure to pesticides among individuals with a positive family history of prostate cancer. Pooled OR was 2.23(1.05-3.41), I^2 = 0.00%, p=0.646.

We have conducted another meta-analysis to further examine the potential association between specific organochlorine pesticides and prostate cancer. Pooled estimates that we obtained for high exposure to DDT, DDE, hexachlorobenzene, oxychlordane and transnonachlor among the general population were weak and insignificant. On the other hand, pooled estimates for high occupational exposure to DDT, heptachlor and lindane showed a positive yet insignificant association.

Based on the available epidemiological literature, we conclude that there is no concrete evidence for associating exposure to specific pesticides to an increased prostate cancer risk. Although a weak yet significant association was observed for high exposure to pesticides among farmers, an impact of exposure assessment methodology and the quality of the studies was also observed. There are still gaps in the available

epidemiological data that makes the association unclear. These include deficiencies in exposure assessment methods and in selection of control populations, as well as lack of adjusting for important confounders including family history of prostate cancer and PSA testing variability.

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INTRODUCTION

I PROSTATE CANCER

1) Epidemiology of prostate cancer

Prostate cancer is the second most common cause of cancer worldwide. There are more than 1.1 million new cases every year, constituting 15% of cancer diagnosed in men. Prostate cancer is also the fifth leading cause of cancer mortality worldwide. More than 300,000 deaths have been recorded in 2012, which accounts for 6.6% of causes of death in men. (GLOBOCAN 2012) In Europe, North America, and some parts of Africa, it is considered the most common cancer in men. (Gronberg 2003) Moreover, according to the most recent study addressing international variations in incidence and mortality rates, there was an increased incidence of prostate cancer in nearly all the countries considered except for a few high income countries.(Center et al. 2012)

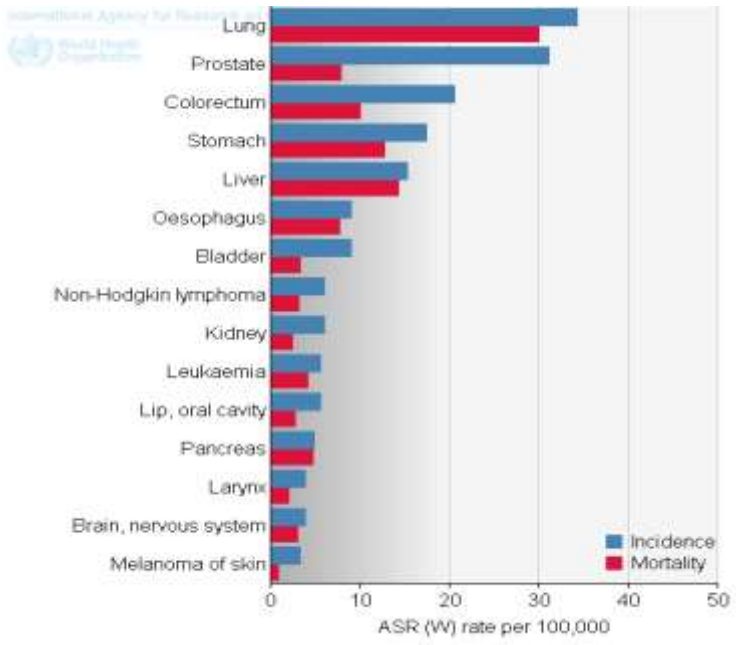


Figure 1: Estimated age-standardized incidence and mortality rates of different cancer types in men worldwide

(WHO, International Agency for Research on Cancer)

http://globocan.iarc.fr/Pages/fact_sheets_population.aspx

1. Incidence rates of prostate cancer

Prostate cancer is a worldwide health burden with an estimated age-standardized incidence rate of 31.1 per 100,000 men, according to the estimates provided by the International Agency of research on Cancer (IARC), as compiled in GLOBOCAN 2012. (GLOBOCAN 2012)

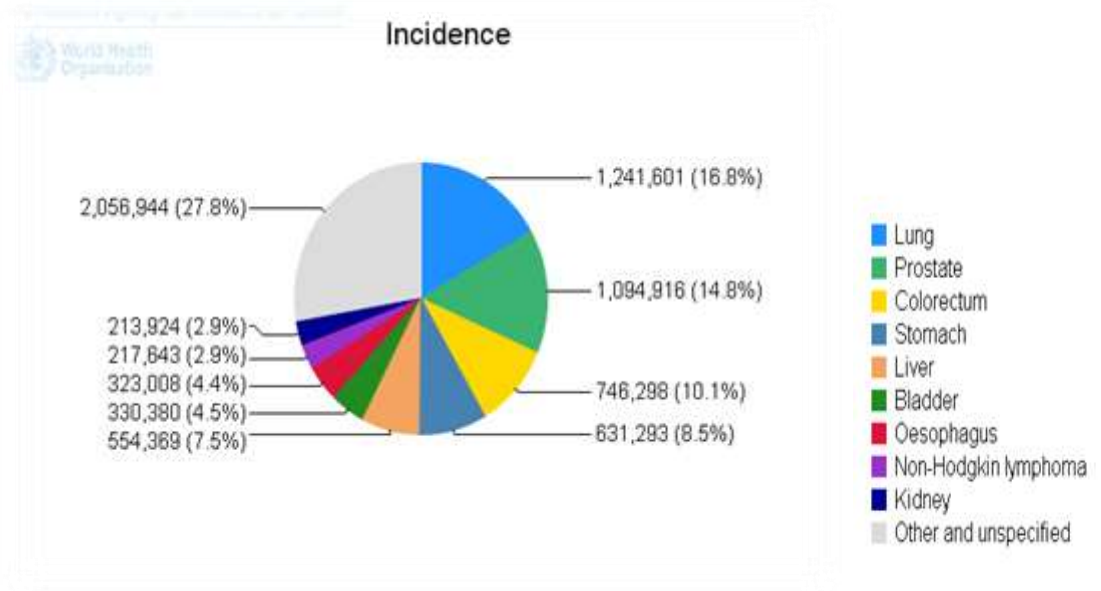


Figure 2. Incidence of prostate cancer worldwide in 2012 compared to other types of cancer in men

However, prostate cancer appears to impact the world's populations differently. Incidence rates vary by more than 25-fold internationally, with the highest rates found in Australia, New Zealand, Northern and Western Europe, and North America. On the other hand, the lowest rates are reported in southern and eastern Asia and North Africa.

This international variation for prostate cancer rates is mainly attributed to differences in detection practices. The widespread use of prostate-specific antigen (PSA) testing, routine histology from transurethral resections of the prostate, as well as sophisticated diagnostic methods have lead to the identification of early or latent cancers.(Kvale et al. 2007)

Furthermore, lifestyle and genetic factors have been suggested to explain some of the diversity in prostate cancer incidence rates. (Center et al. 2012) This diversity may also provide meaningful insights into the etiology of the disease and may help in generating new hypotheses for further research.(Hsing and Chokkalingam 2006)

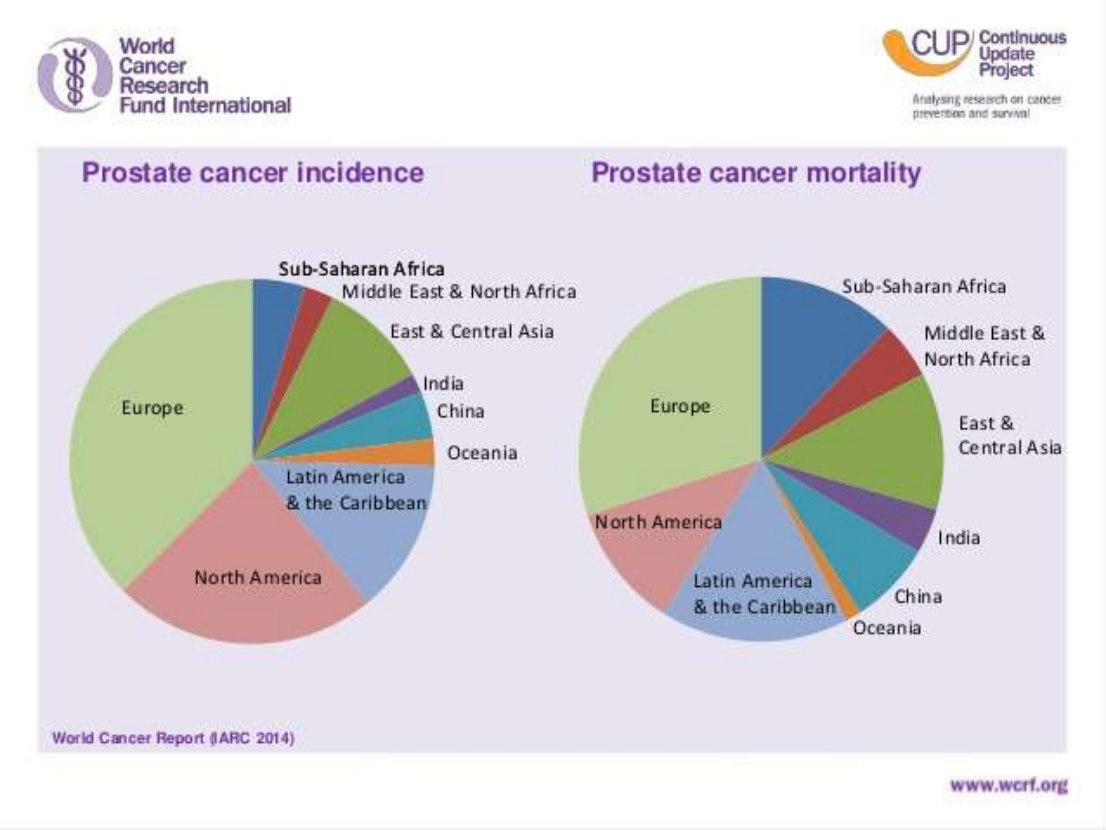


Figure 3. International variations in the incidence and mortality rates for prostate cancer (IARC 2014)

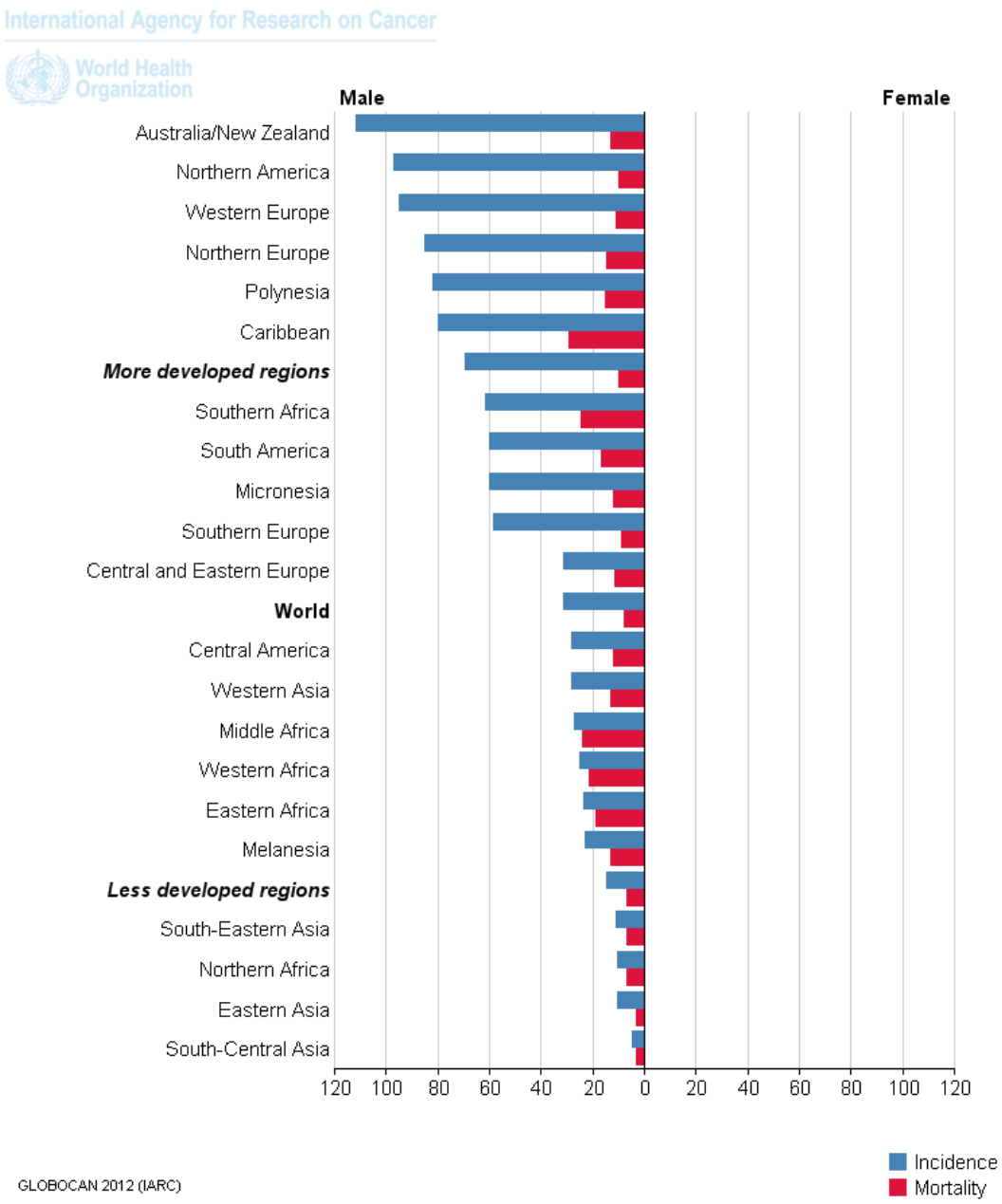


Figure 4: Estimated age-standardized incidence and mortality rates worldwide (per 100,000)

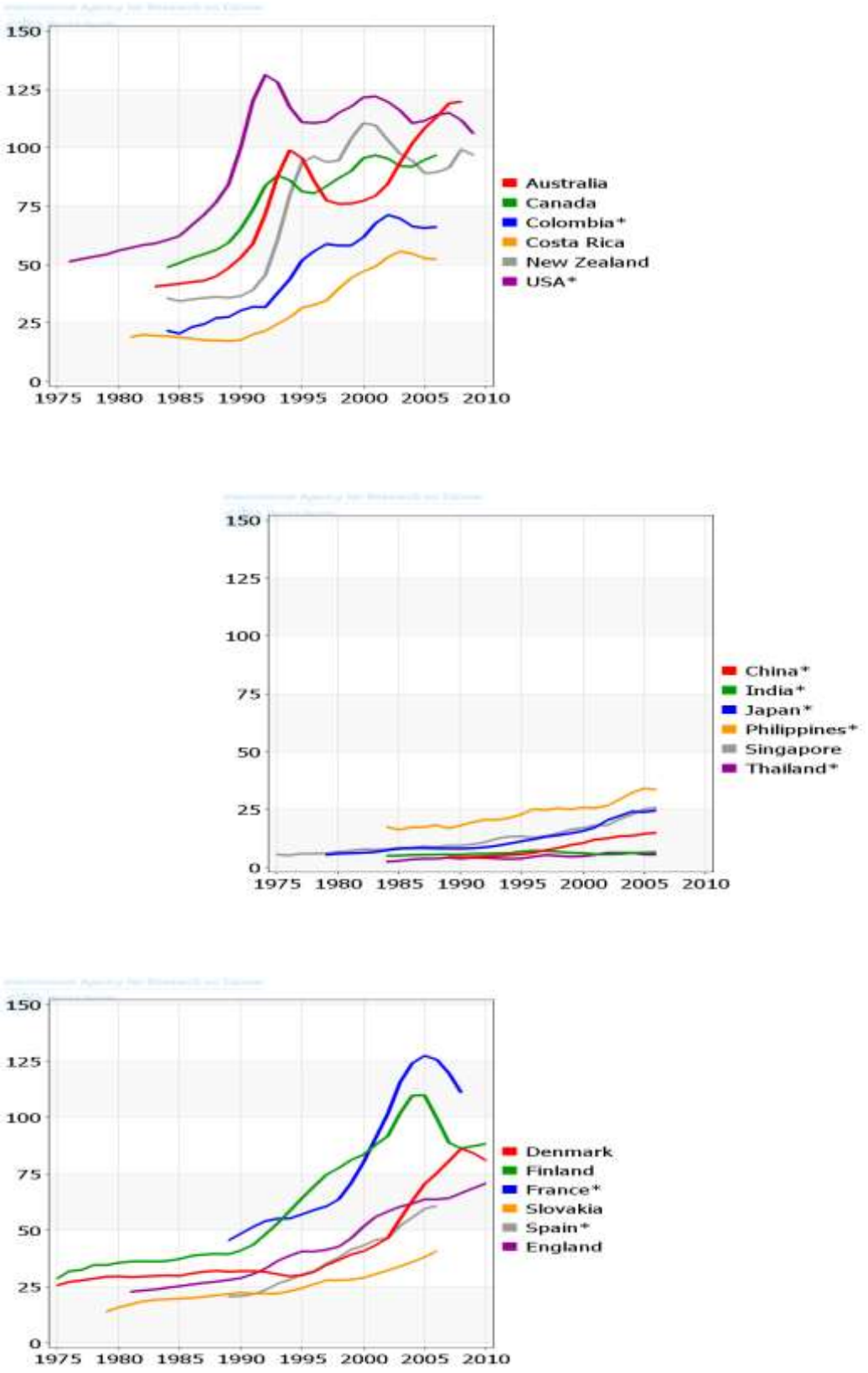


Figure 5: Trends in incidence of prostate cancer in selected countries: age-standardized rate (W) per 100,000 (IARC, * Regional data sources)

1.1. Incidence of prostate cancer in European Countries

In 2012, the incidence of prostate cancer in European countries was 64 cases per 100,000 adults, a total of 417,137 new cases (12.1% of the total incidence for all cancer types). http://www.cancerresearchuk.org/cancer-info/cancerstats/world/incidence

However, there is a notable variation between European countries. Comparison of prostate cancer incidence in 24 European countries revealed a fivefold variation in the age-adjusted rates, where the incidence is highest in Northern and Western Europe (> 200 per 100,000), and lowest in Eastern and Southern Europe.(Arnold et al. 2013)

Nevertheless, rates have shown a continuous increase in almost all of 37 studied European countries. (Bray et al. 2010) According to Great Britain statistics, prostate cancer incidence has almost tripled from 33 per 100,000 in 1975 to 97 per 100,000 in 2007. In Spain, for the same year, the estimated number of prostate cancer cases was 65.18 per 100,000 adults (an estimated 27,853 new cases).

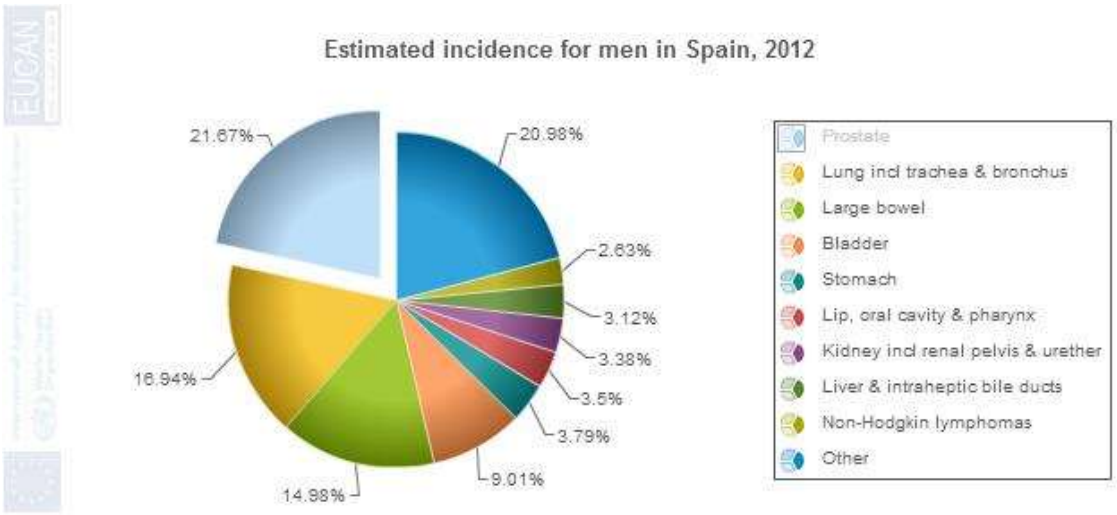


Figure 6: incidence of prostate cancer in Spain compared to other types of cancer in men 2012 (IARC/EUCAN) http://eco.iarc.fr/eucan

In a huge study that followed 15 million people in five Nordic countries, prostate cancer was the most common cancer in the study (340,000 prostate cancer cases) accounting for nearly 1/3 of all incident cancer among the studied population. (Pukkala et al. 2009)

1.2. Incidence of prostate cancer in USA

On the other hand, according to the National Cancer Institute, USA, the estimated number of age-adjusted new cases of prostate cancer was 147.8 per 100,000 men per year, while the estimated number of new cases in 2014 is 240,000 (14% of all new cancer cases). The lifetime risk of developing prostate cancer is high, as approximately 15 % of men will be diagnosed with prostate cancer at some point during their lifetime, based on 2009-2011 data. http://seer.cancer.gov/statfacts/html/prost.html

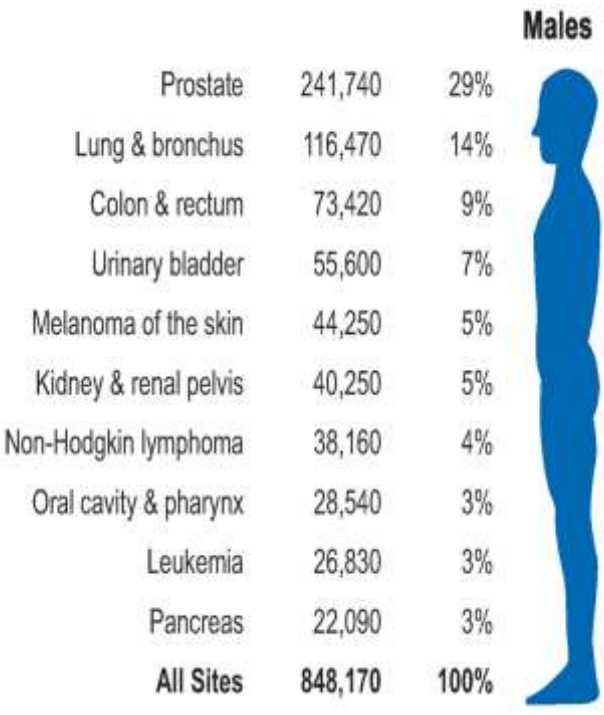


Figure 7. Estimated numbers of new cases of prostate cancer in comparison to other types of cancer (USA/ 2014)

1.3. Incidence of prostate cancer in less developed regions

In countries where no screening is available, information is sparse regarding the incidence and management of the disease. However, differences in prostate cancer diagnostic practices are most likely the greatest contributor to the variation in incidence rates worldwide. (Zlotta et al. 2013) Comparative geographic–pathologic studies suggest that genetic, epigenetic, and environmental factors may also be responsible for ethnic variations in the post induction progression of prostate cancer (Sim and Cheng 2005;Watanabe et al. 2000)

According to Globocan 2012, the incidence of prostate cancer in less developed regions is lower than that reported in developed countries. Prostate cancer comes fourth after lung, liver and colorectal cancer. In spite of that, it has to be noted that incidence of prostate cancer in less developed countries is also increasing rapidly. (Delongchamps et al. 2006;Gu 2000)

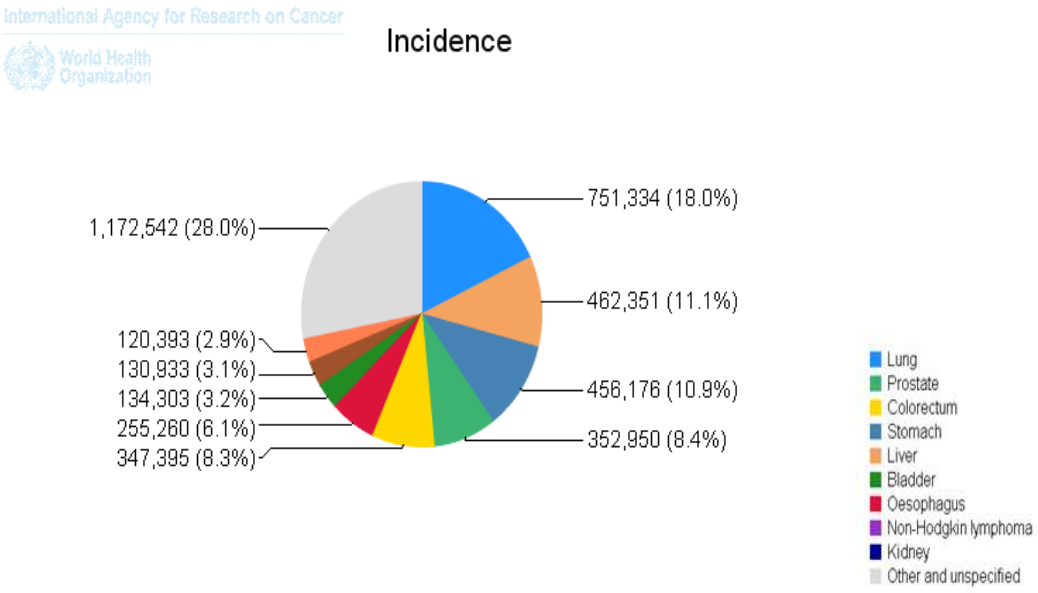


Figure 8. Incidence of prostate cancer in developing countries compared to other types of cancer in men

2. Prevalence rates of prostate cancer

The far-reaching effects of PSA screening in the nineties were a direct consequence of the large prevalence of the previously undiagnosed prostate cancer cases. (Jahn et al. 2014) However, traditional epidemiological studies are based on diagnosed cases, due to the fact that the asymptomatic cases are obtained from autopsy studies that detect latent prostate cancer.(Delongchamps et al. 2007) For that reason, the contemporary prevalence of latent prostate cancer globally is not well known. (Zlotta et al. 2013)

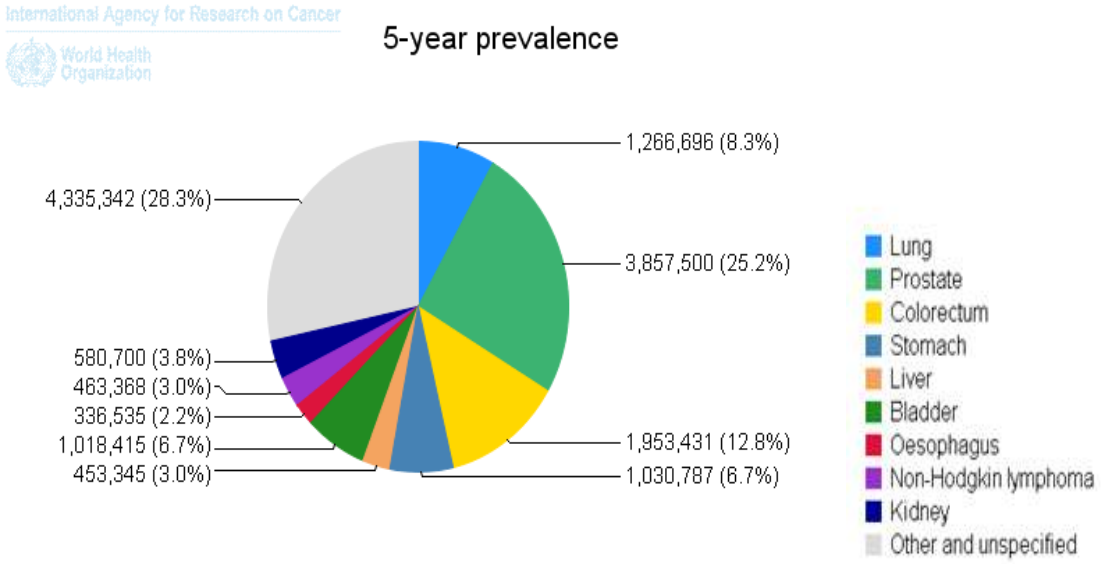


Figure 9. Five year prevalence of diagnosed prostate cancer worldwide in 2012 compared to other types of cancer in men

Nevertheless, based on autopsy studies, prostate cancer prevalence information according to age has been published by several authors. (Sakr et al. 1993;Sanchez-Chapado et al. 2003;Soos et al. 2005;Stamatiou et al. 2006;Yatani et al. 1988) As seen in table 1, microscopic foci of prostate cancer diagnosed by autopsy includes younger age groups than incidence data and most men in the older age groups were found to be affected.(Haas et al. 2008)

Table 1. Autopsy prevalence of prostate cancer in various parts of the world (Haas et al. 2008)

Age	US Whites	US Blacks	Japan	Spain	Greece	Hungary
21-30	8	8	0	4	0	0
31-40	31	31	20	9	0	27
41-50	37	43	13	14	3	20
51-60	44	46	22	24	5	28
61-70	65	70	35	32	14	44
71-80	83	81	41	33	31	58
81-90			48		40	73

2.1. Prevalence rate of prostate cancer in European countries

As seen in figure 10, there is an obvious variation in the 5 year-prevalence of diagnosed prostate cancer cases between European countries.

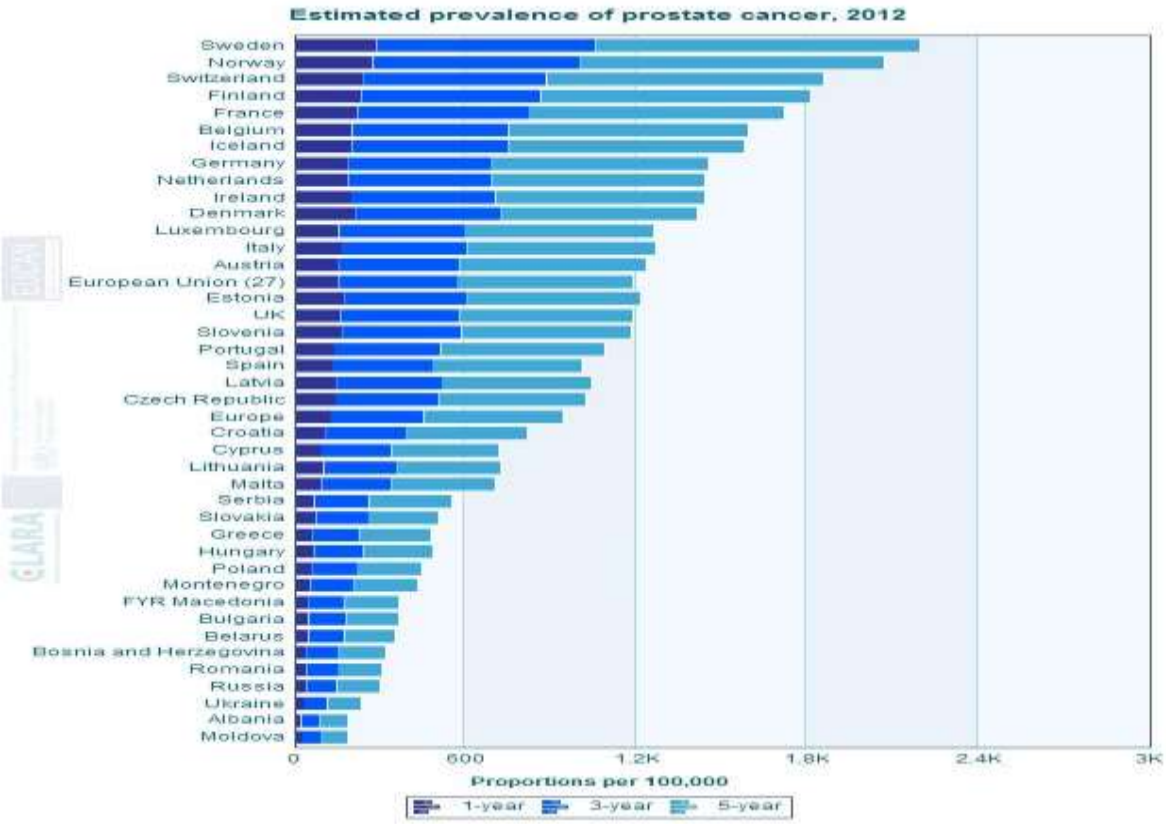


Figure 10. Estimated 5 year prevalence of prostate cancer in different European countries, 2012(IARC/EUCAN) http://eco.iarc.fr/eucan/

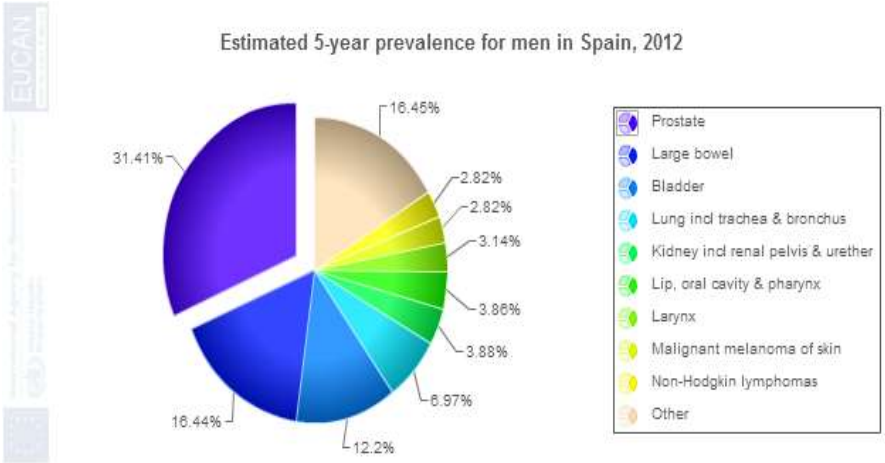


Figure 11. Estimated 5 year prevalence of prostate cancer in Spain compared to other cancer types in men, 2012(IARC/EUCAN), http://eco.iarc.fr/eucan/

2.2. Prevalence rate of prostate cancer in USA

The estimated number of men living with prostate cancer in 2011 was 2,707,821. According to Globocan 2012, the 5 year prevalence of prostate cancer in the USA constitutes about 40% of all cancer types diagnosed among men.

3. Mortality rates of prostate cancer

Prostate cancer is the fifth leading cause of cancer death worldwide, but there is a tenfold variability in mortality rates between different countries. (Bouchardy et al. 2008) It is the second most common cause of cancer death in Australia (2011)(Australian Institute of Health and Welfare. 2012), Britain (2011)(Office of National Statistics Release. 2011) and the United States (2009) (U.S.Cancer Statistics Working Group. 2013) ; and third most common in Canada (2008) (Canadian Cancer Society's Advisory Committee on Cancer Statistics. 2013), the European Union (2012) (Ferlay et al. 2013)and New Zealand (2010). (Ministry of Health. 2012)

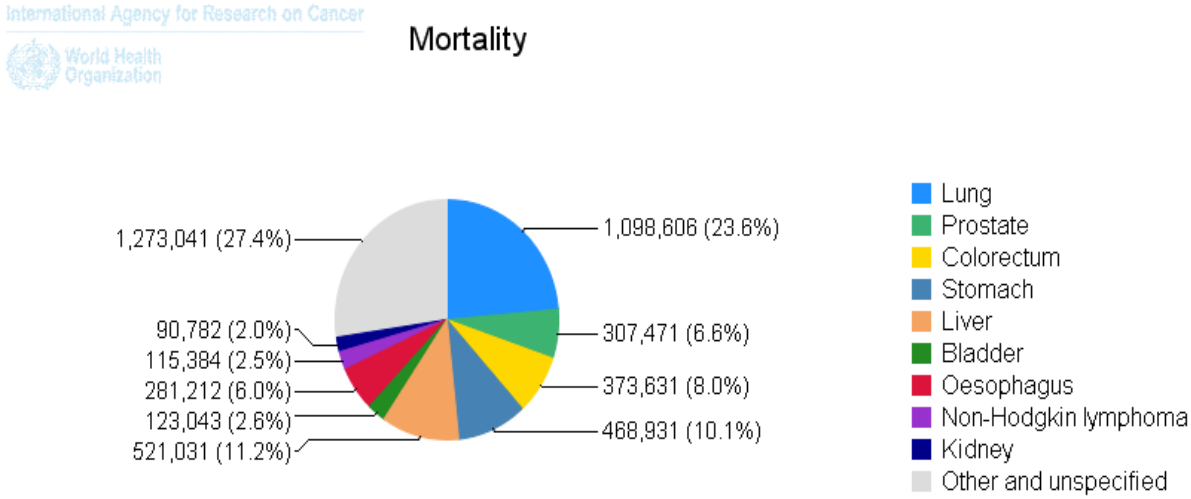


Figure 12. Mortality rates for different cancer types worldwide
http://globocan.iarc.fr/Pages/fact_sheets_population.aspx

Mortality rates due to prostate cancer tend to be highest in low-to middle-income settings including parts of South America, the Caribbean, and sub-Saharan Africa. (Center et al. 2012)

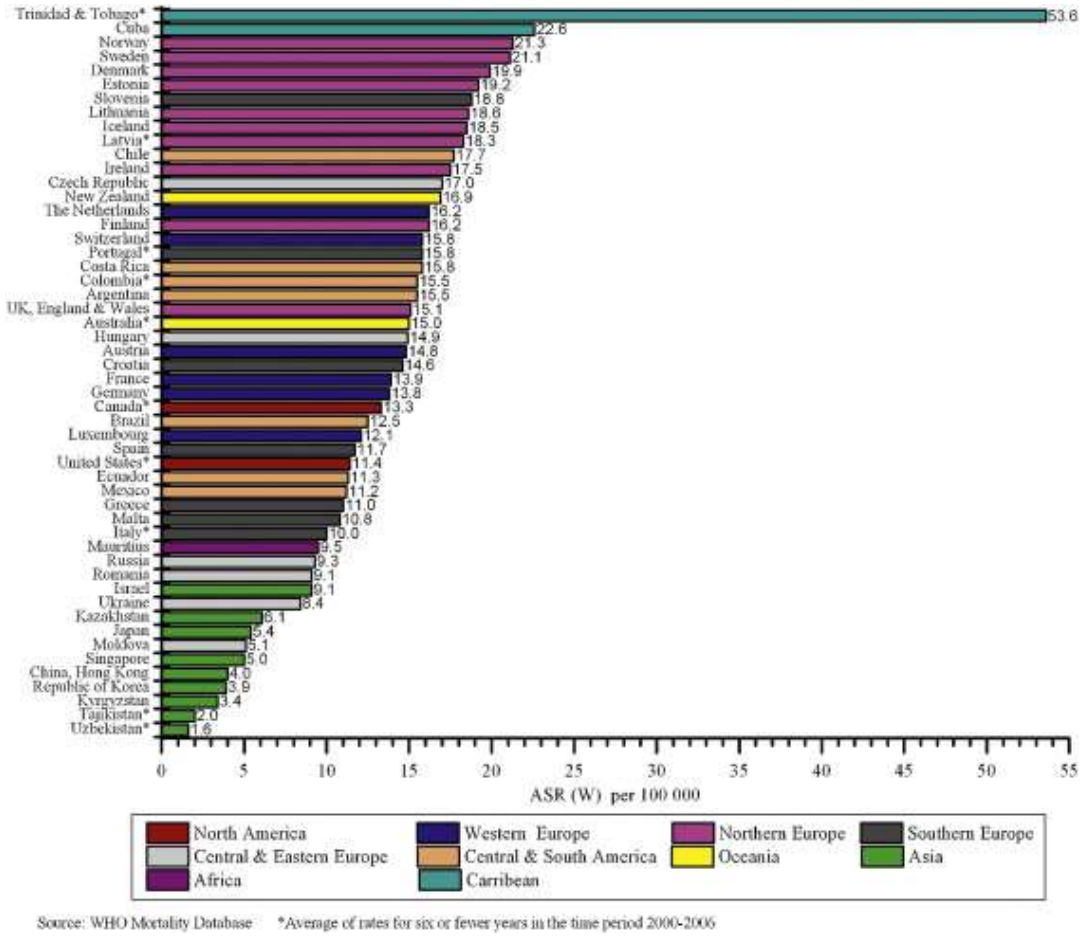


Figure 13. Prostate cancer mortality rates among a selected group of countries worldwide

Moreover, there are changing trends in mortality from prostate cancer. In several countries, mortality decreased to a level lower than before the introduction of PSA screening. These countries include USA, and to a lesser extent in Germany, Switzerland, Canada, France, Italy and Spain. In other countries, mortality from prostate cancer decreased but rates remain higher than before the introduction of PSA

screening. These include Australia, New Zealand, Austria, Finland, Netherlands, Norway, United Kingdom, Singapore, Sweden and Portugal. The third trend observed is a continued increase in mortality as in Belgium, Denmark, Greece, Ireland, Bulgaria, Czech Republic, Russian Federation, Romania, Poland, Argentina, Chile, Cuba, Mexico, Japan, China and Hong Kong. (Bouchardy et al. 2008)

3.1. Mortality rates of prostate cancer in European Countries

Almost 90,000 deaths from prostate cancer were estimated to have occurred in 2008 in Europe, ranking it the third most common cause of cancer death amongst men, after lung and colorectal cancers. The highest prostate cancer mortality rates are in the Baltic region (Estonia, Latvia and Lithuania) and in Denmark, Norway and Sweden. Prostate cancer mortality has been decreasing in 13 of the 37 European countries considered predominantly the higher-resource countries within each region. (Bray et al. 2010)

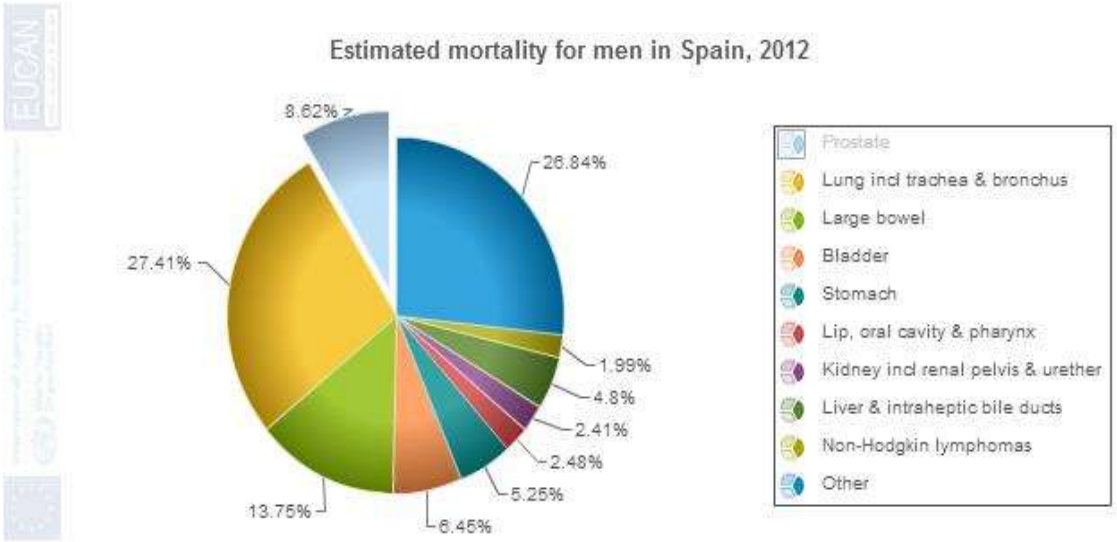


Figure 14. Mortality from prostate cancer in Spain compared to other cancer types in men. http://eco.iarc.fr/eucan/

3.2. Mortality rates of prostate cancer in USA

According to the National Cancer Institute, USA, the estimated number of deaths in 2014 is 29,480 which constitute 5% of all deaths due to cancer. Based on 2007-2011 data, the number of deaths was 22.3 per 100,000 men per year (age-adjusted).

Table 2. Estimated incidence, mortality and 5-year prevalence of different cancer types in men worldwide (http://globocan.iarc.fr/Pages/fact_sheets_population.aspx)

Cancer	Incidence			Mortality			5-year prevalence		
	Number	(%)	ASR	Number	(%)	ASR	Number	(%)	Prop.
Lip, oral cavity	198975	2.7	5.5	97919	2.1	2.7	467157	3.0	18.0
Nasopharynx	60896	0.8	1.7	35753	0.8	1.0	161899	1.1	6.2
Other pharynx	115131	1.6	3.2	77585	1.7	2.2	251118	1.6	9.7
Oesophagus	323008	4.3	9.0	281212	6.0	7.7	336535	2.2	13.0
Stomach	631293	8.5	17.4	468931	10.1	12.8	1030787	6.7	39.7
Colorectum	746298	10.0	20.6	373631	8.0	10.0	1953431	12.7	75.3
Liver	554369	7.5	15.3	521031	11.2	14.3	453345	3.0	17.5
Gallbladder	76844	1.0	2.1	60334	1.3	1.6	90368	0.6	3.5
Pancreas	178161	2.4	4.9	173812	3.7	4.8	114434	0.7	4.4
Larynx	138102	1.9	3.9	73261	1.6	2.0	388593	2.5	15.0
Lung	1241601	16.7	34.2	1098606	23.6	30.0	1266696	8.2	48.8
Melanoma of skin	120649	1.6	3.3	31393	0.7	0.9	452674	2.9	17.4
Kaposi sarcoma	29022	0.4	0.8	17358	0.4	0.5	55337	0.4	2.1
Prostate	**1111689**	**15.0**	**31.1**	**307471**	**6.6**	**7.8**	**3923668**	**25.5**	**151.2**
Testis	55266	0.7	1.5	10351	0.2	0.3	214666	1.4	8.3
Kidney	213924	2.9	6.0	90782	2.0	2.5	580700	3.8	22.4
Bladder	330380	4.4	9.0	123043	2.6	3.2	1018415	6.6	39.3
Brain, nervous system	139608	1.9	3.9	106379	2.3	3.0	190011	1.2	7.3
Thyroid	68179	0.9	1.9	12627	0.3	0.3	271270	1.8	10.4
Hodgkin lymphoma	38520	0.5	1.1	15464	0.3	0.4	108301	0.7	4.2
Non-Hodgkin lymphoma	217643	2.9	6.0	115384	2.5	3.2	463368	3.0	17.9
Multiple myeloma	62469	0.8	1.7	43094	0.9	1.2	124985	0.8	4.8
Leukaemia	200676	2.7	5.6	151317	3.3	4.2	284797	1.9	11.0
All cancers excl. non-melanoma skin	7427148	100.0	205.4	4653132	100.0	126.3	15362289	100.0	592.0

4. Survival rates of prostate cancer

Generally, prostate cancer has high survival rates, but death rates are higher in African Americans and depend mainly on the stage and age at diagnosis.

4.1. Survival rates of prostate cancer in European Countries

Overall, during the last decade, the 5-year relative survival percentages for prostate cancer steadily increased from 73.4% in 1999-2001 to 83.4% in 2005-2007. However, there is still a survival difference between men diagnosed in Eastern Europe and those in the rest of Europe. (De et al. 2014) A pooled analysis of 828 patients from 6 nonrandomized studies found that highly or moderately differentiated tumors yielded a 10-year disease-specific survival rate of 87%, but poorly differentiated tumors were associated with a 34% survival rate.(Chodak et al. 1994)

4.2. Survival rates of prostate cancer in USA

On the other hand, in USA, based on data from SEER for the years 2004-2010, relative 5 year survival of patients diagnosed with prostate cancer compared to the survival of people of the general population who are of the same age and race is 98.9%. However, survival depends primarily on the staging of prostate cancer which ranges from only 28% in case of distant metastasis to 100% for localized cases (Given that 81% of prostate cancer cases are localized, the high survival rate could be explained).

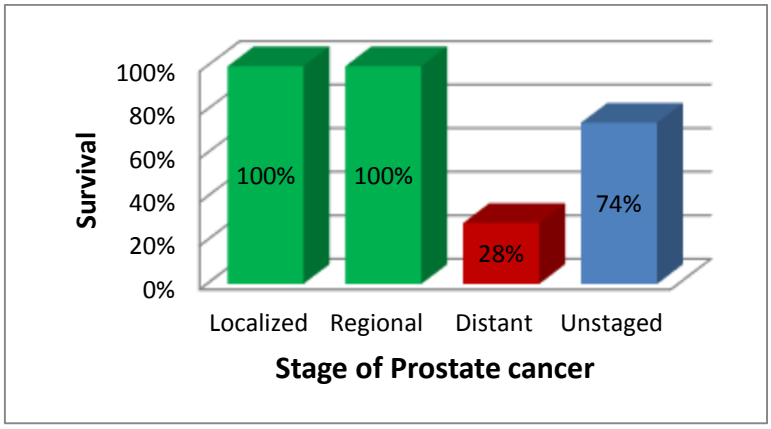


Figure 15. Survival rate of prostate cancer by stage at diagnosis

5. Economic Burden of prostate cancer

With the expected increases in the life expectancy of men and therefore the incidence of prostate cancer, the disease's economic burden is also expected to increase substantially. It is estimated that the total economic costs of prostate cancer in Europe exceed € 8.43 billion with a high proportion of the costs of prostate cancer care occurring in the first year after diagnosis. In European countries with available data (UK, Germany, France, Italy, Spain, the Netherlands), this amounted to € 106.7-179.0 million for all prostate cancer patients diagnosed in 2006.(Luengo-Fernandez et al. 2013)

2) Etiology of prostate cancer

Despite of the high morbidity of prostate cancer, its etiology remains obscure. The only established risk factors are increasing age, race and positive family history of prostate cancer.

1. Established risk factors of prostate cancer

1.1. Age

The disease primarily affects older men, with a median age at diagnosis around age 65-74 years. For that, it is a major health concern in developed countries with their greater proportion of elderly men in the general population. (European Association of Urology, Guidelines, 2014 edition) The incidence of prostate cancer increases exponentially with advancing age - an increase that is faster than that for any other malignancy. It is to be noted that occurrence of prostate cancer among those with a family history is mostly at a younger age. (Saarimaki et al. 2015)

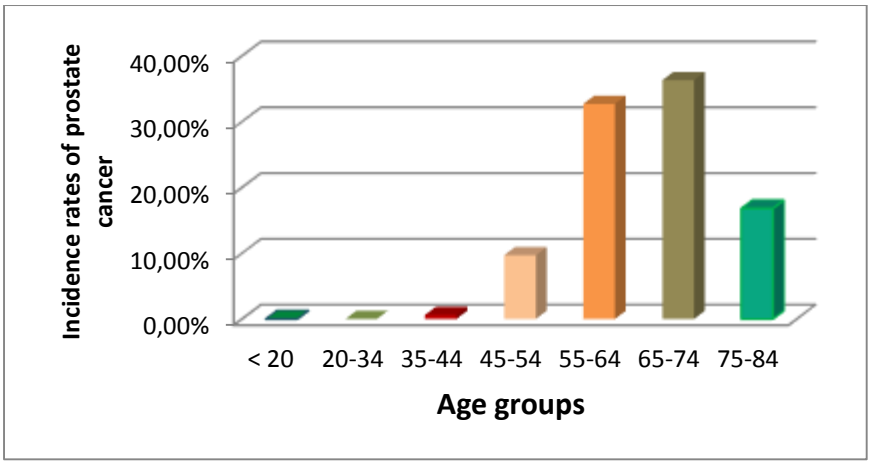


Figure 16. Percent of new cases of prostate cancer by age group

1. 2. Family History

Apart from age and ethnicity, family history is the strongest risk factor of prostate cancer. (Damber and Aus 2008) Hereditary factors contribute to 5 to 10% of all cases of prostate cancer and their effect on early onset cases is notably greater, reaching to 30 to 40%. (Saarimaki et al. 2015)

A recent meta-analysis reported a strong familial clustering of prostate cancer with a pooled rate ratio for first degree family history equal 2.48, 95% CI, 2.25 to 2.74 and an attributable fraction among those having an affected first-degree relative equals to 59.7%, 95% CI, 55.6 to 63.5% for men at all ages and 65.2%, 95% CI, 57.7–71.4% for men younger than 65. (Kicinski et al. 2011)

In the year 2000, a study published in the New England Journal of Medicine that included 44,788 pairs of twins from Sweden, Denmark and Finland, found that 42 percent of prostate cancer cases were attributed to inheritance. (Lichtenstein et al. 2000) Another large population based study including 8,148,737 individuals evaluated the impact of familial cancer and found the highest population attributable fraction (PAF) for all types of cancers to be for prostate cancer (13.94%). (Frank et al. 2014)

An obvious reason for this aggregation is inheritance of genes that cause prostate cancer, some of which show high penetrance, whereas other genes show polymorphism and low penetrance. The first gene locus identified was named hereditary prostate cancer locus-1 (HPC1). (Damber and Aus 2008)

Other genetic variants include variants in the tumor suppressor gene BRCA2, the DNA repair genes PALB2, BRIP1, CHEK2, NBS1 and the transcription factor gene HOXB13, all of which appear to confer a "moderate" excess risk of prostate cancer. Even more, 76 common variants that confer a "small" excess risk have been identified by genome-wide association studies. Thus far, approximately 30 % of the familial risk of prostate cancer has been explained. (Eeles et al. 2014)

A recent study has also confirmed the role of previously reported prostate cancer associated single nucleotide polymorphism (SNPs) for the familial disease, after analyzing 9560 prostate cancer cases.(Teerlink et al. 2014)

1.3. Ethnicity

There is a wealth of evidence demonstrating significant ethnic differences in incidence and mortality rates of prostate cancer. The obvious disparity between African American men and other ethnicities for prostate cancer incidence is particularly striking, as it reached 223.9/100,000 among this particular ethnic group (years 2007-2011). (SEER Stat Fact Sheets 2015)

On the other hand, for mortality rates, the Caribbean population has shown the highest rates in the world (26.3 per 100,000) closely followed by sub-Saharan Africans (10 per 100,000), whereas Asians have the lowest mortality rates worldwide (2.5 per 100,000). (Ferlay et al. 2010;Gronberg 2003;Jones and Chinegwundoh 2014)

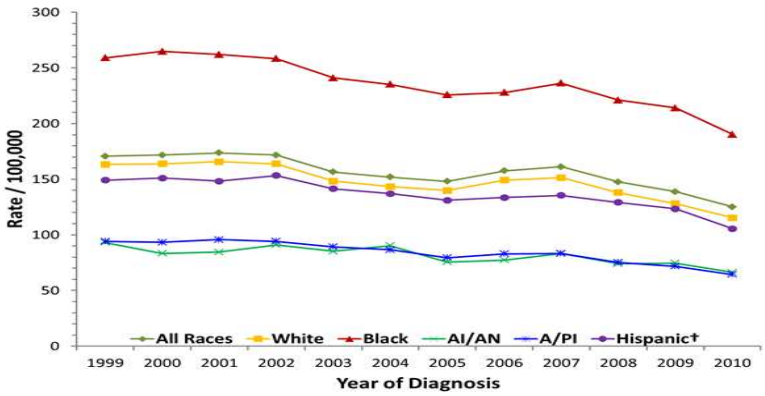


Figure 17. Different incidence rates for different ethnic groups in USA.

(AI/AN= American Indian/ Alaska natives, A/PI= Asian/Pacific Islanders)

In spite of the fact that there is a wide variability in the accuracy of reporting and registration, and obvious disparities in the access to and the quality of the health care systems in different countries, they cannot solely explain the observed substantial differences between different populations. (Jones and Chinegwundoh 2014)

More importantly is that the high prostate cancer incidence and mortality in black Americans are not specific to the United States. Indeed, many reports on prostate cancer incidence in African migrants or descendants from around the world show the same results, suggesting therefore a common genetic susceptibility to prostate cancer development, independently to any other factors.(Delongchamps et al. 2007)

Furthermore, in a recent review that included 19 studies of prostate cancer discovered at autopsy among 6,024 men, authors observed pronounced racial differences: in men 70 to 79 years, the prevalence was 50.5% in United States Blacks, 35.7% of whites, and 21.2% in Asian autopsies. These trends parallel observed incidence rates by race, suggesting that racial discrepancies in prostate cancer incidence are not solely due to different PSA screening patterns.(Jahn et al. 2014)

1.4. Certain genetic variants

During the past decade, molecular studies have provided unexpected clues as to how prostate cancer arises and progresses. There has also been tremendous achievements in identification and characterization of genes associated with inherited susceptibility to prostate cancer.(Nelson et al. 2003)

The role of genes started to emerge after observing of clustering of familial cases. The first gene locus identified was named hereditary prostate cancer locus-1 (HPC1). (Smith et al. 1996) Since this discovery of a genetic link, several other candidate genes have been identified. A recent meta-analysis including 57 studies concluded that men with GSTM1, GSTT1, GSTP1 and A131G polymorphism are associated with high risks of prostate cancer. (Gong et al. 2012) Lately, the role of gene environment interaction has been highlighted in a number of studies and different mechanisms by which carcinogens may interact with host-related gene polymorphism have been suggested. (Koutros et al. 2010;Tabrez et al. 2014)

2. Potential risk factors of prostate cancer

2.1. Occupational and environmental exposures

In search for the ambiguous etiology of prostate cancer, many epidemiological studies have examined the potential role of various environmental exposures. Observing the wide differences in the occurrence of prostate cancer between populations was considered to be a reflection of an important role of environmental risk factors. Some authors suggested that screening practice differences alone are unlikely to explain the nearly substantial difference in prostate cancer risk between high- and low-risk populations. (Hsing and Chokkalingam 2006)

Migration studies have shown that when Asian men (who have the lowest incidence of prostate cancer worldwide) move from their home lands to USA, incidence of prostate cancer among these people increase, implicating the role of environmental and life-style related factors.(Gomez et al. 2013;Lee et al. 2007)

On the other hand, occupational settings have provided the chance to investigate the role of higher levels of exposures. Different types of at job exposures have been proposed to contribute to prostate cancer carcinogenesis. These include arsenic, cadmium, rubber, diesel engine emissions, gasoline, metallic dust, metal working fluids, polycyclic aromatic hydrocarbons, and solvents. However, there is marked inconsistency in the epidemiological data and results remain inconclusive. (Bates 2007;Brown and Delzell 2000;Christensen et al. 2013;de et al. 2009;Hesterberg et al. 2005;Huff et al. 2007;Mirer 2003) In most cases, hypotheses were built on mechanistic data showing tumor promotion due to modifications of cell replication and apoptosis,

mediated through genes and epigenetic mechanisms. (Benbrahim-Tallaa and Waalkes 2008;Hartwig 2013;Treas et al. 2013;Waalkes 2003) Pesticide exposure is of particular interest and therefore will be displayed later in more details.

Excess risk of prostate cancer has been reported among several occupational groups including farmers, metal workers, mechanics and nuclear industry workers (Alavanja et al. 2003;Blair et al. 1992;Dich and Wiklund 1998;Sharma-Wagner et al. 2000), but still there is controversy among published epidemiological data.

2.2. Other factors that have been related to prostate cancer

In spite of many years of rigorous search for possible risk factors of prostate cancer, no convincing results have been obtained. Lifestyle risk factors including diet, physical activity, sexual factors, smoking, alcohol consumption, obesity, as well as other pathological conditions like inflammation, diabetes mellitus, and sexually transmitted diseases have been implicated, but their definite roles in the etiology of prostate cancer remain unclear.

Diet has been extensively studied with respect to prostate cancer risk, with largely inconsistent findings other than inverse associations with lycopene and selenium and a positive association with calcium. However, none of these associations could be accepted as causal. (World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) 2007)

Relating heavy ***alcohol consumption*** or ***tobacco smoking*** to an increased prostate cancer risk is still questionable.(Huncharek et al. 2010;Rota et al. 2012) On the other hand, the role of ***physical activity, adiposity*****, and** ***levels of insulin and insulin-like***

growth factors in prostate cancer development has also been widely studied, but with inconclusive results. (Meyerhardt et al. 2010;Norman et al. 2002)

Many aspects of ***sexual behavior*** have been related to prostate cancer including sexual activity (Spence et al. 2014) and sexually transmitted diseases (Caini et al. 2014;Huang et al. 2008;Strickler and Goedert 2001) but results have also been inconsistent. Very limited evidence is reported for less studied factors as ultra violet exposure, (Yu et al. 2014) marital status, anthropometry, circumcision (Krause et al. 2006) and vasectomy.(Dennis et al. 2002;Kohler et al. 2009)

Uncontrolled confounding by these un-established risk factors is not likely to be a major source of bias. Instead, potential differences in diagnostic intensity are of greater concern in most epidemiologic studies of prostate cancer risk.(Chang et al. 2014) What is more conclusive is that the pathogenesis of prostate cancer reflects complex interactions between environmental and genetic factors. This has been displayed in a number of recent studies that detected various genetic variants modifying pesticides associations with prostate cancer risk. (Gong et al. 2012;Karami et al. 2013;Koutros et al. 2011;Kumar et al. 2010;Tabrez et al. 2014)

II PESTICIDES: Carcinogenicity and Endocrine Disrupting Potential

Pesticides constitute a diverse class of xenobiotics that are extensively used for the protection of crops and for increasing the yield of agricultural products. Pesticides include various classes notably organochlorines, organophosphates, carbamates and pyrethroids.

Exposure to pesticides occurs as a result of occupational as well as environmental factors such as contamination of drinking water and food and may elicit various effects on human health. (Vakonaki et al. 2013)

Evaluating the effect of pesticide exposure on prostate cancer development has lately received much attention which is attributed to two important characteristics; the postulated carcinogenic effects of many types of pesticides as well as their potential for endocrine disruption.

1) Pesticides as carcinogens

Several carcinogenic mechanisms including genetic, epigenetic modification as well as oxidative stress have been demonstrated for a number of pesticides. (Collotta et al. 2013) These effects have been associated with de-regulated oncogenes and tumor suppressor genes. *Oncogenes* control mainly cellular proliferation and are either mutated or expressed at higher levels than normal in cancer cells. *Tumor suppressor genes* are genes that protect the integrity of the genome by inhibiting the cell cycle when substantial errors or mutations have occurred and are usually down-regulated or inactivated in cancer cells. *Oncogenes* encode proteins that are involved in signal transduction from the extracellular environment and the cytoplasmic region toward

the nucleus, where transcription is initiated. These proteins include growth factor receptors, cytoplasmic proteins involved in signal transduction or cellular proliferation, and transcription factors regulating the transcription of certain genes. Tumor suppressor genes encode for proteins mainly involved in cell cycle arrest or apoptosis. Environmental exposures including pesticides have been found to cause mutations in the coding regions of *Oncogenes* and *tumor suppressor genes*, as well as direct effect on the genome. (Vakonaki et al. 2013)

Mainly on the basis of experimental data from animal and cell line studies, several pesticides have been classified as class 2B carcinogens.(Rogan and Chen 2005) (N.B. There are four groups that define the carcinogenicity of a chemical: group 1 refers to compounds that are definitely carcinogenic to humans, group 2A refers to compounds that are probably carcinogenic to humans, while class 2B refers to substances that are possibly carcinogenic, group 3 are those not classifiable, whereas group 4 includes compounds that are probably not carcinogenic to humans). (IARC Monographs 2012)

Most studies have focused on the carcinogenicity caused by organochlorine pesticides in animal and in vitro models.(Mladinic et al. 2012) For example, the pesticide beta-hexachlorocyclohexane (β-HCH), a contaminant of the pesticide lindane has been reported to increase mRNA expression of MMP-13, a marker of invasiveness in vitro, and the expression of a number of proto-oncogenes (normal gene which, when altered by mutation, becomes an oncogene).(Wong and Matsumura 2007)

The structurally similar compound hexachlorobenzene (HCB) is known to cause liver tumors in animals through a mechanism involving activation of proto-oncogenic proteins.(Randi et al. 2003) The organochlorine pesticide methoxychlor has been found to increase the rate of ovarian atresia in mice (Borgeest et al., 2004).

Organochlorine pesticides as HCB, DDT and heptachlor epoxide have also been found to activate erbB2 kinase in prostate cancer cell lines (Over expression of erbB2 occurs in some types of cancer including prostate cancer). (Tessier and Matsumura 2001)

Evidence regarding the carcinogenic effect of other classes than organochlorines is limited. However, some carcinogenic mechanisms have been displayed for diazinon, a common organophosphate that has been associated with many cancer types. Experimental evidence indicates that diazinon modifies gene promoter DNA methylation levels, which may play a pathological role in cancer development. (Zhang et al. 2012) Figure 18 outlines a scheme underlying possible mutagenic effects of pesticides on oncogenes and tumour suppressors.(Vakonaki et al. 2013)

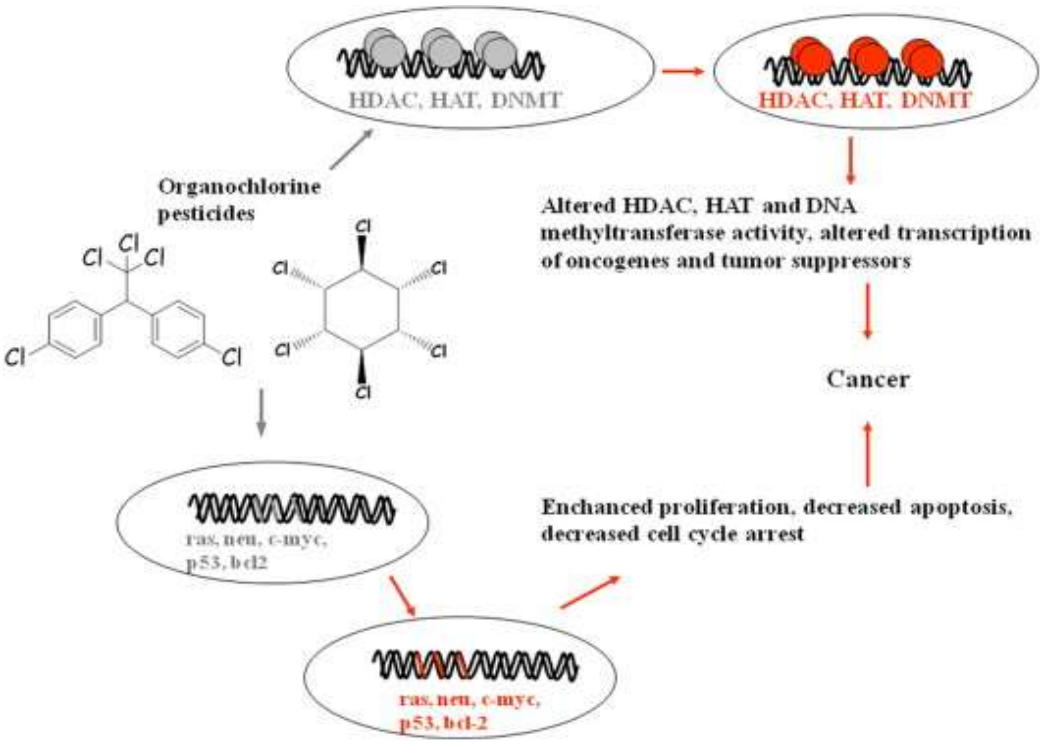


Figure 18. The effect of organochlorine pesticides on the genome and the epigenome. (Vakonaki et al, 2013)

Derivatives of lindane and methoxychlor can induce genetic alterations in the structural integrity of oncogenes and tumor suppressor genes. Red stripes indicate mutations occurring after pesticide exposure. In addition pesticides can promote enhanced or decreased expression of epigenetic enzymes such as histone deacetylases, Histone acetyltransferases and DNA methyltransferases and in turn regulate the transcription of genes involved in cell cycle control and apoptosis. Red circles indicate altered epigenetic regulation of oncogenes and tumor suppressor genes

2) Pesticides as potential endocrine disruptors

Endocrine disruptors are defined as exogenous chemical agents that interfere with the synthesis, secretion, transport, binding, transfer, action or elimination of natural hormones (US Environmental Protection Agency 1997). During the last century, many endocrine disrupting compounds have been synthesized and released into the environment. Among the most important of which are a large group of pesticides, where up to 105 different pesticides have been identified as endocrine disruptors.(Mnif et al. 2011;US Environmental Protection Agency 2012)

Pesticides, as endocrine disruptors, exert their effects by mimicking or antagonizing the effect of endogenous hormones and/or disrupting the synthesis and metabolism of endogenous hormones or hormone receptors. (Sonnenschein and Soto 1998) All of these mechanisms have been postulated to interfere with the normal levels of circulating hormones. (Mnif et al. 2011)

Examples of endocrine disrupting mechanisms of some pesticide types

DDT and its metabolite DDE exhibit hormonal activity through mechanisms involving the steroidogenic pathway, receptor mediated changes in protein synthesis or anti-androgenic and estrogenic actions. (Mrema et al. 2013) High concentrations of *p*,*p*-DDE has been found to function as an inhibitor of 5α-reductase, the enzyme that converts testosterone to dihydro-testosterone (DHT) (Androutsopoulos et al. 2013;Luccio-Camelo and Prins 2011) Other organochlorine pesticides such as lindane may act as androgens antagonist due to inhibition of DHT binding to androgen receptors.(Androutsopoulos et al. 2013) Diverse endocrine disrupting mechanisms for

a large list of pesticides have been displayed in details elsewhere. (McKinlay et al. 2008;Mnif et al. 2011)

III ASSOCIATING PESTICIDE EXPOSURE TO PROSTATE CANCER

1) Pathophysiological background of prostate cancer

Experimental evidence has indicated the role of testosterone in the development of the normal prostate gland. (Crawford 2009) However, the relationship between testosterone levels and the rate of cell proliferation in the prostate is controversial, and epidemiological studies of circulating testosterone levels and prostate cancer risk yielded conflicting results. (Isbarn et al. 2009;Morgentaler 2006)

Nevertheless, it has long been established that the active metabolite DHT controls mitotic activity in the prostate. This takes place by binding to the androgen receptor and being then trans-located to the nucleus of prostate cells for DNA binding and activation of androgen responsive genes. The androgen receptor is crucial for androgen activity in the prostate and it has even been suggested that the frequency of androgen receptor gene polymorphisms vary between populations consistently to variations in prostate cancer incidence. Also, the levels of 5α reductase that is responsible for transforming testosterone into the active DHT was found to be different in various ethnic groups and greater among groups with greater rates of prostate cancer. (Crawford 2009;Ross et al. 1992) Nonetheless, in recent years, many authors have been consistently against supporting a direct effect of androgens on prostate cancer development and opposed the longstanding androgen hypothesis. (Morgentaler 2014)

On the other hand, the role of estrogen in the normal development of the prostate gland has emerged only lately after it has been found that prostate tissue is a target for direct regulation by estrogens.(Coffey 2001;Harkonen and Makela 2004;Williams 2010) After a substantial amount of studies, it became clearer that estrogen markedly synergizes androgen effects, and this induces more than fourfold increase in the total prostate weight and DNA content. This enhancement of prostate growth requires the specific combination of estrogens with activated testosterone; DHT. Changing of estrogen levels that occurs with aging is believed to be responsible for promoting abnormal prostatic growth.(Coffey 2001)

Accordingly, the role of estrogen in the development of prostate cancer has received much attention. (Soronen et al. 2004;Williams 2010) It has been postulated by animal studies that estrogens are involved in local cell proliferation and prostate carcinogenesis in a manner analogous, yet different, to that of androgens.(Risbridger et al. 2007) but whether this is directly applicable to the human prostate had not been clarified.

Prostate gland has been found to possess estrogen receptors, ER α and ER β. (Harkonen and Makela 2004) As reported by experimental studies, intracellular estrogen directly induces the proliferation of aberrant prostatic basal cell hyperplasia to promote the formation of squamous metaplasia.(Risbridger et al. 2007) It was also found that ER-α activation is essential for prostate cancer development while a reduction of ER-β has been noted in prostate cancer. (Bardin et al. 2004;Ellem and Risbridger 2007) The ratio between free testosterone and free oestradiol has been postulated to determine the degree of ER-α stimulation.(Prins and Korach 2008)

2) Demonstrated effects of pesticides on prostate cancer cells from experimental studies

As a result of the postulated role of estrogens in the pathogenesis of prostate cancer as previously discussed and the endocrine disrupting potential demonstrated for many pesticides types, linking pesticide exposure to prostate cancer has received much attention. However, in spite of the huge amount of experimental studies about the possible effects of pesticides as carcinogens and endocrine disruptors, only few studies have demonstrated a direct effect on the prostate gland in particular. (Hu et al. 2012) Some authors still believe that the exact mechanisms of action of a number of pesticides remain largely obscure. (Wang et al. 2010)

Notwithstanding, very recent studies have shown that the pesticide DDE activates tumor-derived AR mutants, frequently present in prostate cancer tumors. The authors thus concluded that exposure to relevant doses of this pesticide can promote cellular proliferation in tumor cells expressing mutant AR. (Androutsopoulos et al. 2013;Shah et al. 2008)

A possible gene-environment interaction has also been hypothesized and a role of susceptibility genes has been demonstrated to be involved in the prostate cancer-pesticide association. (Koutros et al. 2010;Koutros et al. 2011;Tabrez et al. 2014) Another important point that has been reported in a number of studies is the heightened sensitivity of the prostate gland to the endocrine disruptors during the critical developmental windows. Thus, infants and children may be considered a highly susceptible population for endocrine disrupting exposures.(De and van 2012;Prins 2008)

3) Epidemiological studies assessing the association between pesticide exposure and prostate cancer

Available epidemiological studies comprise different designs, various exposed populations and locations, discrete types of studied pesticides and numerous methodologies applied for assessment of pesticide exposure. Moreover, inconsistentency of reported results is an obvious feature of the epidemiological literature.

The potential association between pesticide exposure and prostate cancer has been handled in three distinct ways: (1) referring to populations known to be exposed to pesticides, where authors hypothesize that farming is a surrogate of pesticide exposure and therefore assume that an increased risk of prostate cancer among farmers may indirectly point to a potential association between pesticide exposure and prostate cancer; (2) examining the association between exposure to overall use of pesticides or (3) more specifically assessing the exposure to a particular type of pesticide.

Blair A et al, in the late eighties, was the first to highlight an increased risk of prostate cancer among farmers compared to the general population. This was interesting as the authors also observed a lower incidence of the majority of the studied cancer types among this particular group of workers. (Blair et al. 1985) This was consistently reported in a number of consequent studies. (Alavanja et al. 2005;Dich and Wiklund 1998) In spite of the fact that there was no assessment of potential exposures, the authors hypothesized that the exposure to hormonally active chemicals was responsible for the increased risk.

Since that date, authors have attempted to identify specific occupational exposures that might account for the cancer patterns observed among farmers. With further understanding of the pathophysiological mechanisms underlying prostate cancer, and the possible mechanisms by which certain types of pesticides may act as endocrine disruptors, studying specific types of pesticides has replaced the older attitude of generalization in assessing exposure to pesticides.(Koutros et al. 2008;Mahajan et al. 2007;Multigner et al. 2010)

In the year 1993, a large prospective cohort study, the Agricultural Health Study (AHS), started which is a collaborative effort between the National Cancer institute, National Institute of Environmental Health Sciences and Environmental Protection Agency. (Agricultural Health Study 2015)This huge study included more than 55,000 pesticide applicators in North Carolina and Iowa. Exposure to a substantial number of pesticide types have been evaluated, and positive associations with prostate cancer risk have been reported for a specific number of studied pesticides, especially among individuals that had a history of family history of prostate cancer. (Alavanja et al.2003;Christensen et al. 2010;Koutros et al. 2013;Mahajan et al. 2006)

Moreover, many case control studies have examined if pesticide exposure could be related to an increased risk of prostate cancer, but results were controversial. A two-fold excess prostate cancer risk among United States farmers was restricted to short-term workers and workers in crop production, and it was not limited to those who began farming after the widespread introduction of pesticides use.(Krstev et al. 1998) Also, Dutch farm laborers who worked between 1960 and 1970 had a significant

increase in prostate cancer risk, and they significantly differed from workers who had sprayed pesticides more days per year. (Van Der Gulden et al. 1995)

Farming employment was the most prominent positive association in another United States case-control study, where patients affected by benign prostate hyperplasia were the controls (Checkoway et al. 1987) , and in an occupational survey in British Columbia, where controls were cancer patients.(Band et al. 1999) However, although exposure to pesticides was more common among prostate cancer patients, ages when starting farm work, years worked, hours of farm work per week, and proportion of exposed to pesticides and herbicides did not differ between cases and controls who reported ever being employed on a farm. Therefore, the authors concluded that occupational exposures in farming did not account for the observed association (Checkoway et al. 1987).

Another study conducted in Netherlands that examined if prostate cancer risk might be related to exposure to different occupational carcinogens, authors found negative results for cases that have reported exposure to pesticides. (Boers et al. 2005)Similar results were obtained in an Australian study, and negative results were even reported for exposure to organochlorine pesticides. (Fritschi et al. 2007)

On the other hand, positive associations have been reported by a nested case-control study for exposure to some types of pesticides. This study compared new cases of prostate cancer among a cohort of predominantly Hispanic labor union farm workers to cancer free cohort. A large number of pesticides was assessed in a partially ecological exposure assignment (linking information about employment dates & location to records of pesticides used kept by department of pesticide regulation).

A dose response association was detected for lindane and heptachlor exposure and a suggestive increase for dichlorvos and methyl bromide, but no increased risk for other pesticides.(Mills and Yang 2003)

Another hospital based study covering five rural areas in Italy assessed exposure to different pesticides, assuming that in areas under study crop infestations were treated according to established protocols. A positive significant association was observed for exposure to organochlorine pesticides including DDT, dicofol and tetradifon. For the last two pesticides, there was tendency for the estimate to increase with duration of exposure. (Settimi et al. 2003) Furthermore, farming was associated with increased risk of prostate cancer among Caucasians (OR=1.8), but not among African-Americans in a population-based case-control study in South Carolina. This study also found a 60% increased risk of prostate cancer among farmers who mixed or applied pesticides. (Meyer et al. 2007) Thus, it is quite evident that available epidemiological data concerned with evaluating the role of occupational pesticide exposure on prostate cancer risk have reported inconsistent results.

On the other hand, some authors have been interested in the long term low exposure level experienced by the general population. Methodologies applied varied considerably from ecological approaches depending solely on geographical inference of exposure (Cockburn et al. 2011;Parron et al. 2014) to sophisticated biological monitoring of serum and adipose tissue levels of specific pesticides.(Aronson et al. 2010;Hardell et al. 2006;Ritchie et al. 2003;Sawada et al. 2010)

Interestingly, a series of studies conducted in Guadeloupe, a French archipelago in the Caribbean have consistently reported a very high incidence of prostate cancer among the general population of these islands. On the basis of mapping analysis of soil pollution, the authors demonstrated that water has been contaminated by pesticides originating from banana plantations. (Belpomme et al. 2009) Authors have also highlighted that this might be a reflection of the role of ethnicity (as 90% of the inhabitants were of African origin) and a Caribbean genetic susceptibility to prostate cancer. (Mallick et al. 2005) More recent studies examining this phenomenon demonstrated that high exposure to organochlorine pesticides may be causally implicated in the high incidence of prostate cancer in these islands. (Belpomme and Irigaray 2011; Landau-Ossondo et al. 2009)

Moreover, in another study conducted on the general population of these islands, where authors applied measuring serum levels of chloredecone, a significant increase in the risk of prostate cancer with increasing plasma chlordecone concentration was reported. (Multigner et al. 2010) A recently published study also found a significant positive association between high serum levels of DDE and prostate cancer risk among inhabitants of Guadeloupe. (Emeville et al. 2015)

Similar results have been reported in a study in India, where authors reported significantly higher levels of β-HCH, γ- HCH and p,p´-DDE in the serum of prostate cancer cases compared to controls. (Kumar et al. 2010) However, other studies did not find increased prostate cancer risk among individuals with higher serum levels of pesticides. These included studies conducted in Canada, Japan, Sweden and USA where authors applied measuring serum level for many pesticide types. No significant

association could be detected for the majority of the studied pesticides. (Aronson et al. 2010;Hardell et al. 2006;Ritchie et al. 2003;Sawada et al. 2010)

As for ecological assessment of exposure, a recent study conducted in the south of Spain reported a significantly higher prevalence of prostate cancer in districts with higher environmental pesticide exposure relative to those with low exposure, thus supporting the hypothesis that environmental exposure to pesticides may be a risk factor prostate cancer at the level of the general population. (Parron et al. 2014)

Another population-based case-control study was conducted in California's intensely agricultural central Valley where authors evaluated ambient pesticide exposure from residential history and independently recorded pesticide and land-use data, using novel geographic information systems approach. The study provided evidence of an association between prostate cancer and ambient pesticide exposures in and around homes in intensely agricultural areas but the associations appeared to be for specific organochlorine pesticides. (Cockburn et al. 2011)

Methodologies used by epidemiological studies for quantification of exposure

Assessment of exposure to pesticides is an indispensible yet the most challenging part in epidemiological studies dealing with the association between pesticide exposure and prostate cancer. As previously displayed, epidemiological studies have adopted various methodologies to assess or quantify exposure to pesticides.

In a number of studies, researchers depended on job titles, which might be a simple way that avoids recall bias. However, this cannot be of value if quantifying of exposures to specific agents is aimed and results would only be of hypothesis

generating potential.(Stewart et al. 2001) However, comparing incidence of different types of cancers among different jobs is still applied in huge cohort studies, as a base document for subsequent studies that focus on associations between specific work-related factors and cancer with the aim to identify exposure-response patterns. (Pukkala et al. 2009)

On the other hand, a large proportion of the available epidemiological studies have been based on self-reporting, where questionnaires have been utilized in order to obtain information about past exposure to pesticides. This is especially of relevance in case of large studies where other methods would be less feasible. (Agricultural Health Study 2015)

However, concerns about the recall bias associated with self-reporting have directed some authors to develop other methodologies. (Engel et al. 2001)These include Job exposure matrices and expert assessment of exposures. There is some overlap between these procedures since both are typically developed by industrial hygienists who base their decisions on literature reviews and/or knowledge of industrial processes and occupational tasks. Different types of Job exposure matrices have been developed, where coded job titles and industries are linked to different scores of intensities and probabilities of pesticide exposure.(Kauppinen et al. 1998;Kauppinen et al. 2014;Young et al. 2004)

The validity of job exposure matrices remains to be explored, although a study comparing job exposure matrix to expert judgment has concluded that the evaluation of exposure with an unbiased job-exposure matrix in studies of the association

between exposure and disease had a statistical power close to that expected in practice with a good expert judgment. (Bouyer et al. 1995)

Expert assessment involves the evaluation of detailed information about every job held by each subject through combining information from subject questioning and workplace measurement data, where experts review all the available data to assign exposure levels.(Boers et al. 2005;Fritschi et al. 2007) It has been suggested to be the best method available for retrospective occupational exposure assessment especially in community based studies, as experts can account for within-job variability of exposures.(Fritschi et al. 2003) In spite of the observed accuracy of this method, it has been applied minimally, which is expected to be attributed to its being costly and a time consuming process.(Bhatti et al. 2011)

Lastly, biological monitoring has been lately applied and may provide a potentially optimal index of exposure that reflects the internal dose of pesticides and offer the advantage of integrating aggregate pesticide intake across multiple exposure routes. (Checkoway H. et al. 2004;Ott 2005) Nevertheless, its feasibility and cost restrict its use in large epidemiological studies. (Nieuwenhuijsen 2015)

4) Previously published reviews and meta-analyses

A number of reviews and meta-analyses have been previously published, the majority of which were concerned with the association between farming and prostate cancer. (Acquavella et al. 1998;Blair et al. 1985;Blair et al. 1992;Keller-Byrne et al. 1997;Ragin et al. 2013;Van Der Gulden and Vogelzang 1996)

Reviews of the epidemiological literature on the occupational risk for prostate cancer have indicated an excess among farmers (Blair et al., 1985; Keller-Byrne et al., 1997; van der Gulden & Vogelzang, 1996). The first review was published in 1985, as previously mentioned and included studies of farming and cancer published from 1949 till 1985, which were mainly surveys of occupational mortality and thus relied mainly on death certificates. The authors found that 77% of studies showed excess risk of prostate cancer among farmers. (Blair et al. 1985)

A decade later, a meta-analysis of 24 studies which examined the association of prostate cancer with farming found a weak positive association overall. The observed excess resulted were mainly from retrospective case-control studies, and was not related to the year of publication or explained by the possible confounders.(Keller-Byrne et al. 1997) The interpretation to be given to these findings also varied by the attitude of the author as in spite of the fact that no exposure-outcome association has firmly been established as causal, the carcinogenic effect of agrochemicals has been consistently proposed. (Keller-Byrne et al. 1997;Van Der Gulden and Vogelzang 1996)

Another meta-analysis was published one year later to update the review of Blair et al, 1985 and to examine the sources of heterogeneity between studies. According to the included studies that were published till 1994, authors reported a marked variation in results by geographical location and design of the study. The weak but significant association was only detected for studies providing proportionate mortality rates and for case-control studies conducted in the USA, but no association was detected for studies conducted in European countries. Overall, the authors were against the

hypothesis that farmers are at increased risk of prostate cancer. (Acquavella et al. 1998)

In 2003 and 2004, van Maele Fabry et al published two consecutive meta-analyses. A substantial amount of literature was evaluated but authors concluded that there was no adequate provided exposure information to draw firm conclusion in spite of the slightly increased pooled estimate displayed. (Van Maele-Fabry and Willems 2003;Van Maele-Fabry and Willems 2004)

For the most recent meta-analysis, Ragin et al studied the association between farming and prostate cancer risk. Studies were exclusive for case-control studies that quantified pesticide exposure. The effect of type of control group as an evident source of heterogeneity among the included articles has been highlighted, although authors depended solely on two studies for reporting this observation. (Ragin et al. 2013)

JUSTIFICATION

The rapidly increasing incidence of prostate cancer, yet unknown etiology, calls for attention to investigate possible risk factors. Identification of potential risk factors is an essential part for prevention as well as treatment of prostate cancer. From a public health perspective, modifiable risk factors are of specific importance due to the potentiality of implementing primary prostate cancer prevention strategies, if they are found to be associated with prostate cancer risk. Moreover, it is necessary to assess the possible role of occupational exposure to substances that have a carcinogenic potential. Greater levels of prevention would lead to a reduction in incidence, morbidity and consequently mortality from prostate cancer.

The eminent role of endogenous hormones in the pathogenesis of prostate cancer necessitates investigating if pesticides, as endocrine disrupting agents, may be related to the development of prostate cancer. On the other hand, pesticides are of specific importance due to several reasons. First, they are among the most widely spread of the available endocrine disruptors. Over the last 50 years, agriculture has deeply changed with a massive utilization of pesticides and fertilizers to enhance crop protection and production, food quality and food preservation. Pesticides are also increasingly employed for public health purposes and for domestic use.

Second, according to a new list of chemicals evaluated for carcinogenic potential by the Environmental Protection Agency´s pesticide program published in 2010 as well as the International Agency of research on cancer monographs, more than 70 pesticides have been classified as probable or possible carcinogens. This classification has been accomplished based on information extracted from animal and toxicological studies as

well as available epidemiological studies. (http://www.epa.gov/pesticides/carlist 2013;Mostafalou and Abdollahi 2013)

Third, pesticides are unique chemicals as a large number of pesticides are persistent in nature for decades and are deliberately spread in the environment; therefore their effects remain even after years of cessation of application.

Fourth, nearly 50% of the world labor force is employed in agriculture. Occupational exposure to pesticides in agriculture concerns various groups and includes product distributors, mixers and loaders, applicators, bystanders, and rural workers re-entering the fields shortly after treatment.

Assessing and managing the occupational health risks posed by the use of pesticides in the agriculture is a complex but essential task for occupational health specialists and epidemiologists. Although several studies have reported an increased risk of prostate cancer among farmers compared to the general population, the hypothesis that such an excess is directly related to pesticide use has not yet been formally demonstrated. (Mnif et al. 2011)

In spite of the fact that mechanistic studies have demonstrated a basis for biological plausibility between several pesticide types and hormone dependant cancers, epidemiological studies provide markedly conflicting results. This inconsistency in an important issue that necessitates conducting a systematic review to summarize these discrepant findings and a meta-analysis to combine data obtained from similar studies. Moreover, identifying sources of heterogeneity between the available epidemiological studies is considered even more relevant.(McElvenny et al. 2004)

We assume that the controversial results displayed by epidemiological studies might be attributed to the complicated nature of the association that led to differences in the approaches of the authors as regards (1) studied pesticides which encompass diverse groups that might be handled generally or individually; (2) exposure assessment and quantification, a main challenge for epidemiological studies; (3) design of the study especially sample selection; (4) adjusting for confounders that is dealt with differently due to the unclear etiology of prostate cancer and (5) studied populations that include both occupational and environmental exposures. Accordingly, this variability is expected to influence the results obtained.

To inform risk assessment and regulatory decision making, the potential relationship between pesticide exposure and prostate cancer requires clarification. For that, we have conducted a systematic review and meta-analysis to; (1) explore the previously highlighted increased risk of prostate cancer observed among farmers as an occupational group; (2) examine if there is an association between pesticide exposure and prostate cancer and; (3) assess if there is a specific role for organochlorine pesticides on the development of prostate cancer.

OBJECTIVES

Main objective

To systematically review epidemiological studies on pesticide exposure and prostate cancer.

Specific Objectives

1. Determining if farmers have an increased risk of developing prostate cancer by systematically reviewing available epidemiological studies and analyzing provided data.

2. Exploring and analyzing potential sources of heterogeneity between studies evaluating prostate cancer risk among farmers.

3. Evaluating the magnitude of the potential association between prostate cancer and pesticide exposure by calculating pooled estimates for homogenous groups of studies, for different levels of exposures as well as for specific categories of pesticides (organochlorine pesticides).

4. Exploring and analyzing potential sources of heterogeneity between studies on pesticide exposure and prostate cancer.

5. Assessing the quality of the available epidemiological studies to examine its impact on the obtained results.

6. Examining the potential role of different variables including study designs and the methodologies adopted by the studies as sources of bias that might explain the inconsistency between studies.

7. Comparing our results with previously conducted meta-analyses.

8. Critically reviewing available studies in order to identify gaps and limitations in the currently available epidemiological literature on pesticide exposure and prostate cancer risk.

These objectives would imply three approaches for assessing the available epidemiological data; (1) systematically reviewing of the available epidemiological data, (2) criteria based approach for assessing quality of available epidemiological studies and (3) meta-analyses of different estimates provided for the studied association.

METHODOLODGY

General outlines of the methodology applied

We have conducted three consecutive meta-analyses following PRISMA and MOOSE guidelines.(Moher et al. 2009;Shamseer et al. 2015;Stroup et al. 2000) Primarily, we carried out a systematic review and a meta-analysis to explore the previously highlighted increased risk of prostate cancer among farmers. The second meta-analysis was to assess the potential association between pesticide exposure and prostate cancer. The third was to more specifically examine the association between organochlorine pesticides and prostate cancer, as a further evaluation of the results that we have obtained from the second meta-analysis.

In the following section we will display the methodology adopted in the first meta-analysis in details, followed by commenting on specific parts that was particular for each of the other two meta-analyses. This is because the general outlines adopted were very similar for the three meta-analyses.

1. Design and eligibility criteria:

1.1. First meta-analysis (Farming and prostate cancer risk)

We conducted a systematic review and meta-analysis of epidemiological data relating farming to prostate cancer risk. Studies were considered for inclusion if they complied with the following criteria: (1) originality; (2) design included case-control or cohort studies; (3) including outcome measures–odds ratios (OR), relative risks (RR), Standardized incidence ratio (SIR) – and their confidence intervals (CI) relating farming and prostate cancer or providing sufficient data from which they could be calculated; (4) written in English, Spanish or French; (5) published between 1986 and April 2014.

Definition of farming included being a farmer, a farm laborer or an agricultural worker, as indicated by self reporting or by registers or membership in farmers associations.

Exclusion criteria were: (1) studies based solely on mortality rates; (2) clinical studies about treatments or interventions; (3) reviews, previous meta-analyses, editorials, or letters were also not included. In case of availability of updates of the same study, we included the most recent one.

1.2. Second Meta-analysis (Pesticide exposure and Prostate cancer)

This meta-analysis was to study the potential association between pesticides exposure and prostate cancer. The predefined inclusion criteria were: (1) original studies; (2) case-control or cohort studies; (3) containing information about association measures – odds ratios (OR), relative risks (RR) – and their confidence intervals (CI) relating pesticides exposure and prostate cancer or providing sufficient data from which they could be calculated; (5) written in English, French or Spanish. We searched for articles published from 1985 till April 2014.

Exclusion criteria were: (1) in vitro experimental studies; (2) those based solely on mortality rates; (3) studies addressing pesticide manufacturing workers (given the particular nature of exposure in production plants); (4) reviews, previous meta-analyses, editorials, or letters were also not included. In case of articles in which subjects were included in a more recent publication, we used the results provided by the most recent one.

1.3. Third meta-analysis (Specific organochlorine pesticides and Prostate cancer risk)

In this meta-analysis, it was confined to studies that analyze a potential association between specific organochlorine pesticide and prostate cancer. This meta-analysis was performed at a later stage than the first two meta-analyses and therefore the search period extended to include studies published till March 2015.

2. Search strategy and Selection of articles

We searched PubMed (U.S. National Library of Medicine, Bethesda, Maryland, USA), Web of Science (Thomson Reuters) and Scopus (Elsevier) databases.

For the first meta-analysis, we have used different combinations of the following medical subject heading terms and keywords: "farming", "farmers", "farm workers", "farm laborers", " agricultural workers", "occupational", "Job", "prostate cancer", "prostatic carcinoma", "prostatic neoplasm", "incidence", "case-control studies", "cohort studies", "occupational cancer".

For the second meta-analysis, we have stressed on pesticide as an exposure and used "pesticide exposure""pesticides", "agricultural exposure", "pesticides application", "pesticide applicators", "farmers", "farm workers", "farming", "agricultural workers", "prostate cancer", "prostatic carcinoma", "prostatic neoplasm", "incidence", "case-control studies", "cohort studies", "occupational cancer", "exposure assessment", "risk factors". We adapted the search tool to the database searched.

For the third meta-analysis, we have applied Mesh terms more specific to organochlorine pesticides as "organochlorine pesticides", "DDT", "DDE",

"hexachlorocyclobenzene", "lindane", "chlordecone", "dicofol", "dieldrin", "endosulfan", "heptachlor", "methoxychlor", "toxaphene".

This was followed by hand searching the references of the included articles and the previously published reviews. Publications that were not found online were obtained by e-mailing the authors or requested from the central library of the university.

Titles and abstracts of the identified articles were reviewed independently by two researchers. Those not considered relevant for further checking of the full text article were excluded and the reasons for that were listed.

Relevant articles were read and analyzed independently by the two researchers. A standardized procedure was followed for extracting and tabulating relevant data as follows.

3. Data extraction

Data collected for each study included: (1) *geographical and temporal variables*: country and area of the study, period of recruitment of cases and controls, or recruitment and follow up periods of cohort studies; (2) *characteristics of the study*: design (case-control or cohort study), study population; sample size; participants' selection; (3) *evaluation of exposure*: type of studied pesticides and method applied for exposure assessment; self reporting using a self or an interviewer administered questionnaire, expert judgment, JEMs, biological samples including serum or adipose tissue pesticide level measurement, or grouped (non-individualized) exposure assessment; (4) *magnitude of the association*: OR/RR estimators and 95% CI by exposure level (For the first meta-analysis, estimator also included Standardized

incidence ratio (SIR)). We have converted the 90% CI provided by four studies into 95%. Where both crude and adjusted ORs were provided, we used the latter; (5) *confounding factors* that were adjusted for in each study.

Lastly, all the information obtained initially by the two researchers was compared and disagreements were resolved by two senior epidemiologists. This procedure lasted from March 2013 till April 2014, and extended to march 2015 for the third meta-analysis.

4. Assessment of the quality of the included articles

To systematically assess the quality of the included articles, we applied the Newcastle Ottawa Scale (NOS) proposed by Wells *et al.* (Wells G 2013) The NOS contains eight items, each is given a star point if fulfilled (except for comparability, maximum of two stars can be given). Accordingly, an overall minimum score of one and a maximum of nine stars can be given for each article.

These items cover three main quality dimensions: (1) selection of the study population; (2) comparability among the groups; and (3) outcome or exposure measures for cohort and case-control studies respectively. (See Appendix Pages212-213)

We utilized the overall scores given for the included studies to categorize them into high (8-9 stars), medium (6-7) and low quality (≤ 5 stars). We also categorized studies according to exposure assessment quality into high (3 stars), medium (2 stars) and low quality (1 star).

Lastly, all the information obtained initially by the two researchers was compared and disagreements were resolved by two experienced epidemiologists.

5. Statistical analysis

In the following section, we refer to the estimates extracted from the included studies in the meta-analysis as odds ratio (OR) for simplicity. After we had extracted ORs and confidence intervals from the articles included in the meta-analyses, the ORs were weighted and pooled. The *pooled estimate* is the weighted sum of the results divided by the sum of the weights. (Sterne 2009)

$RR_p = \Sigma\, w_i\, RR_i / \Sigma\, w_i$

where RR_p *is the overall estimate of the pooled effect*

w_i *= weight given for each study*

and RR_i *is the odds ratio/relative risk for the* i^{th} *study*

The *weight* of each study is the reciprocal of the variance.

5.1. Statistical pooling of data

There are two models that might be used for pooling the results of different studies. When there is little variation between studies ($I^2 \leq 25\%$; as assigned by Higgins et al., 2003), the pooled estimate could be calculated according to a fixed model (Mantel-Haenszel Method). This model assumes a common effect size for all the studies, with any differences seen between the studies considered to be due to sampling error. (Michael Borenstein. 2010)

The study variance (Vi) is calculated using the CI given according to the equation:

$Vi = [(\ln (CI\ upper) - \ln (CI\ lower)) / 3.92]^2$.

The estimate of the pooled RR in the fixed effect model is the exp ln $(RR)_p$.

The pooled ln $(RR)_p = \Sigma [\ln(RR)_i / V_i] / [\Sigma(1/V_i)]$.

This is a variance-weighted least square mean.

The variance of the pooled ln $(RR)_p$, Var (ln $(RR)_p$) or V_p is given by:

$V_p = [SE (\ln (RR_p)]^2 = [\Sigma(1/V_i)]-1$, where SE is the standard error.

The pooled variance is used to calculate a 95% CI around the pooled RR estimate.

Alternatively, random-effects models assume that the different studies are estimating different, yet related, effects. Therefore, it adjusts not only for the within-study variance but also the between-study variance. Thus, under the random-effects model each study is estimating an effect size for a unique population, and so must be given appropriate weight in the analysis. (ie, where the small study has almost no impact under the fixed effect model, it has a larger impact in the random effects model) This results in wider confidence intervals when using a random-effects model than under a fixed-effect model, and correspondingly claims of statistical significance will be more conservative. (The Cochrane Collaboration 2008)

Given the expected heterogeneity between the available studies, we have decided a priori to use the random-effects model. (Michael Borenstein. 2010) This is because the

practice of starting with a fixed-effect model and switching to a random-effects model if significant heterogeneity is detected has been discouraged.

Under the random-effects model, which was described by DerSimonian and laird,(DerSimonian and Laird 1986) the point estimate of the pooled effect measure and its CI incorporate an estimate of between-study variation in the weighting (τ^2).

A non-iterative estimator of τ^2 has been proposed and is defined as

$$(\tau^2) = [Q - (k - 1)] / [\Sigma w_i - (\Sigma w_i^2) / \Sigma w_i] \text{ or } 0$$

Where Q is the heterogeneity statistic, k is the total number of studies, & w_i are the inverse variance weights for ln (RR). (Sterne AC 2009)

We have displayed the meta-analyses that we have conducted in forest plots with the studies ordered according to publication dates to provide a visual impression of the effect of time on the estimates. In the forest plot, the confidence interval for each study is represented by a horizontal line and the point estimate by a square. The size of the square corresponds to the weight of the study in the meta-analysis. They are inversely proportional to the study specific estimate variance. The combined effect estimate and its confidence interval are symbolized by a diamond. Its middle corresponds to the risk estimate and the width represents CI. The vertical dashed red line provides a visual comparison of the pooled estimate with the study specific estimates. (Judith Anzures-Cabreraa and Julian P.T.Higgins 2010)

5.2. Evaluation of homogeneity

As Cochran's Q test has been criticized for having a low power to detect heterogeneity with relatively small number of studies included in the analysis, we have utilized an alternative approach, the I^2 test. (Hardy and Thompson 1998;Ioannidis 2008)

This test quantifies the effect of heterogeneity, providing a measure of the degree of inconsistency in the studies' results. (Higgins et al. 2003) The quantity called I^2 describes the percentage of total variation across studies that is due to heterogeneity rather than chance.

I^2 is calculated as follows: $I^2 = 100\% \times (Q - df) / Q,$

Where Q is Cochran's heterogeneity statistic and df the degree of freedom.

Negative values of I^2 are put equal to zero, so that I^2 lies between 0% and 100%.
A value of 0% indicates no observed heterogeneity and larger values show increasing heterogeneity.

Heterogeneity was considered as low for values between 25% and 50%, moderate for 50% to 75% and high for >75%. (Higgins et al. 2003)

Note: Cochran´s Q statistics test has a χ2 distribution with degrees of freedom equals to the number of studies pooled minus 1. The applied formula is:

$\chi 2 = \Sigma wi\ [ln(RR)i - ln(RR)p]^2$,

For i = 1 to N, where N is the number of studies combined,

ln(RRp) is the overall pooled RR estimate,

ln(RRi) is the RR for the I th study

and wi = 1/Vi where Vi is the variance of the ln(RR)i.

A low P value for this statistic indicates the presence of heterogeneity, which undermines the validity of the pooled estimates.

All statistical analyses were performed using Stata version 12 statistical software (Stata Corp, College Station, TX, USA).

5.3. Methods of exploring heterogeneity

In case of detected heterogeneity, I^2 > 25%, we explored heterogeneity between studies by one or more of the following methods:

- Stratification (Sub-group analysis)
- Sensitivity analysis and analysis of influential studies.
- Meta-regression
- Cumulative analysis

5.3.1. Stratification (Sub-group Analysis)

We explored heterogeneity by stratifying studies based on several potential variables that we assume might have produced the detected heterogeneity. These priori defined variables included the following: overall quality of the studies according to NOS, quality of exposure assessment, methods applied for quantification of exposure, adjusting for family history of prostate cancer, pesticide category, exposed population, type of control population for case-control studies, year of publication, decade of prostate cancer diagnosis and geographical location. All subset analyses were

performed taking into account study design, owing to the expected differences between case-control and cohort studies.

5.3.2. Sensitivity analyses

We conducted sensitivity analysis in order to (1) determine the robustness of the findings; (2) appraise whether some of the selections made had a major effect on the results of the meta-analyses; (3) examine the disproportionate influence that a specific study may impose on the combined summary statistics.

Sensitivity analyses were conducted by:

- Deleting studies that reported extreme ORs.
- Removing the studies that reported extreme precision values.
- Eliminating studies with the lowest quality.
- Performing a meta-analysis including only studies reporting specific data, or those of high quality.
- Re-evaluating pooled estimates on using both fixed and random-effects models.
- Omitting from the subset analysis studies that are different from the others, for eg. Having a unique exposure assessment methodology, studied population is different or sample selection is exquisite, etc.
- We have also re-calculated pooled estimates after excluding one study in a sequential manner, when we found difficulty in obtaining homogeneity by sub-stratification or by applying the previously displayed sensitivity analysis techniques (analysis of influential studies).

5.3.3. Meta-regression

We also conducted a meta-regression analysis to explore the relative importance of variables that we have detected from sub-stratification to explain heterogeneity between studies.(Baker et al. 2009) Regression of the estimated OR on the indicator variables, allowed evaluation of heterogeneity by several study characteristics simultaneously.

5.4. Publication bias

The trend for studies with more favorable or statistically significant results to be published more than studies with less favorable or non significant results is referred to as publication bias. (Peters and Mengersen 2008)

The possible influence of publication bias was graphically assessed with contour enhanced funnel plots, where the natural logarithm of the estimator of OR was plotted against the inverse of the SE (estimate's precision).(Peters et al. 2008) The SE of the log of the estimate was utilized. This method examines whether any funnel plot asymmetry is likely to be due to publication bias compared with other underlying causes of funnel plot asymmetry.

The contours help to indicate whether areas of the plot, where studies are perceived to be missing, are where studies would have statistically significant effect sizes or not and thus decrease or increase the evidence that the asymmetry is due to publication bias. We also tested forest plot asymmetry by applying the model proposed by Egger,(Egger et al. 1997) to confirm if there is a sort of small study effect.

RESULTS

General Outlines

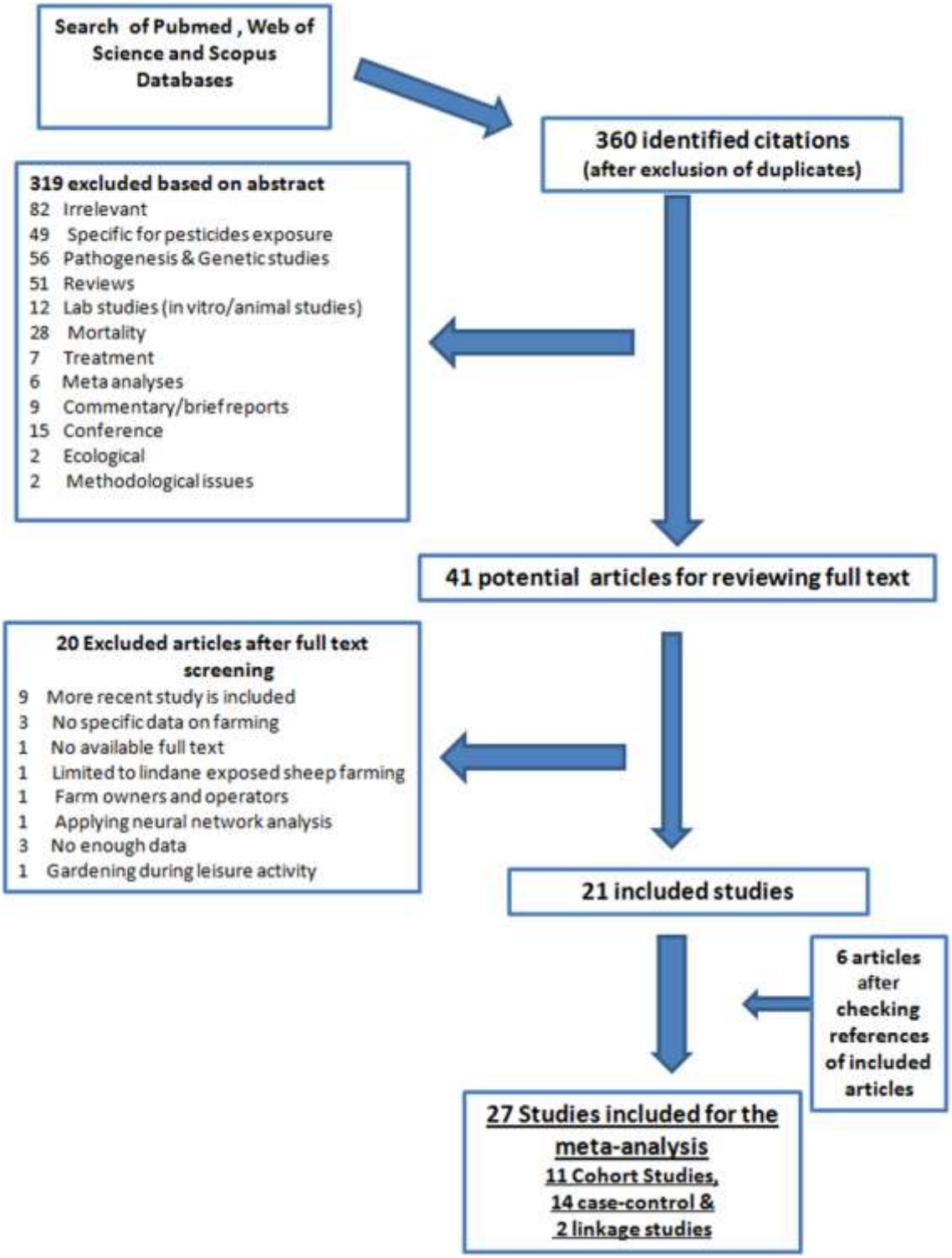
For more clarity, we display the results of each of the studied associations separately and in a consequent manner. ie. Results are displayed first for the systematic review and meta-analysis of studies relating farming to prostate cancer then for the association between pesticide exposure and prostate cancer risk, and lastly for the results of the association between organochlorine pesticide exposure and prostate cancer.

A) FARMING AND PROSTATE CANCER

1) Selection of the studies addressing the association between farming and prostate cancer

As displayed in figure 19, our search strategy applied to find studies that provided estimates for prostate cancer among farmers yielded a number of 360 articles (after omitting duplicates). 319 papers were obviously irrelevant from title or abstract. After reviewing the full texts of 41 potentially eligible articles, 20 were excluded for not complying with the inclusion criteria as listed in the flowchart. Hand searching of the references of the included articles as well as previous reviews resulted in six more articles that fit our inclusion criteria. Finally, we systematically reviewed 27 articles (covering fourteen case-control, eleven cohort and two linkage studies)

Figure 19. Flow chart summarizing the selection process of the articles for the first meta-analysis (association between farming & prostate cancer risk)



2) Characteristics of the studies that addressed the association between farming and prostate cancer

Tables A and B (Appendix) display characteristics of the case-control (Aronson et al. 1996;Band et al. 1999;Brownson et al. 1989;Ewings and Bowie 1996;Fincham et al. 1992;Franceschi et al. 1993;Keller and Howe 1994;Krstev et al. 1998;Meyer et al. 2007;Pearce et al. 1987;Reif et al. 1989;Settimi et al. 2003;Talamini et al. 1986;Van Der Gulden et al. 1995),cohort (Bouchardy et al. 2002;Frost et al. 2011;Gunnarsdottir and Rafnsson 1991;Koutros et al. 2010a;Kristensen et al. 1996;Laakkonen and Pukkala 2008;Mills and Shah 2014;Parker et al. 1999;Pukkala et al. 2009;Wiklund and Dich 1995;Zeegers et al. 2004) and linkage studies (Olsen and Jensen 1987;Sharma-Wagner et al. 2000) included in the meta-analysis. These characteristics can be summarized as follows:

1. Location:

More than half of the studies were conducted in Europe (n=15), of which a large proportion were in Scandinavian countries (n= 9). Ten studies were carried out in USA or Canada, while the remaining two studies were conducted in New Zealand.

2. Sample Size:

The smallest cohort included 1177 individuals (Parker et al. 1999) while the largest incorporated 15 million people from five Nordic countries (Pukkala et al. 2009). For case-control studies, number of prostate cancer cases ranged from 124 (Settimi et al. 2003) to 981 (Krstev et al. 1998) individuals.

3. Methodology applied for collecting information about farming:

Half of the studies utilized questionnaire including detailed job history, while six studies depended on the job recorded in the cancer registry. On the other hand, other registries as census data or farmers' pension data were used by the remaining eight studies.

4. General design adopted by the studies

Almost half of the studies (n=12) compared incidence of prostate cancer among different job categories including farmers (evaluated occupational variation in prostate cancer risk). Twelve studies evaluated different types of cancers among farmers. Only three studies studied specifically prostate cancer among farmers. On the other hand, the methodology applied by two studies was based solely on linking data from cancer registries to data about job held by the individuals.

3) Assessment of the quality of the studies about farming and prostate cancer

A large percentage of the studies were observed to have potential sources of selection and/or information bias. For example, general healthy population constituted the controls for only two of the included thirteen case-control studies. This is because a large proportion of the studies used cancer controls from cancer registries.

Moreover, depending on the job recorded in the cancer registries which was the job held at the time of the registration may have also resulted in selection bias. This was adopted by five of the included studies. For the overall quality according to NOS criteria, about half of the studies (n=13) had a score of ≥ 6 points. For scores given for

selection, half of the case control studies got only one or two NOS scores, while the other half got three to four points.

Regarding adjustment for potential confounders, age was adjusted for in all studies but family history of prostate cancer was adjusted for in only five studies. Other variables as smoking, socioeconomic status, alcohol consumption, marital status and area of study were adjusted for occasionally in some of the studies.

4) Results reported by the included articles

4.1. Qualitative analysis of the results

Of the fourteen included case-control studies, six found a significant positive association between farming and incidence of prostate cancer.(Band et al. 1999;Brownson et al. 1989;Fincham et al. 1992;Meyer et al. 2007;Mills and Shah 2014;Reif et al. 1989) However, the estimates were modest for all of the studies. Four studies reported insignificant associations, (Keller and Howe 1994;Parker et al. 1999;Pearce et al. 1987;Settimi et al. 2003) four reported no association (Ewings and Bowie 1996;Franceschi et al. 1993;Mills and Shah 2014;Van Der Gulden et al. 1995), while a positive association was restricted to short term employment in one study. (Krstev et al. 1998)

For almost all the cohort studies, SIR was close to unity as only two (Bouchardy et al. 2002;Koutros et al. 2010a) reported a weak yet significant association. Worth mentioning is that the recruitment of individuals for these studies was different from the others. Men above 65 years old were excluded in one study,(Bouchardy et al.

2002) while inclusion was restricted to licensed pesticide applicators in the other one.(Koutros et al. 2010a)

4.2. Quantitative analyses of the results

4.2.1. Exploring Heterogeneity by stratification and sensitivity analysis

There was a high degree of heterogeneity when pooling the estimates provided by the 27 included studies (Pooled estimate = 1.07, 95% CI, 1.03 –1.12, I^2 = 84.8%, p = 0.00). As it is fundamental to differentiate between the estimates derived from the different study designs, we have stratified studies accordingly. As observed in figure 20, the heterogeneity decreased among case-control but not for cohort studies.

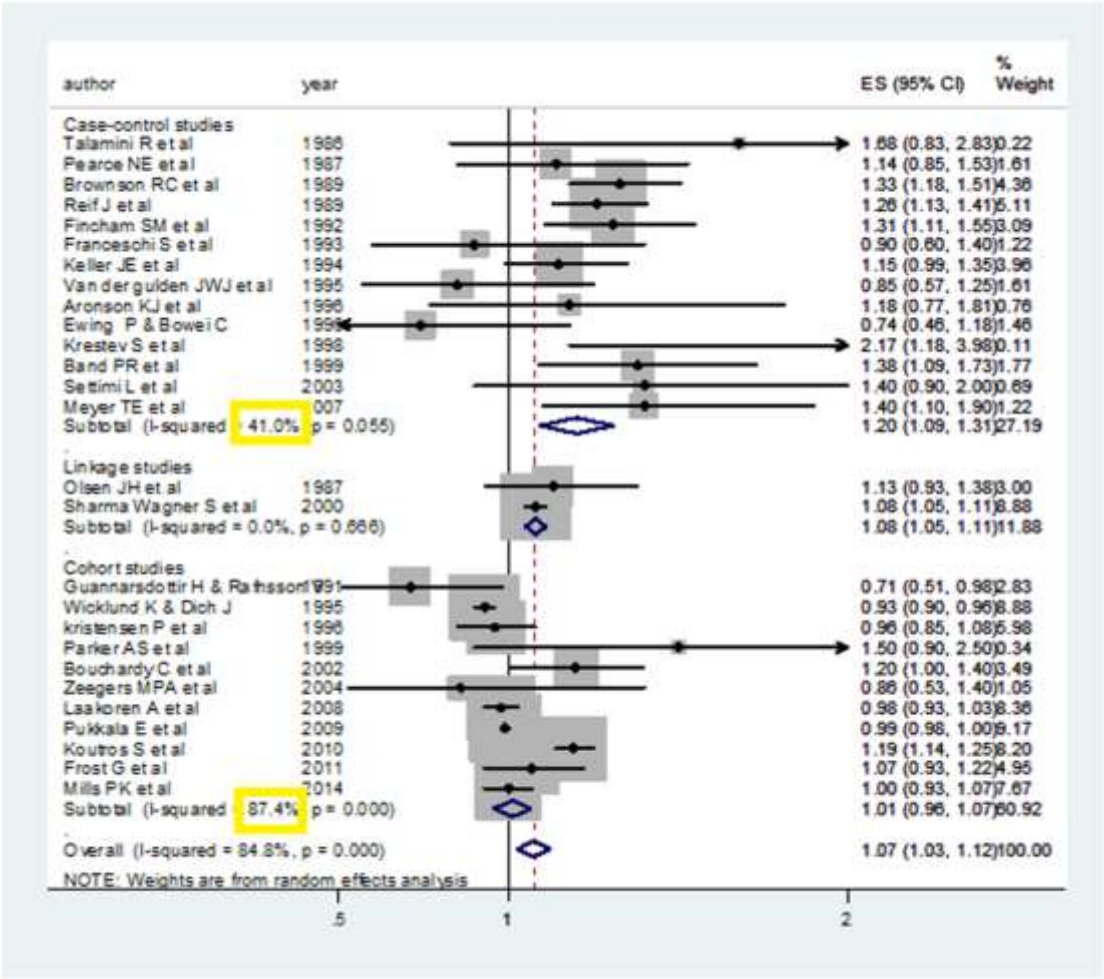


Figure 20. Forest plot displaying sub-stratification of studies relating farmers to prostate cancer by study design

Homogeneity could not be revealed when stratifying cohort studies by many variables as country of study, year of publication or quality of the studies. However, heterogeneity was resolved on eliminating three studies. One study was unique for studying specifically licensed pesticide applicators, while another was the only study that excluded men > 65 years due to lack of data about occupation for this age group. However, in spite of achieving homogeneity, the pooled estimate changed minimally from 1.01, 95% CI, 0.96-1.07, I^2 = 87.4%, p = 0.00 to 0.99, 95% CI, 0.96-1.02, I^2 = 22.3%, p = 0.252. (Table 3)

On the other hand, for the case-control studies, homogeneity was achieved when eliminating the two studies that were the only ones conducted in UK and Netherlands and also provided the lowest ORs.(Ewings and Bowie 1996;Van Der Gulden et al. 1995) However, the pooled estimate was weak and changed only minimally which might add to the robustness of the results.

Table 3. Exploring heterogeneity among the included studies by Sensitivity analysis

Sensitivity Analysis	Studies included in the analysis	No of Studies	Pooled OR	CI (95%)	Heterogeneity I^2 (%)	P value
Sub-grouping by design	Cohort studies	11	1.01	0.96 – 1.07	87.4%	0.000
	Case-control	14	1.20	1.09 – 1.31	41.0%	0.056
	Linkage studies	2	**1.08**	**1.05 – 1.11**	**0.00%**	0.666
Eliminating 3 cohort studies [β]	Cohort Studies	8	**0.99**	**0.96 – 1.02**	**22.3%**	0.252
Eliminating 2 case-control studies [¥]	Case-control studies	12	**1.26**	**1.19 – 1.33**	**0.00%**	0.570

Pooled estimates are in bold if they are significant and there is homogeneity between the studies

[β] These studies are Wicklund K & Dich J 1995, Bouchardy C et al 2003, Koutros S et al 2010.

[¥] Eliminating two studies, Ewings P & Bowie C, 1996 & Van der gulden JWJ, 1995 that provided the most extreme ORs

As seen in Table 4, homogeneity was also reached when we stratified case-control studies by a number of variables including location, publication years, decades of prostate cancer diagnosis, type of control population and quality of the studies.

Table 4. Exploring heterogeneity among the included case-control studies by Sub-group analysis

Variable of Subgroup Analysis	Studies included in the analysis	No of studies	Pooled OR	CI (95%)	Heterogeneity I^2 (%)	P value
Location	USA/Canada	7	**1.28**	**1.19 – 1.38**	**0.00%**	0.564
	UK & Netherlands	2	0.80	0.55 – 1.05	0.00%	0.663
	New- Zealand	2	**1.24**	**1.11 – 1.37**	**0.00%**	0.552
	Italy	3	1.20	0.76 – 1.64	40.4%	0.187
Years of publication	1986 – 1994	7	**1.24**	**1.16 – 1.33**	**6.50%**	0.378
	1995 – 1997	3	0.87	0.65 – 1.09	0.00%	0.391
	1998 – 2007	4	**1.41**	**1.19 – 1.63**	**0.00%**	0.760
Years of diagnosis of prostate cancer	Late 70s till late 80s	8	**1.27**	**1.19 –1.35**	**0.00%**	0.640
	Late 80s till early 90s	4	0.91	0.68 – 1.14	25.3%	0.260
Type of control population©	Cancer Patients	7	**1.27**	**1.19 – 1.34**	**0.00%**	0.734
	BPH or diseases other than cancer	2	0.80	0.55 – 1.05	0.00%	0.663
	Hospitalized for other diseases	3	1.08	0.75 –1.42	12.8%	0.318
	Healthy Men	2	**1.48**	**1.02 –1.94**	**6.90%**	0.300
Design of the study	Comparing incidence of different cancer types among farmers	5	**1.24**	**1.14-1.34**	**24.9%**	0.508
	Comparing ORs of PC for different jobs	7	1.12	0.87-1.37	52.1%	0.051
	Specifically evaluating PC risk among farmers	2	**1.40**	**1.08-1.72**	**0.00%**	1.00
Quality of the study	Lower quality studies	5	**1.25**	**1.17 – 1.34**	**0.00%**	0.594
	Higher quality studies	9	1.15	0.93 –1.38	52.0%	0.033

Pooled estimates are in bold if they are significant and there is homogeneity between the studies

©One study is missing from this analysis, as the controls included both cancer patients as well as healthy population

In the following section, we will display the results of subset analysis by forest plots for better visualizing of the results.

1. Sub-stratification of case-control studies by location

Homogeneity was revealed when we grouped studies by the geographical location, except for studies conducted in Italy. A significant yet weak association was observed for studies conducted in USA/ Canada as well in New-Zealand, but not for those carried out in European countries as UK and Netherlands.

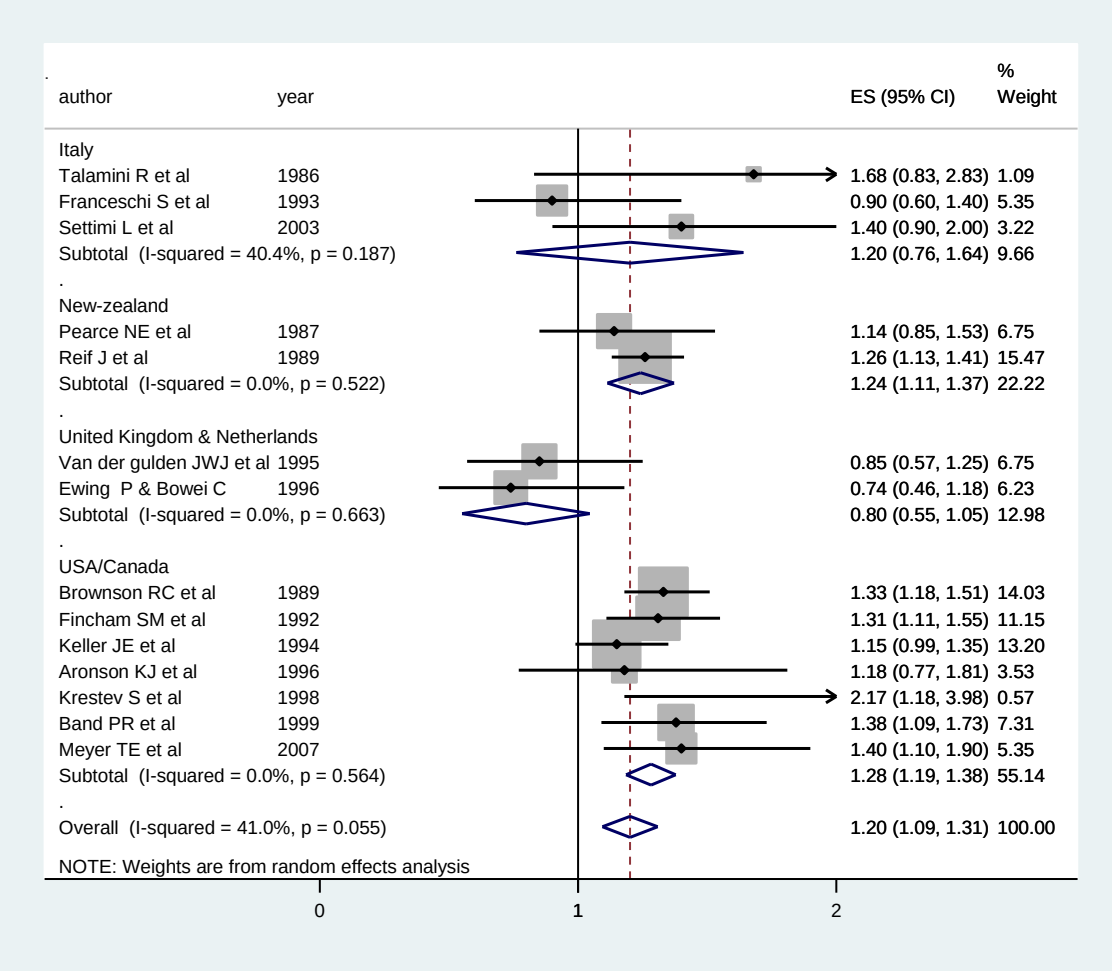


Figure 21. Forest plot displaying sub-stratification of case-control studies relating farmers to prostate cancer by geographical location

2. Sub-stratification of case-control studies by publication years

Homogeneity among case-control studies was also revealed when stratifying by years of publication. What was interesting is that for the three studies that demonstrated no association, they shared in common that controls were not cancer patients. Also, the design was comparing the incidence of prostate cancer among different job categories including farming, which might indicate less chance of publication bias. On the contrary, for the four studies that were published more recently and provided the positive significant association, the controls were cancer patients in three out of the four studies and the design was exclusively studying prostate cancer among farmers, which might indicate a higher probability of publication bias.

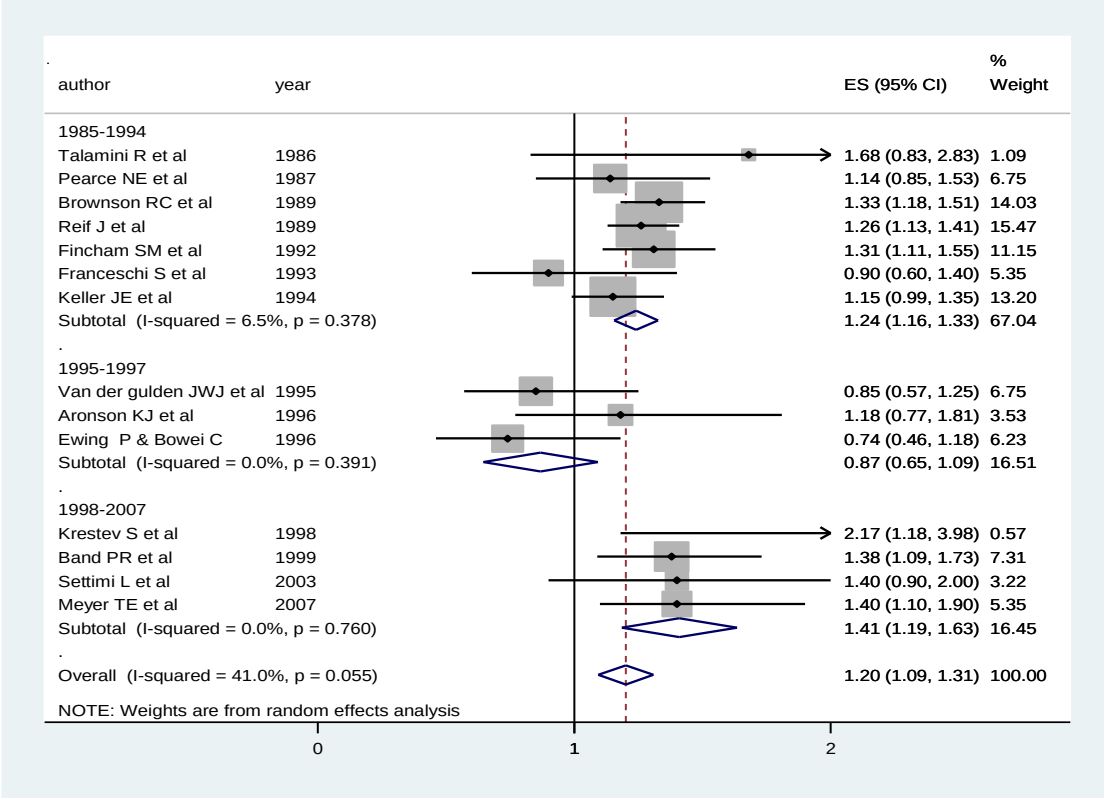


Figure 22. Forest plot displaying sub-stratification of case-control studies relating farmers to prostate cancer by publication years

3. Sub-stratification of case-control studies by quality

Stratifying studies by quality revealed homogeneity only for lower quality studies. However, the pooled estimate was very close to that of pooling the higher quality studies but it was significant only for lower quality studies.

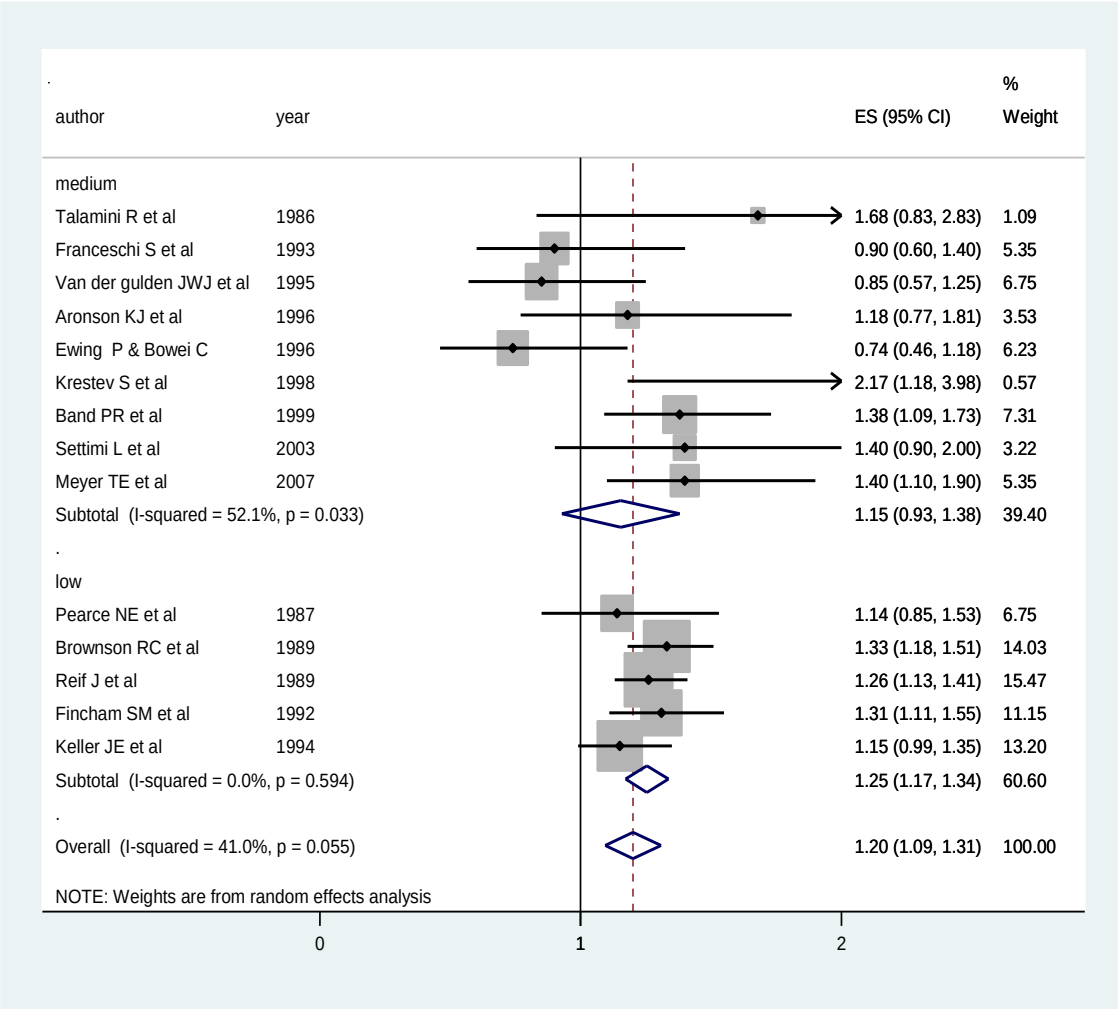


Figure 23. Forest plot displaying sub-stratification of studies relating farmers to prostate cancer by quality of the articles

4. Sub-stratification of case-control studies by type of control population

Grouping studies by type of controls used in the study revealed homogeneity for all study sub-groups.

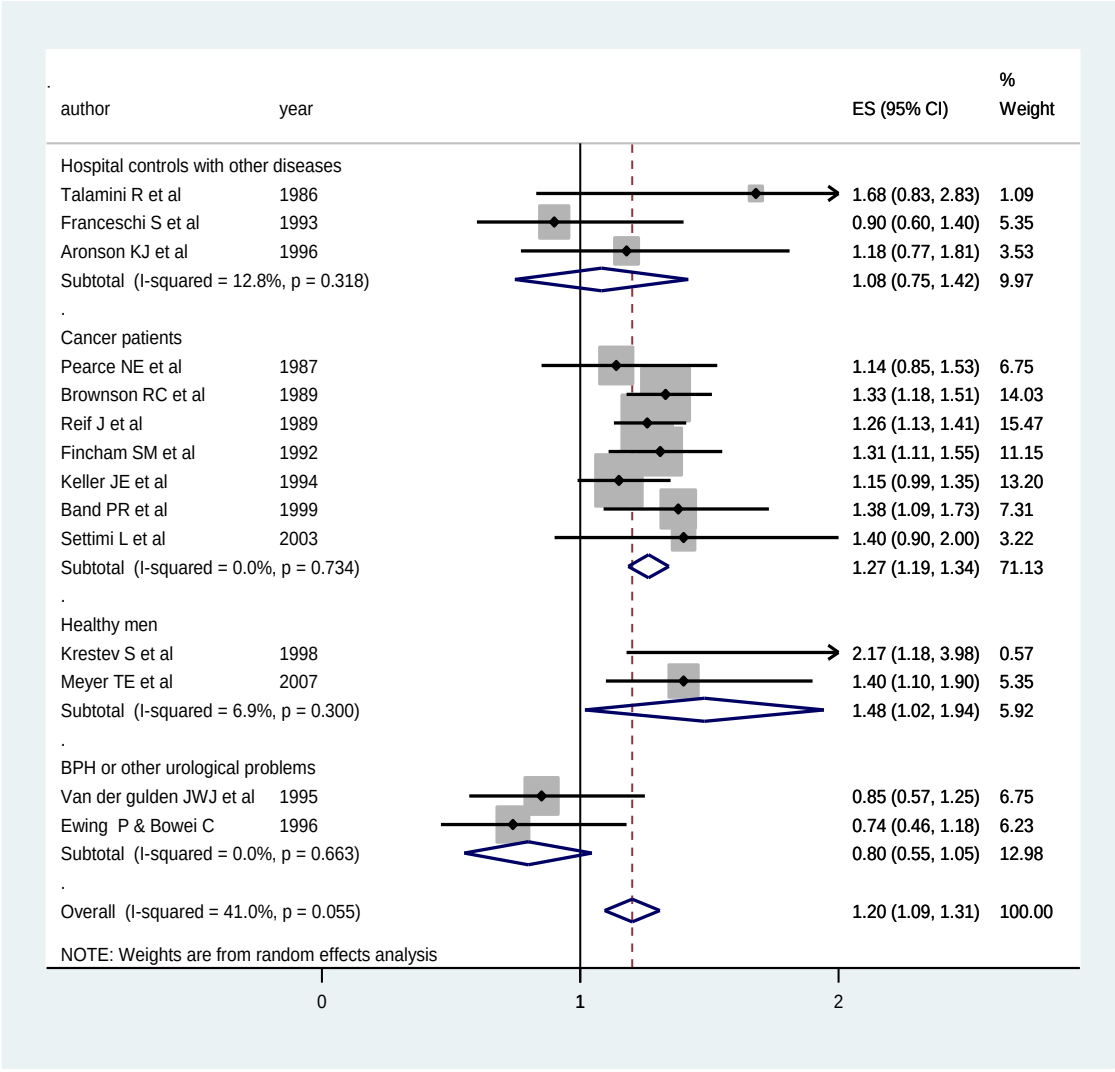


Figure 24. Forest plot displaying sub-stratification of studies relating farmers to prostate cancer by type of control population

5. Sub-stratification of case-control studies by the design of the study

Homogeneity was revealed among case-control studies also by stratifying by the general design adopted. Pooling the four case-control studies that examined different types of cancers (including prostate cancer) among farmers provided a significant association. These four studies share in common lower quality, controls were cancer patients and three out of the four studies collected information about farming from cancer registries that included the job recorded at the time of registration.

The pooled estimate was even higher for the two studies that evaluated only prostate cancer risk among farmers, where publication bias might be expected. On the other hand, the overall estimate of studies that compared different occupations by incidence of prostate cancer did not reveal any association. This is the same methodology adopted by the cohort studies.

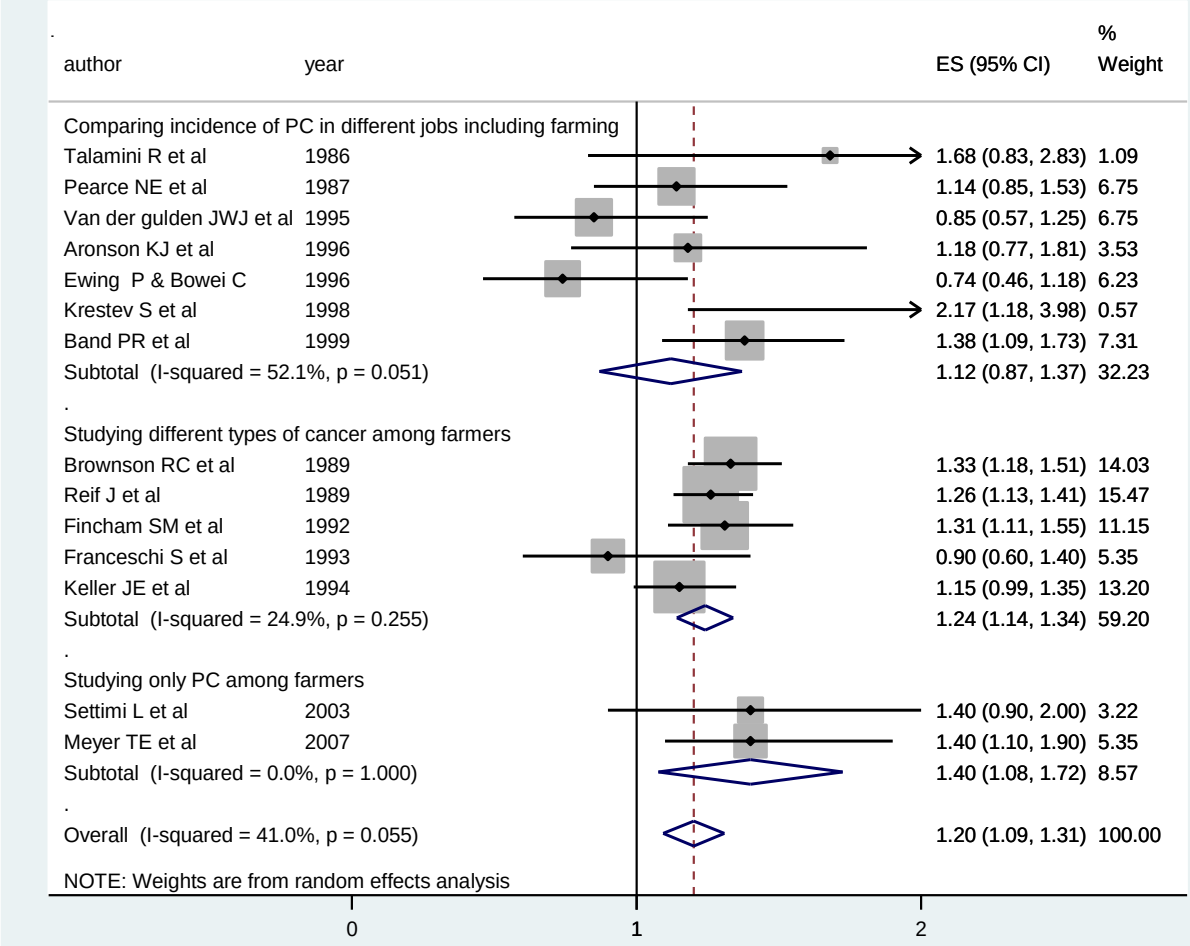


Figure 25. Forest plot displaying sub-stratification of case-control studies relating farmers to prostate cancer by design adopted by the study

4.2.2. Detection of Publication bias

Figure 26 displays contour enhanced funnel plots with corresponding random-effects pooled estimates across studies. Although it is not noticeable from visual inspection of the funnel plot that small sized studies are dispersed more in parts of the plot that indicates statistical significance, according to the results of Egger´s test (estimated bias coefficient = 1.205, standard error = 0.567, p = 0.043), there seems to be a sort of small study effect.

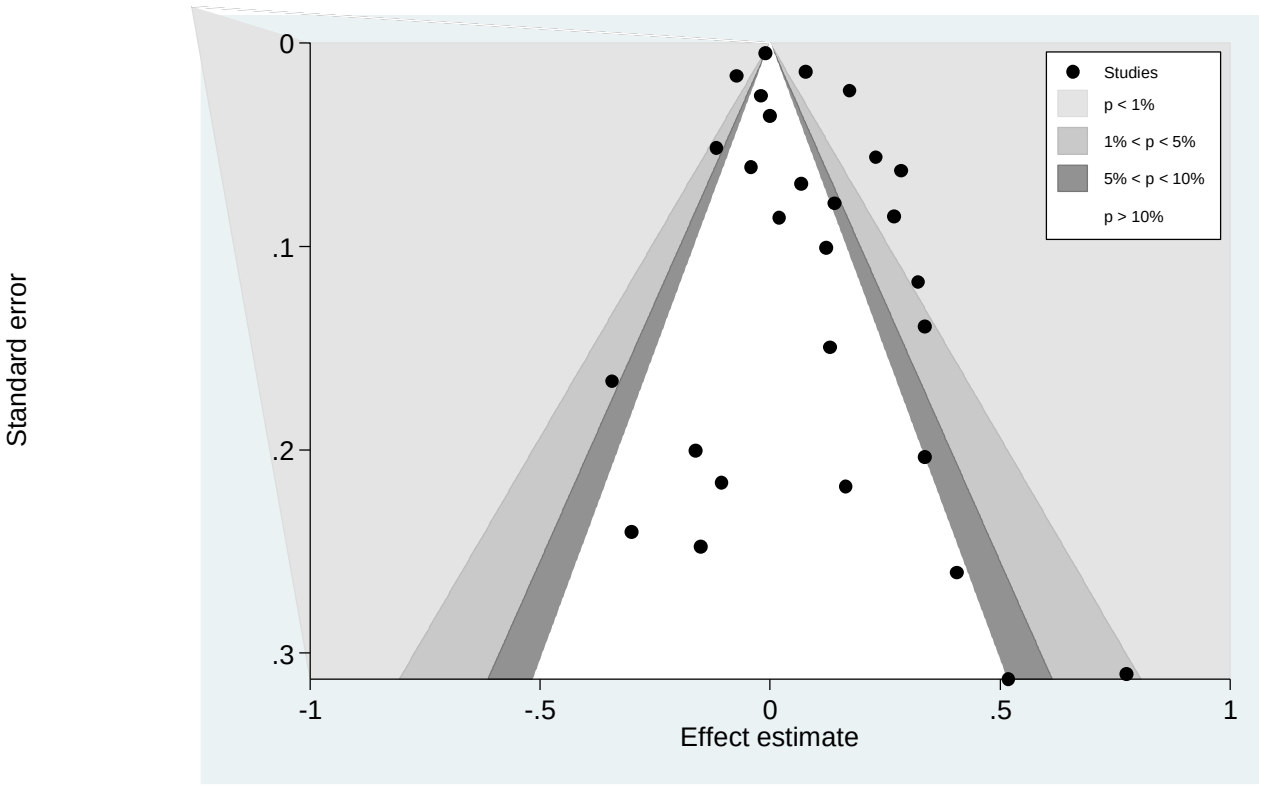


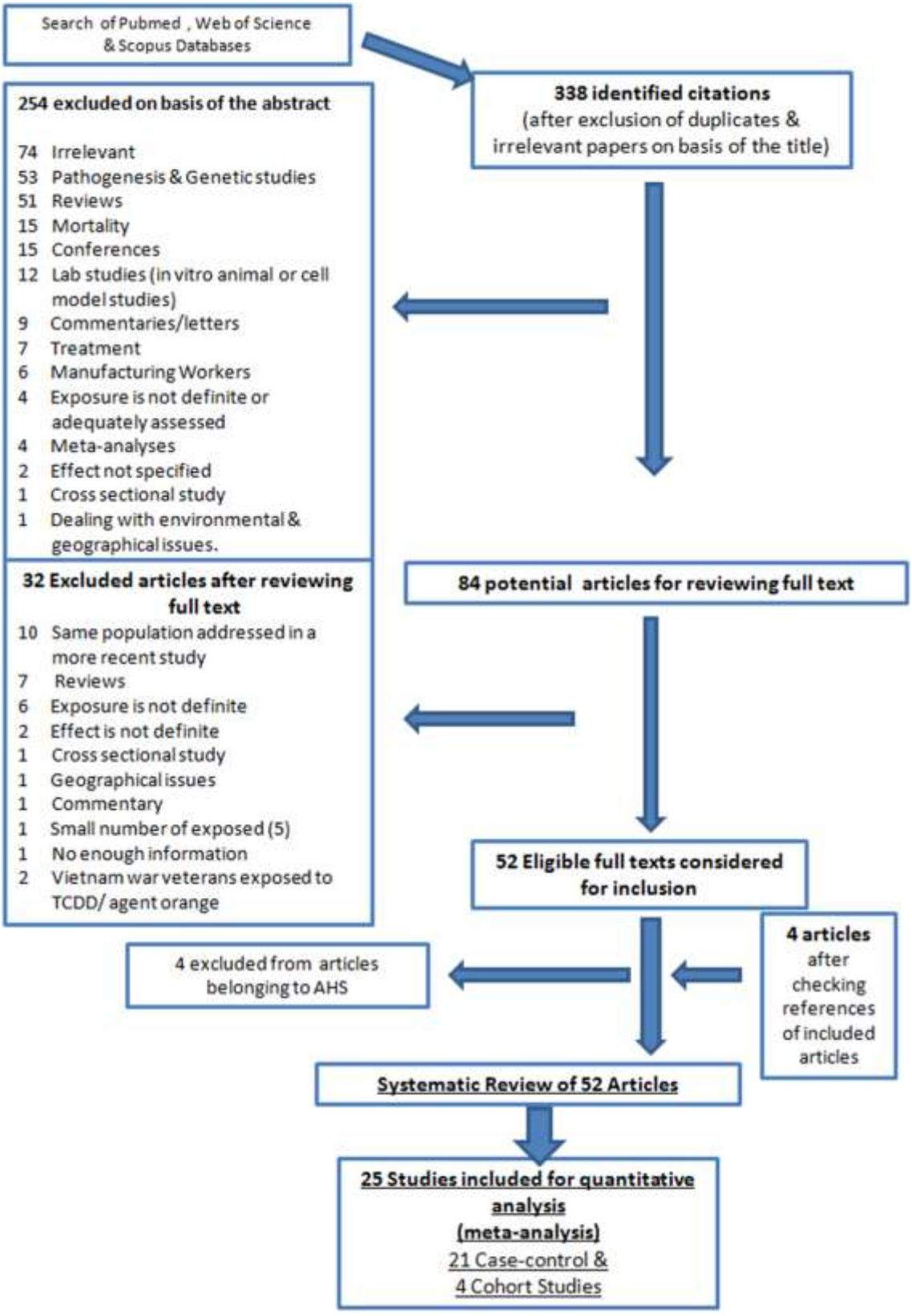
Figure 26. Contour enhanced funnel plots for the natural logarithm of prostate cancer risk among farmers versus their standard errors.

B) Pesticide exposure and Prostate cancer

1) Selection of the studies addressing pesticide exposure and prostate cancer

As seen in Figure 27 (flow chart summarizing the selection process of the articles), our search strategy yielded 338 articles after omitting duplicates and irrelevant articles. 254 were ineligible after reviewing the abstracts for not complying with our inclusion criteria. After reviewing full text of the 84 potentially eligible articles, 32 were excluded for various reasons as presented in the flowchart. 32 articles belonged to the same cohort, the AHS, but four were excluded for not providing estimates for the association under question. Hand searching of the references of the included articles resulted in four more articles that fit our inclusion criteria. Finally, we systematically reviewed 52 articles while 25 articles were included in our meta-analysis (covering 21 case-control and four cohort studies) as only the most recent article belonging to the AHS was included in the meta-analysis.

Figure 27. Flow chart for the selection process of the articles for the second meta-analysis (association between pesticide exposure & PC risk)



2) Characteristics of the studies addressing pesticide exposure and prostate cancer

Tables C and D (Appendix) display characteristics of the case-control (Aronson et al. 1996;Aronson et al. 2010;Band et al. 2011;Boers et al. 2005;Cockburn et al. 2011;Ewings and Bowie 1996;Forastiere et al. 1993;Fritschi et al. 2007;Hardell et al. 2006;Krstev et al. 1998;Meyer et al. 2007;Mills and Yang 2003;Multigner et al. 2010;Parent et al. 2009;Ritchie et al. 2003;Sawada et al. 2010;Settimi et al. 2003;Sharpe et al. 2001;Strom et al. 2008;Subahir et al. 2009;Van Der Gulden et al. 1995) and cohort studies (Dich and Wiklund 1998;Fleming et al. 1999;Koutros et al. 2013a;Zhong and Rafnsson 1996) that were included in the meta-analysis of pesticide exposure and prostate cancer. **Table E (Appendix)** displays articles belonging to the AHS that constituted part of the systematic review.(Alavanja et al. 2003;Barry et al. 2012;Beane Freeman et al. 2005;Bonner et al. 2007;Bonner et al. 2010;Christensen et al. 2010;De Roos et al. 2005;Delancey et al. 2009;Greenburg et al. 2008;Hou et al. 2006;Kang et al. 2008;Koutros et al. 2008;Koutros et al. 2009;Koutros et al. 2010a;Lee et al. 2004;Lynch et al. 2006;Lynch et al. 2009;Mahajan et al. 2006a;Mahajan et al. 2006b;Mahajan et al. 2007;Mozzachio et al. 2008;Purdue et al. 2007;Rusiecki et al. 2004;Rusiecki et al. 2006;Rusiecki et al. 2009;Samanic et al. 2006;van Bemmel et al. 2008)

For simplicity, we will comment in the following part on the 27 articles derived from the AHS simultaneously, due to their sharing common characteristics, followed by the other included studies. We could not include all the studies in the meta-analysis to avoid duplication of the results, and therefore we only included data reported by the most recent study. (Koutros S. et al, 2013) However, we have put much effort in

analyzing all of the studies that used data of the AHS, due to its being the most recent cohort study conducted that assessed exposure to a large number of pesticides.

Moreover, articles derived from this large prospective cohort are of high quality as regards many aspects. These include selection of the cohort members, detailed assessment of exposure, long follow up period and adjustment for many potential confounders. Strengths of articles derived from this study also include the huge size of the exposed individuals, prospective nature, and assessing different and specific pesticides.

2.1. Characteristics of the articles belonging to the Agricultural Health Study

The AHS is a large prospective cohort study that was established in 1993–1997. Persons applying for certification to use restricted-use pesticides in Iowa or North Carolina were enrolled. The whole cohort included 89,658 people, among which were 52,395 private applicators and 4,916 commercial applicators.(Agricultural Health Study 2015;Alavanja et al. 2003)

Since the start of the AHS, many articles have been published studying the association of tens of pesticides and many types of cancers and diseases. In our systematic review, we have included those that provided estimates for the association between any type of pesticide and prostate cancer risk. These constituted 28 articles; only the most recent one was included in our meta-analysis to avoid duplication of data.(Koutros et al. 2013a)

For the other 27 articles, number of exposed ranged between 3,657(Mozzachio et al. 2008) and a maximum of 41,035, (De Roos et al. 2005) while numbers of non-exposed ranged from 2,042 (Alavanja et al. 2003) and 45,774. (Barry et al. 2012) We have included articles published from 2003 till 2013.

1. Pesticides assessed by articles belonging to AHS

In case of 24 of the included 27 articles belonging to the AHS, a single specific pesticide was studied in each article. (Table E, appendix) Pesticides in general, OC pesticides and a number of specific pesticides were assessed by the remaining three studies.

2. Means of collecting pesticide exposure information by the AHS

Pesticide applicators were enrolled when they completed an enrollment questionnaire which sought information on the use of a large number of pesticides (ever/never), crops grown, personal protective equipment (PPE) used, pesticide application methods, other agricultural activities and exposures, nonfarm occupational exposures, smoking, alcohol consumption, a history of prostate cancer in first degree relatives and other data.(Alavanja et al. 2003)

For many pesticides, information was obtained on the duration of use (years) and frequency of use (days per year). Another take-home questionnaire included more detailed use information on the 28 pesticides reported as ever/never use in the enrollment questionnaire.(Alavanja et al. 2003)

In addition to the self-reported exposure information on pesticide use from questionnaires, data utilized included pesticide monitoring data form literature, the Pesticide Handlers Exposure Database, as well as results of Environmental Protection

agency pilot pesticide monitoring surveys, to estimate lifetime intensity of exposure to specific pesticides.

3. Quantification of exposure in the AHS

An exposure intensity score developed by Dosemeci et al was utilized by almost all the articles deriving data from the AHS.(Dosemeci et al. 2002) This score weights aspects of pesticide use that may modify the intensity of exposure, including whether an applicator personally mixed or prepared the pesticides for application, the repair of pesticides application equipment and the use of PPE during these activities. The

Intensity Weighted number of Lifetime exposure Days (IWLDs) was calculated by multiplying exposure intensity score by lifetime exposed days.

4. Adjustment for confounding factors in the AHS

All of the included studies belonging to the AHS adjusted for age, all except for three articles adjusted for family history of prostate cancer, sixteen adjusted for smoking and seven articles adjusted for race. Other factors that were adjusted for in different articles included alcohol consumption, use of other correlated pesticides, residence and education.

5. Effect estimators provided for the 27 included AHS articles

Twenty three out of the included 27 articles belonging to the AHS presented rate ratios for the association between the pesticide studied and prostate cancer. Two studies provided SIR, one study used OR and another one presented Hazard ratio.

2.2. Characteristics of the studies included in the meta-analysis

Publication Dates:

More than half of the retrieved studies were published in more recent years (2005-2013). The oldest article included dates back to the year 1993.(Forastiere et al. 1993) However, collecting information dates back as early as 1965 in the Swedish Study. (Dich and Wiklund 1998)

Geographical Distribution:

Half of the studies were conducted in USA or Canada (n = 13), nine were carried out in Europe (including 1 study in France, 1 in Great Britain and another in Iceland) as well as two studies for each of the following countries: Netherlands, Italy, and Sweden), while the remaining three studies were carried out in Australia, Japan and Malaysia.

Sample Size:

For the cohort studies included in our meta-analysis, sample size ranged from 2,449 (Zhong and Rafnsson 1996) to 54,412.(Koutros et al. 2013a) Number of cases ranged from a minimum of 49 (Parent ME *et al* 2009) and a maximum of 1,386 (Boers D *et al* 2005) studied prostate cancer cases. Controls ranged from 20 (Hardell L *et al* 2006) to 3,999 (Band PR *et al* 2011) in number. Thus, our meta-analysis included 8,688 cases and 15,381controls, as well as 126,757 subjects from cohort studies.

Pesticides assessed:

Fourteen studies assessed exposure to any pesticide, (Forastiere et al. 1993; Van der Gulden et al.1995; Aronson et al. 1996; Ewing and Bowei 1996; Krestev et al. 1998; Sharpe et al. 2001; Boers et al. 2005; Meyer et al. 2007; Strom et al. 2008; Parent et al. 2009; Subahir et al. 2009; Zhong and rafnsson 1996; Dich and Wicklund 1998; Fleming et al. 1999) nine examined a number of specific pesticides, (Mills et al. 2003; Ritchie et al. 2003; Settimi et al. 2003; Hardell et al. 2006; Fritschi et al. 2007; Aronson et al. 2010; sawada et al. 2010; Cockburn et al. 2011; Band et al 2011)while one study addressed chlordecone. (Multigner et al 2010) For the studies that have assessed a large number of pesticides, we have utilized OR presented for OCs (including Lindane, DDT, DDE or Organochlorines in general) as this was the common pesticide group among all the studies.

Methodology applied for assessment of pesticide exposure:

Five studies (20%) applied biological monitoring of studied pesticides, of which four measured serum level of the studied pesticides (Ritchie JM. et al 2003; Aronson K. et al 2010; Multigner L. et al 2010; Sawada N et al 2010), one study measured pesticides level in adipose tissue (Hardell L. et al 2006).

Seven (28%) relied only on self-reporting of pesticide exposure through questionnaires (Van der Guden et al 1995; Ewings P. and Bowei C. 1996; Krestev S et al 1998; Sharpe CR et al 2001; Meyer E et al 2007; Subahir MN. et al 2009; Koutros S. et al 2013), two studies utilized mainly Job exposure matrices (JEM) (Band PR et al 2011; Strom SS. et al

2008) while four (16%) depended on expert assessment of at job exposure (Boers D. et al 2005; Fritschi L. et al 2007; Aronson K. et al 1996; Parent ME. et al, 2004).

For the remaining seven studies (28%), assignment of exposure was less individualized as they depended mainly on similarities between groups of farmers or pesticide applicators as regards types, dates, patterns or locations of crops grown, or similarities in workplace conditions (from employment records) for assigning of probability and/or levels of pesticide exposure. (Forastiere F *et al* 1996, Zhong et Rafnsson 1996, Dich et Wicklund 1998, Fleming LE *et al* 1999, Mills PK et Yang 2003, Settimi L *et al* 2003, Cockburn M *et al* 2011).

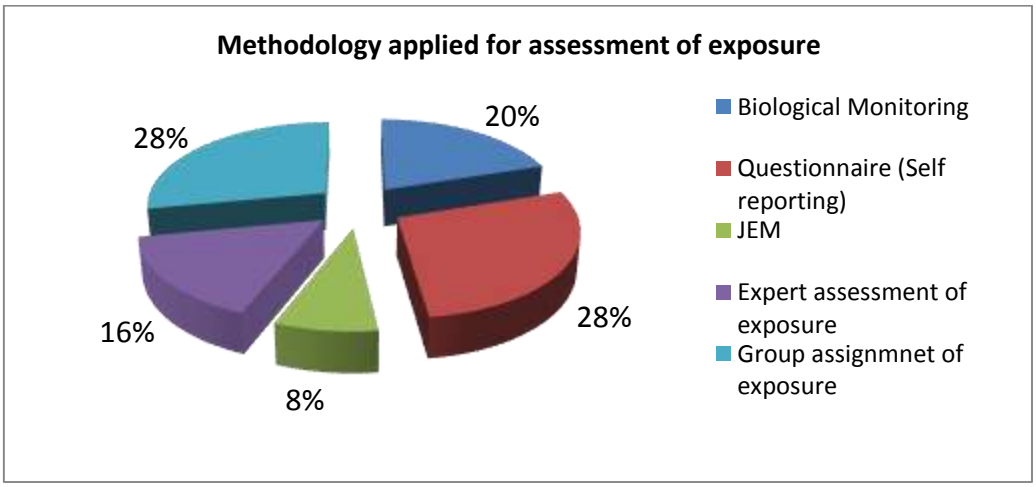


Figure 28. Pie chart showing the percentages of the different methodologies applied for assessment of exposure to pesticides

Therefore, as far as quantification of exposure is concerned, only five studies applied precise quantification and measured serum-or adipose- level of pesticides as was previously mentioned. Fifteen studies applied various semi-quantitative measures including six studies that compared the exposed by duration of lifetime exposure,

while the other nine studies evaluated life time cumulative exposure. They utilized different models that share in common comparing intensity levels, duration and/or probability of exposure. On the other hand, five studies where exposure was assessed dichotomously (exposed versus unexposed).

Population for which risk estimates were presented:

Four studies provided prostate cancer risk estimates for pesticide applicators while eleven were specific for farmers and six for general population. For the remaining four studies, they were constructed in a way so that information about at job exposure was collected from cancer patients and controls without specifying a single specific job in the results provided.

Types of control population for case-control studies:

General healthy population constituted the controls in case of half of the studies , while the other half used hospital controls that were diagnosed with other types of cancer (Forastiere F *et al* 1993 *,* Band PR *et al* 2011 , Settimi *et al* 2003) or Benign prostatic hyperplasia(BPH) or other urological complaints (Van der Gulden *et al 1995*, Aronson K *et al* 2010 and Hardell L *et al* 2006) or used two groups of controls including patients diagnosed with BPH as well as those hospitalized and treated for other diseases (Ewings P & Bowie C1996), or population controls as well as cancer patients. (Aronson K et al. 1996)

Effect estimators calculated:

Those provided in twenty of the studies were Odds Ratios (OR), while two studies presented Relative Risk (RR). Three out of the four included cohort studies calculated Standardized Incidence Ratio (SIR), comparing the incidence of prostate cancer among the studied cohort to its incidence among the general population of the area of the study. For simplicity, we refer to the effect estimator as OR.

Adjustment for potential confounders:

Almost all articles adjusted for age, as it was adjusted for in all except for two studies (Dich et Wicklund 1998, Zhong et Rafnsson 1996). However, only 5 studies adjusted for family history of prostate cancer (Settimi L *et al* 2003, Boers D et al 2005, Hardell L *et al* 2006, Multigner L *et al* 2010, Koutros S *et al* 2013). Other factors that were adjusted occasionally in some studies included race, smoking, alcohol intake, duration of employment and response to questionnaire.

Follow up of the cohort studies & recruitment periods of case-control studies:

Follow up of cohort studies ranged from 12 years (Fleming LE et al. 1999) and up to 38 years (Zhong Y et Rafnsson V 1996) while recruitment period for case-control studies ranged from 1 year (Cockburn M *et al* 2011) to 12.8 years (Sawada N et al. 2010), (mean = 4.75, median = 4 years).

3) Assessment of the quality of the articles included in the meta-analysis

As we were basically interested in detecting selection as well as information bias, therefore we have applied items proposed by the New-castle Ottawa Assessment Scale. Table F (Appendix) displays the scores given for each study according to assessing four sources of selection bias, three sources of information bias as well as adjustment for confounders (reflecting comparability between groups). Table G (Appendix) displays rating of the included case-control studies according to NOS scale. Table H (Appendix) displays grouping of these studies by different quality aspects.

We hypothesize a priori that a number of criteria might indicate accuracy, validity and thus quality of the studies. We consider that measuring serum levels of the studied pesticide potentially provide the possibility to obtain exposure indicators with higher specificity compared to indicators of external exposure. (Vlaanderen et al. 2008a) We also assume that considering expert judgment about probability, intensity, duration and frequency of exposure would produce more valid results than uniquely depending on information retrieved from Questionnaire. (McGuire et al. 1998) Stratifying the exposed into more categories according to exposure level would be expected to be more accurate than dichotomous stratification.

For case-control studies, potential sources of selection bias were observed. For example, general healthy population constituted the controls for only half of the studies. Also, 67% of case-control studies (n=14) suffered from potential sources of information bias mainly arising from lack of efficient assessment of exposure. Regarding comparability between cases and controls, age was adjusted for in all studies. However, only 5 studies (24%) adjusted for family history of prostate cancer.

(Boers et al. 2005;Hardell et al. 2006;Multigner et al. 2010;Settimi et al. 2003;Strom et al. 2008) We have also observed that Prostate specific antigen (PSA) screening rates differences was not commented on except in three studies. (Meyer et al. 2007;Multigner et al. 2010;Strom et al. 2008)

Methodological deficiencies were more pronounced for the three cohort studies (other than the AHS) that studied the association between pesticide exposure and prostate cancer risk. Potential sources of selection bias included comparing the observed incidence of prostate cancer among the exposed cohort to that expected in the general population, which inherently introduces bias due to the healthy worker effect. Also, ascertainment of exposure was not from a secure record (Zhong and Rafnsson 1996) or was relatively crude and based solely on licensure year. (Fleming et al. 1996) Outcome assessment was not adequate in the three studies. (Zhong and Rafnsson 1996; Fleming et al. 1996; Dich and Wiklund 1998) Regarding comparability between exposed and unexposed, there was no adjustment even for age in two studies (Zhong and Rafnsson 1996; Dich and Wiklund 1998)

4) Results reported by studies addressing pesticide exposure and prostate cancer

4.1. Results reported by articles of the AHS

There was an excess risk of prostate cancer among pesticide applicators of the AHS, compared to the general population, in spite of the low incidence of all cancers combined. However, Standardized Incidence Ratio (SIR) obtained was only modestly elevated; 1.14, 95% CI, 1.05−1.24.(Alavanja et al. 2003) Seven years later, similar results were updated (SIR 1.19, 95% CI, 1.14− 1.25). Nevertheless, authors also

calculated relative SIR, that puts into account the low incidence of all cancers combined among the pesticide applicators, and higher values were reported (1.66, 95% CI, 1.57- 1.77).(Koutros et al. 2010a)

On the other hand, no clear association could be detected when studying exposure to a large number of specific pesticides. In twenty (71.4%) of AHS articles, authors did not find an increased prostate cancer risk for exposure to all of the following pesticides: alachlor, atrazine, glyphosate, diazinon, pendimethalin, cyanazine, phorate, metachlor, dicamba, malathion, carbaryl, organochlorines, captan, trifluralin, chlorothalonil, thiocarbamate herbicide (EPTC), metribuzin, imazethapyr and metachlor.(Lee et al. 2004; Rusieck et al. 2004; Beane et al. 2005; De Roos et al. 2005; Hou et al. 2006; Lynch et al. 2006; Mahajan et al. 2006; Rusieck et al. 2006; Samanic et al. 2006; Bonner et al. 2007; Mahajan et al. 2007; Purdue et al. 2007; Greenburg et al. 2008; Kang et al. 2008; Koutros et al. 2008; Mozzachio et al. 2008; van Bemmel et al. 2008; Delancey et al. 2009; Koutros et al. 2009; Rusieck et al. 2004)

A significant association was found for only very limited types of pesticides as in case of butylate, RR for the highest exposed categories compared to non-exposed 2.09(1.27-3.44). A suggestive association was reported for terbufos, RR for tertile 2 and 3 were 1.28(1.06-1.55) and 1.21(0.99-1.47) respectively. (Rusieck et al 2009; Lynch et al. 2009) For the most recent study belonging to the AHS that examined the association between lifetime cumulative exposure to 48 different pesticides and risk of prostate cancer, (Koutros et al. 2013a) a positive association was found for the highest quartile of exposure to only three organophosphates (fonofos, malathion and terbufos) and the OC aldrin.

As seen in Table 5, a consistent finding of the AHS is an observed significant association between exposure to certain pesticides and prostate cancer among those with a positive family history. A family history-pesticide exposure interaction was reported for several specific pesticides that include Phorate, Fonofos, Butylate and Coumaphos.(Alavanja et al. 2003;Christensen et al. 2010;Koutros et al. 2013a;Mahajan et al. 2006a)

Table 5. Relative Risks, Interaction odds ratios, 95% CI for prostate cancer associated with different levels of pesticide exposure among pesticide applicators with a positive family history of prostate cancer

Study, Year	Pesticide	RR, 95% CI for those with a family history	Interaction OR¶	Intensity of exposure	Referent Category
Alavanja MCR, 2003	Alachlor	1.36 (0.88 – 2.10)	1.50 (0.93 – 2.41)	Ever exposed	Those with a family history (FH) of PC but never exposed to the studied pesticides
	Aldicarb	1.60 (0.83 – 3.09)	2.01 (0.95 – 4.23)		
	Atrazine	1.28 (0.77 – 2.12)	1.52 (0.80 – 2.62)		
	Butylate	**1.78 (1.16 – 2.73)**	**1.93 (1.19 – 3.11)**		
	Carbofuran	**1.81 (1.18 – 2.77)**	1.58 (0.98 – 2.55)		
	Chlorpyrifos	1.29 (0.84 – 1.98)	**1.65 (1.02 – 2.66)**		
	Coumaphos	**2.17 (1.24 – 3.82)**	**2.58 (1.29 – 5.18)**		
	Dicamba	1.35 (0.88 –2.08)	1.51 (0.95 – 2.43)		
	Fonofos	**1.80 (1.14 – 2.84)**	**2.04 (1.21 – 3.44)**		
	Permethrin	**2.38 (1.34 – 4.25)**	**2.31 (1.17 – 4.56)**		
	Phorate	**1.67 (1.09 – 2.56)**	**1.64 (1.02 – 2.63)**		
	Terbufos	1.45 (0.95 – 2.23)	1.52 (0.94 – 2.45)		
Mahajan R, 2006	Fonofos	1.09 (0.61 – 1.95)	**1.27 (1.00 – 1.51)**	T1	Non exposed with no FH
		1.94 (1.16 – 3.25)		T2	
		1.83 (1.12 – 3.00)		T3	
Mahajan R, 2006	Phorate	1.90 (0.80 – 4.50)	1.53 (0.99 –2.37)	T2	Non exposed with no FH
		1.91 (0.86 – 4.24)		T3	
Koutros S, 2008	Dichlorvos	1.18 (0.73 – 1.82)	–	Ever	Non exposed with no FH
		1.29 (0.69 – 2.40)		T1	
		0.72 (0.34 – 1.55)		T2	
		1.42 (0.75 – 2.70)		T3	
Lynch SM, 2009	Butylate	**1.67 (1.01 – 2.78)**	**1.34(1.00 – 1.76)**	High exposed	Non exposed
		2.00(1.07 – 3.74)	1.37 (0.67 – 2.80)	High exposed	Low exposed
Christensen CH, 2010	Coumaphos	**1.65(1.13 –2.38)**	**1.91(1.23 – 2.95)**	Ever exposed	Non exposed with no FH
Multigner L, 2010	Chlordecone	0.97 (0.33 – 2.83)	**<0.001***	T1	Non exposed with no FH
		3.22(1.03 – 10.05)		T2	
		3.00(1.12 – 8.07)		T3	
Barry KH, 2012	Methyl bromide	1.46 (0.97 – 2.20)	0.05*	Ever	Non exposed With no FH
		1.67 (0.98 – 2.84)	0.19*	T1	
		1.28(0.71 – 2.30)		T2	
		1.42(0.78 – 2.59)		T3	
Koutros S, 2013	Fonofos	0.91(0.55 – 1.49)	**0.04***	Q1	Non exposed with no FH
		1.70(1.07 –2.72)		Q2	
		1.22 (0.74 – 1.99)		Q3	
		2.01(1.36 – 2.99)		Q4	
	Dieldrin	1.55 (0.63 – 3.82)		T2	
		1.54 (0.62 – 3.83)	0.73*	T3	

¶ Interaction odds ratio is a method of assessing the influence of a FH of prostate cancer on pesticide associated risk. A cross product term was included in the logistic model: age + FH + pesticide exposure + (FH x pesticide exposure). (Alavanja MCR *et al*, 2003). It can be interpreted as the ratio of the joint effect of exposure and positive family history versus the expected effect of each singly.(Christensen CH *et al*, 2010)

All studies belong to the AHS except for Multigner L *et al*, 2010.

Bold ORs represent the statistically significant associations.

* = P value of interaction

FH = family history

T1, T2, T3= Tertiles by Intensity weighted lifetime days of exposure.

Q1, Q2, Q3, Q4= Quartiles by Intensity weighted lifetime days of exposure

4.2. Results presented by the studies included in our meta-analysis

4.2.1. Qualitative analysis of the results

The findings of the 21 included case-control studies were inconsistent but could be summarized as follows. No association could be detected in five studies, (Aronson KJ. 1996; Ewing P and Bowie C. 1996; Fritschi L. et al 2007; Sawada N. et al 2010; Aronson KJ. et al 2010), four of which applied accurate methodologies, with low potential selection and information bias. Two of these studies depended on expert assessment of exposure based on detailed information of every job held by each subject through combining information from subject questioning and workplace measurement data ((Aronson KJ. 1996; Fritschi L. et al 2007), while two applied measuring serum level of the studied pesticides. (Sawada N. et al 2010; Aronson KJ. et al 2010)

Furthermore, a negative association has been found in a large population-based case-referent study that examined prostate cancer risk for a large number of job exposures utilizing expert assessment of exposure. (Boers D. et al 2005) An insignificant association was reported by three case-control studies. (Forastiere F. et al 1993; Van Der Gulden JW. Et al 1995; Hardell L. et al 2006)

On the other hand, twelve studies (57%) reported significant associations (Krstev et al. 1998; Sharpe et al , Mills et al. 2003 , Ritchie et al. 2003, Settimi et al. 2003 , Meyer et al. 2007 ; Strom et al. 2008 ; Subahir et al. 2009 ; Parent et al. 2009 ; Multigner et al. 2010 ; Cockburn et al. 2011; Band et al. 2011) However, the association was confined to specific conditions in the majority of studies. For example, an increased prostate cancer risk was observed only among farmers employed for short durations (Krstev et al 1998) and among those who farmed less than five years. (Meyer et al 2007)

Also, an association was found among Hispanics (Mills et al. 2003; Strom et al. 2008) and among Caucasians farmers but not for African Americans, (Meyer et al. 2007) which might conflict with the fact that the highest incidences of prostate cancer are observed among Afro-American peoples. An association was also found among farmers with high pesticide expsoure, though types of pesticides were not systemically assessed and the study was limited by the small number of cases (49 farmers). (Parent et al. 2009)

Furthermore, cases were found to be more likely exposed to pesticides when authors depended only on self-report of ever been exposed, (Subahir et al. 2009) or more frequently exposed (defined as once a week or more for ≥ 6 months) during leisure activities (Sharpe et al. 2001) which could be considered as less accurate methodologies.

The association was more evident for certain pesticides; particularly organochlorines. Example for this was the association found in case of exposure to chlordecone, (Multigner L. et al, 2010), DDT and dicofol (Settimi et al. 2003; Band et al. 2011) or long term exposure to specific organochlorine pesticides as lindane and heptachlor (Mills et al. 2003; Ritchie et al. 2003), or environmental exposure to the overall use of organochlorine pesticides. (Cockburn et al. 2011)

Regarding the included cohort studies, other than the AHS, results were as follows: there was no increased incidence of prostate cancer among pesticide applicators in Iceland compared to the general population, contrary to the increased risk of other cancer types among the studied population. (Zhong and Rafnsson 1996) Also, an association was only confined to being licensed for agricultural pesticide applicators

between 1965 and 1976. (Dich and Wiklund 1998) On the other hand, a significant elevated risk of prostate cancer was detected among pesticide applicators in Florida compared to the general population, although exposure measures were solely based on information about pesticide application licensure year and duration of exposure. (Fleming et al. 1999)

4.2.2. Quantitative analyses of the results (Meta-analyses)

Pooled estimate for the lowest exposed group:

Pooled ORs for the lowest exposed groups versus the non-exposed for the case-control studies was 1.02, 95% CI, 0.88–1.17 (I^2=0.00%, p = 0.622).

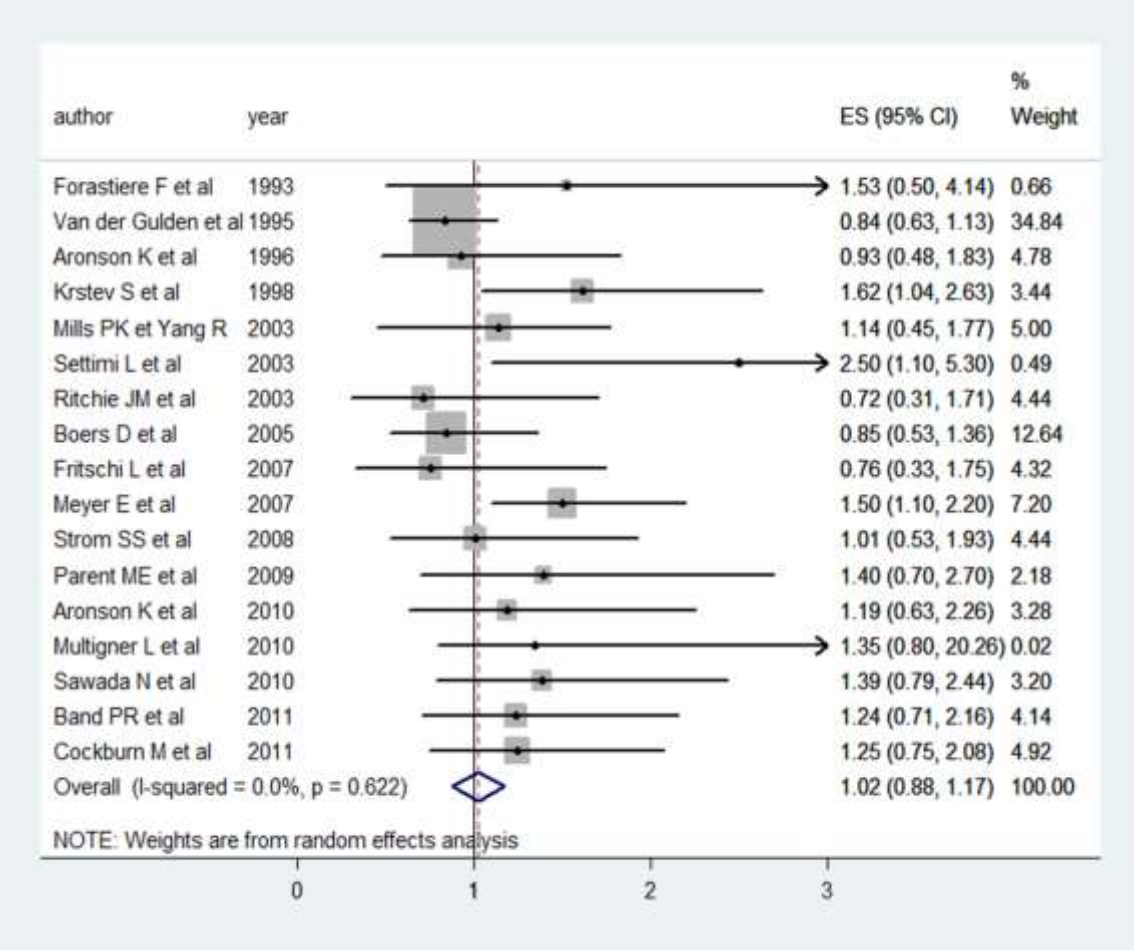


Figure 29. Forest Plot displaying random-effects meta-analysis of the association between low exposure to pesticides and prostate cancer risk (Case-control studies)

Pooled estimate for the highest exposed groups:

However, pooled ORs for the highest exposed categories was 1.33, 95% CI, 1.02−1.63, but heterogeneity was greater than that expected by chance (I^2 = 44.8%, p= 0.024). (NB. Only one cohort study provided RR for high pesticide exposure)

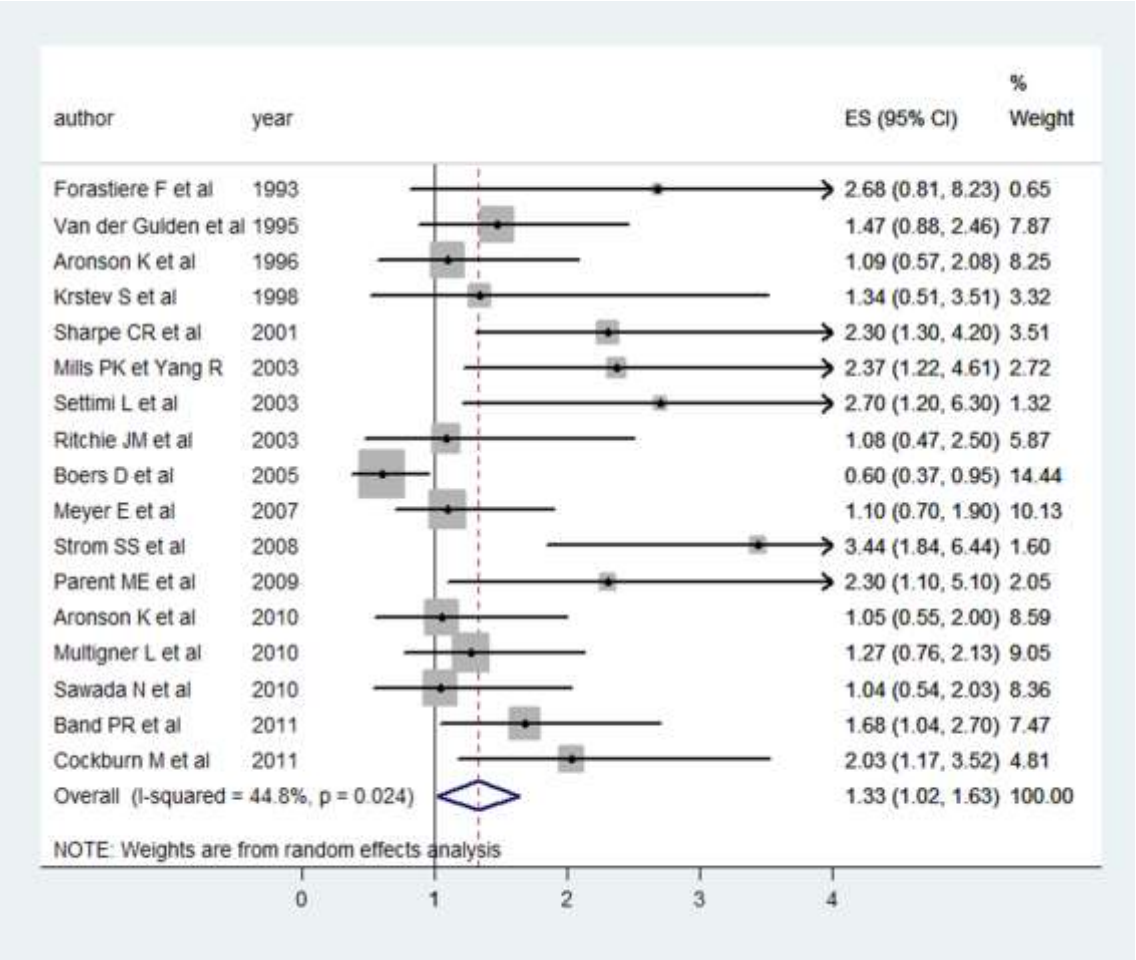


Figure 30. Forest Plot displaying random-effects meta-analysis of the association between high exposure to pesticides and prostate cancer risk
(Case-control studies)

Pooled estimates for ever/never exposed to pesticides:

On the other hand, pooled OR for ever versus never been exposed to pesticides from case-control studies was 1.27, 95% CI, 0.92 −1.63, yet heterogeneity was observed (I^2=54.3%, p = 0.016). For the cohort studies, we obtained the same pooled estimate and high degree of heterogeneity was observed; 1.27, 95% CI, 0.65 −1.89 (I^2=95.9%, p = 0.000).

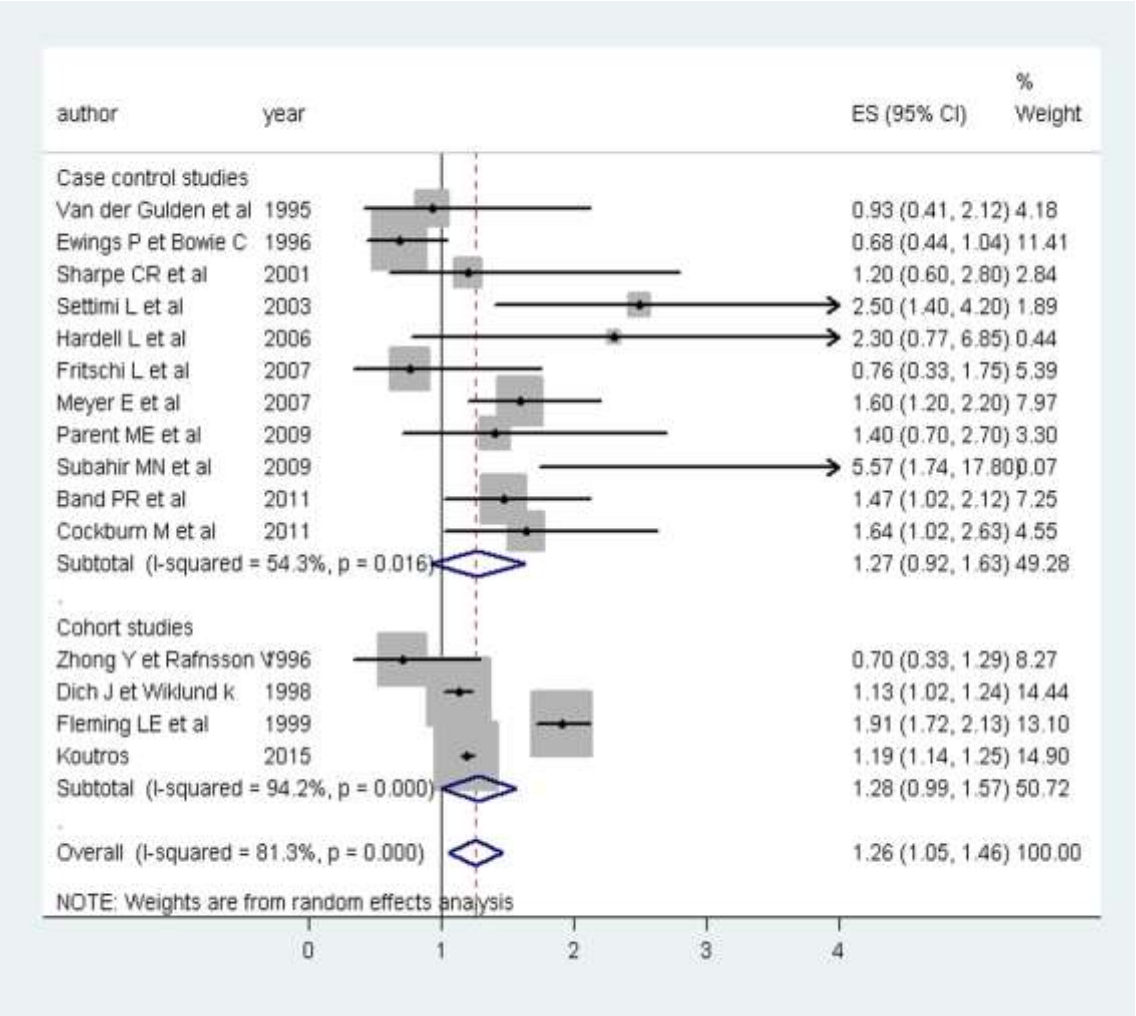


Figure 30. Forest Plot displaying random-effects meta-analysis of the association between ever versus never been exposed to pesticides and prostate cancer risk (Stratification by the design of the studies)

4.2.2.1. Exploring Heterogeneity

Homogeneity was revealed for the highest exposed categories when stratifying case-control studies by a number of variables as follows:

As seen in Table 6, pooled OR of the five studies (Ritchie et al. 2003; Boers et al. 2005; Aronson et al. 2010; Multigner et al. 2010 ; Sawada et al. 2010) that applied more precise exposure assessment (scoring 3 stars, NOS) showed no association; with a pooled OR equal 0.85, 95% CI, 0.57-1.14, I^2 = 16.2%, p = 0.311. This was contrary to the high and significant pooled estimate for the four studies (Forstiere et al. 1993; Sharpe et al. 2001; Parent et al. 2009; Cockburn et al. 2011) that assessed exposure to pesticides less precisely (1star, NOS); pooled OR, 2.19, 95% CI, 1.38-3.00, I^2 = 0.00%, p = 0.982. However, we have noted that four out of the five studies in the first group did not report specific occupation, as these ORs are for the highest serum level of pesticides detected in the general population. On the other hand, farmers were the exposed population in three out of the four studies in the second group.

Table 6 . Pooled estimates, 95% CI, I^2 & p values for homogeneity between case-control studies presenting prostate cancer risk estimates for high exposure to pesticides by grouping by Quality of the studies and methodology adopted for assessment of pesticide expsoure

Grouping	Studies included in the analysis	No of Studies	Pooled OR	CI (95%)	Heterogeneity I^2 (%)	Heterogeneity P value
Overall quality of the studies according to NOS	High quality	4	*0.88*	*0.53–1.23*	34.2%	0.207
	Medium quality	11	**1.43**	**1.12 –1.75**	**2.20%**	0.421
	Low quality	2	*2.09*	*0.97 –3.21*	**0.00%**	0.743
Exposure assessment quality (NOS)	3 Stars	5	*0.85*	*0.57 –1.14*	**16.2%**	0.311
	2 Stars	8	**1.42**	**1.06 –1.77**	**4.90%**	0.392
	1 Star	4	**2.19**	**1.38–3.00**	**0.00%**	0.982
Methodology adopted for assessment of pesticide exposure	Measuring serum level of pesticides	4	*1.12*	*0.74–1.50*	**0.00%**	0.966
	Expert Judgment	3	*0.90*	*0.29–1.51*	49.2%	0.140
	Mainly depended on JEM	2	*2.22*	*0.63–3.81*	49.8%	0.158
	Self reporting	4	*1.34*	*0.91–1.77*	**0.00%**	0.493
	Group-level exposure assessment Ω	4	**2.24**	**1.36–3.11**	**0.00%**	0.955

Pooled ORs are in bold if they demonstrate statistical significance and present homogenous studies, and in italics when statistically insignificant. I^2 is in bold when reflecting homogeneity.

Ω Either depended mainly on aggregate non individualized data or utilized employment records to broadly categorize types of exposure

We have also sub-grouped case-control studies, in order to explore if methodology adopted by the studies could be related to the pooled estimates obtained. The association remained significant only for studies applying grouped assignment of exposure; pooled estimate was 2.24, 95% CI, 1.36 −3.11. For self reporting of exposure (depended only on questionnaire), the pooled estimate was not significant; 1.34, 95% CI, 0.91-1.77.

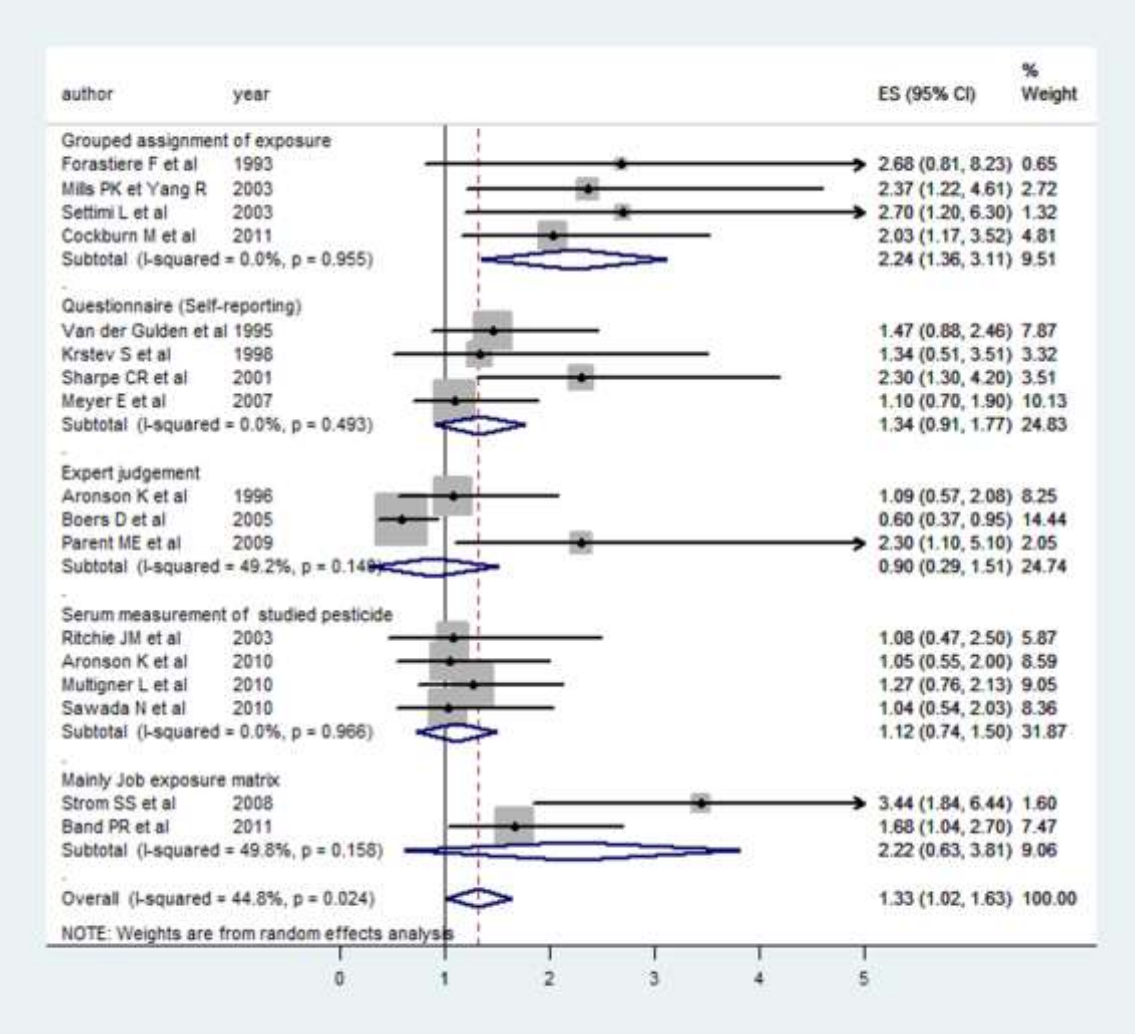


Figure 32. Forest plot displaying stratification of case-control studies by the methodology applied for assessment of pesticide exposure

Stratifying by the studied population has also revealed homogeneity for farmers as well as for the general population. As seen in Figure 32, pooling ORs for high pesticide exposure among farmers have shown a positive significant association, pooled OR, 1.47, 95% CI, 1.05-1.90, I^2 = 0.00%, p = 0.572.

However, on further sub-grouping of these studies that were conducted exclusively for farmers, the association remained high and significant only for studies that applied grouped assignment of exposure, pooled OR, 2.50, 95% CI, 1.18-3.82, I^2 = 0.00%, p = 0.973. On the other hand, studies that applied more individualized exposure assessment by assessing pesticide exposure by self-reporting did not show an association, pooled OR, 1.13, 95% CI, 0.58-1.69, I^2 = 0.00%, p = 0.771

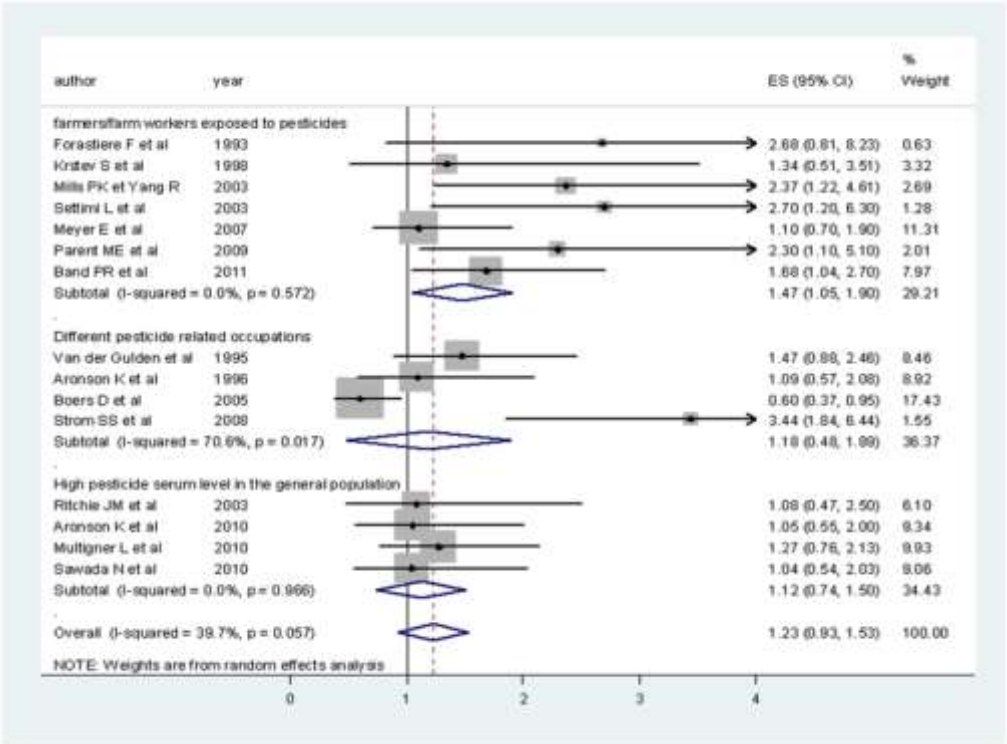


Figure 33. Forest plot displaying stratification of case-control studies by the studied population

Table 7. Pooled estimates, 95% CI, I^2 & p values for homogeneity between case-control studies by grouping by exposed population

Grouping	Studies included in the analysis	No of Studies	Pooled OR	CI (95%)	Heterogeneity I^2 (%)	Heterogeneity P value
Design of the studies as regards exposed population @	Farmers/Farm workers exposed to pesticides	7	**1.47**	**1.05–1.90**	**0.00%**	0.572
	High pesticide serum levels among general population ™	4	*1.12*	*0.74–1.50*	**0.00%**	0.966
	Different pesticide related occupations ∑	4	*1.18*	*0.48–1.89*	70.6%	0.017
Methodology applied for exposure assessment in studies of farmers ÷	Self reporting only (Questionnaire)	2	*1.13*	*0.58–1.69*	**0.00%**	0.771
	Group-level exposure assessment	3	**2.50**	**1.18–3.82**	**0.00%**	0.973
Type of pesticide studied in studies of farmers	OC pesticides	3	**1.88**	**1.17– 2.60**	**0.00%**	0.625
	Any Pesticide	4	*1.25*	*0.72–1.78*	**0.00%**	0.597

Pooled ORs are in bold if they demonstrate statistical significance and present homogenous studies, and in italics when statistically insignificant. I^2 is in bold when reflecting homogeneity.

@ Two studies are not included, one measured pesticide application during leisure activity (Sharpe CR et al, 2001) and the other measured ambient pesticide exposure in agriculturally intensive areas (Cockburn M et al, 2011)

™ These studies reported ORs for men who have the highest levels of serum pesticides compared to those with the lowest detected levels, without relating it to a specific occupational exposure.

∑ The occupationally exposed include different groups of pesticide applicators as farmers, horticulturalists, dock workers, ranchers, lawn care workers, etc.

÷ Two studies were not included in this stratification, one study used JEM (Band PR et al 2011) and the other one applied expert assessment of exposure (Parent M et al, 2009).

Moreover, as observed in table 8, sub-stratifying studies by type of studied pesticide revealed an impact of the quality of the studies. Lower overall quality as well as quality of exposure assessment seems to consistently exaggerate the association.

Table 8. Pooled estimates, 95% CI, I^2 & p values for homogeneity between case-control studies by grouping of studies by type of evaluated pesticides

Grouping	Studies included in the analysis	No of Studies	Pooled OR	CI (95%)	Heterogeneity I^2 (%)	Heterogeneity P value
Type of pesticide studied	Organochlorine (OC) pesticides	8	**1.35**	**1.02–1.67**	**0.00%**	0.539
	Pesticides in general	9	*1.32*	*0.83–1.80*	55.9%	0.020
Exposure assessment quality of studies about OC*	3 Stars	4	*1.12*	*0.74– 1.50*	**0.00%**	0.966
	2 Stars	3	**1.88**	**1.17–2.60**	**0.00%**	0.625
Overall quality (NOS) of studies about pesticides in general(Any pesticide)	Higher quality studies	2	*0.72*	*0.31–1.12*	29.1%	0.235
	Medium quality studies	6	**1.55**	**1.03–2.06**	**19.6%**	0.285
Exposure assessment quality of studies about pesticides in general ¶	2 Stars	5	*1.27*	*0.87– 1.67*	**4.80%**	0.380
	1 Star	3	**2.33**	**1.22–3.45**	**0.00%**	0.982

Pooled ORs are in bold if they demonstrate statistical significance and present homogenous studies, and in italics when statistically insignificant. I^2 is in bold when reflecting homogeneity.

* Only one study received one star.

¶ One study only has three stars, therefore not included in the sub-stratification.

Sub-stratifying studies by other variables that we thought might be responsible for the observed heterogeneity have also showed some interesting results. This includes a higher pooled estimate for studies where control group were cancer patients. Also, pooling results of USA and Canada provided higher and significant ORs than those conducted in European countries.

We have observed that publication dates of the studies did not coincide with the dates of prostate cancer diagnosis and collecting of data. Therefore, when we explored the effects of these dates on the pooled estimates, it was interesting to note that there was no effect of publication dates on the pooled estimates (Figure 34). However, stratifying studies by decades of prostate cancer diagnosis showed that those diagnosed in the eighties provided the higher and significant associations.

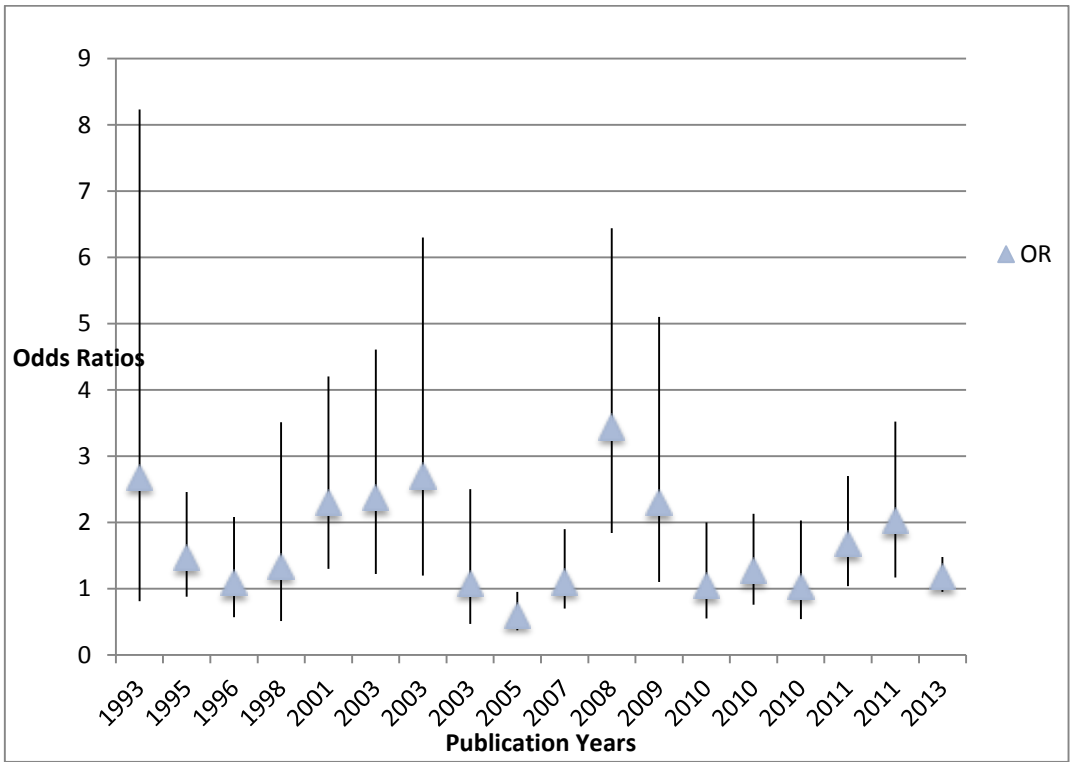


Figure 34. Plot of years of publication against OR of prostate cancer for high pesticide exposure (Detection of the effect of publication date)

Table 9. Pooled estimates, 95% CI, I^2 & p values for homogeneity between case-control studies by various grouping of studies (subset analyses)

Grouping	Studies included in the analysis	No of Studies	Pooled OR	CI (95%)	Heterogeneity I^2 (%)	Heterogeneity P value
Type of control #	Hospital based ¥	7	**1.41**	**1.01– 1.80**	**0.00%**	0.680
	Population based	9	*1.36*	*0.89 –1.83*	61.4%	0.008
Adjustment for family history of PC	Not adjusted	13	**1.33**	**1.07–1.60**	**0.00%**	0.696
	Adjusted	4	*1.38*	*0.47–2.28*	72.1%	0.013
Geographical location of the study α	European Countries	5	*1.16*	*0.56–1.77*	58.0%	0.049
	USA or Canada	11	**1.41**	**1.09 – 1.73**	**8.80%**	0.360
Publication Years	1993-2001	5	*1.41*	*0.93 – 1.89*	**0.00%**	**0.624**
	2003-2008	6	*1.30*	*0.66 –1.95*	63.1%	0.019
	2010-2013	6	*1.34*	*0.99 –1.69*	**0.00%**	0. 531
Decade of PC diagnosis τ	Eighties	8	**1.54**	**1.14 – 1.95**	**0.00%**	0.740
	Nineties	5	*0.94*	*0.57 – 1.32*	42.3%	0.139
	After year 2000	4	*1.56*	*0.88 – 2.24*	34.6%	0.205

Pooled ORs are in bold if they demonstrate statistical significance and present homogenous studies, and in italics when statistically insignificant. I^2 is in bold when reflecting homogeneity.

One study used two used two distinct groups of controls, population and cancer controls (Aronson KJ et al, 1996)

¥ Hospital controls included: Cancer controls (4 studies), Benign prostatic hyperplasia (2 studies), other diseases (1 study).

α One missing study (Sawada Net al, 2010), Japan.

τ We have observed that years of prostate cancer diagnosis did not coincide with publication years.

Consistent results were obtained from the meta-regression analysis, as quality of exposure assessment and adjusting for family history were significant factors ($P = 0.003$ and $P = 0.041$, respectively), with these two variables explaining most between-study variability (adjusted $R^2 = 58\%$).

4.2.2.2. Sensitivity Analysis

Results of sensitivity analyses are displayed in Table 10. Homogeneity was observed after deleting studies reporting extreme ORs, but with no change in the pooled estimate which might add to the robustness of our results. Worth mentioning is the high significant association when pooling estimates of high exposure among those with a positive family history of prostate cancer as well as for developing aggressive forms of the disease.

Since we have previously conducted subset analysis without including cohort studies, we re-evaluated the results after including the cohort study that provided RR for high pesticide exposure, (Koutros S. et al 2013) but results did not change.

We have also observed that homogeneity for ever/never exposed to pesticides was revealed when eliminating one study from case-control studies. However, the high degree of heterogeneity observed between the three cohort studies that reported SIRs could not be resolved.

Table 10. Pooled estimates, 95% CI, I^2 & p values for homogeneity between studies: Sensitivity Analysis

Grouping	Number of studies	Pooled OR	CI (95%)	Heterogeneity I^2 (%)	P value
Pooled ORs for high exposure to pesticides					
- All	18	1.27	1.01– 1.52	42.6%	0.029
- Case-control studies	17	1.33	1.02 – 1.63	44.8%	0.024
- Eliminating two studies providing the most extreme ORs Ω	15	**1.34**	**1.09 – 1.58**	**0.00%**	0.746
Pooled ORs for high exposure to pesticides, Case control studies using population controls					
- All	9	1.36	0.89 – 1.83	61.4%	0.008
- Eliminating two studies providing the most extreme ORs Ω	7	**1.34**	**1.00 – 1.67**	**0.00%**	0.746
Pooled ORs for ever/never exposed to pesticides (case-control studies)					
- All	11	1.27	0.92 – 1.63	54.3%	0.016
- Eliminating one study with an extreme OR ¥	10	**1.40**	**1.14 – 1.67**	**1.90%**	0.422
- Pooled ORs for high exposure to pesticides and having a positive family history of prostate cancer	3	**2.23**	**1.05 – 3.41**	**0.00%**	0.646
- Pooled ORs for high exposure to pesticides and developing aggressive forms of PC	3	1.80	1.03 – 2.57	29.5%	0.242

Pooled ORs are in bold if they demonstrate statistical significance and present homogenous studies.

ORs = Odds ratios, SIRs = Standardized incidence ratios.

Ω Boers D *et al*, 2005 & Strom SS *et al*, 2008.

¥ Ewings P & Bowie C, 1996.

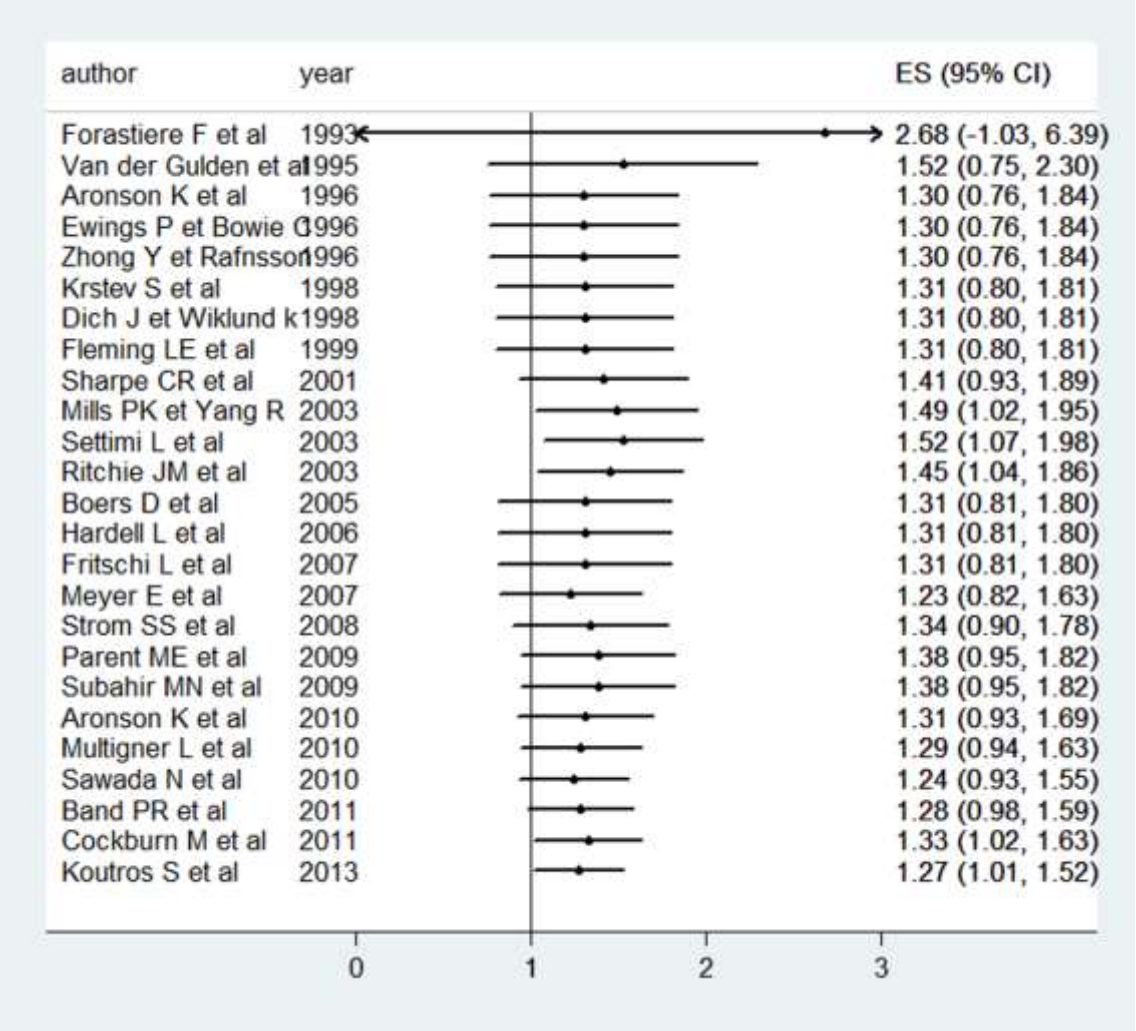


Figure 35. Forest plot displaying cumulative pooled estimates of Prostate cancer for high pesticide exposure

4.2.3. Detection of Publication bias

Figure 36 and 37 display contour enhanced funnel plots with corresponding random-effects pooled estimates across studies for low and high pesticide exposure respectively.

As seen in Figure 36, there was no visual evidence of asymmetry when pooling estimates for low exposure to pesticides (Egger's Test: bias coefficient = 0.572, SE = 0.593, p = 0.348). However, asymmetry could be observed for high pesticide exposure (Egger's Test: bias coefficient = 1.547, SE = 0.808, p = 0.072) (Figure 37).

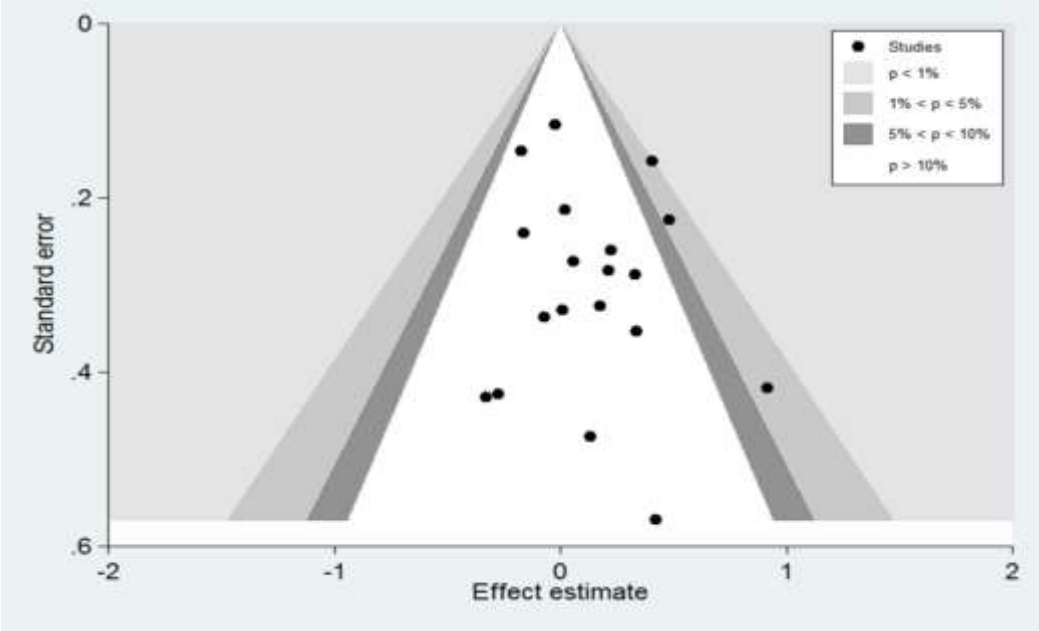


Figure 36. Contour enhanced funnel plots for the natural logarithm of prostate cancer risk estimates for low exposure to pesticides versus their Standard errors

(Each circle presents estimate presented by a study. Contour lines differentiate the significance and non significance regions at 1%, 5% and 10% significance levels. Note the almost symmetrical dispersion of studies)

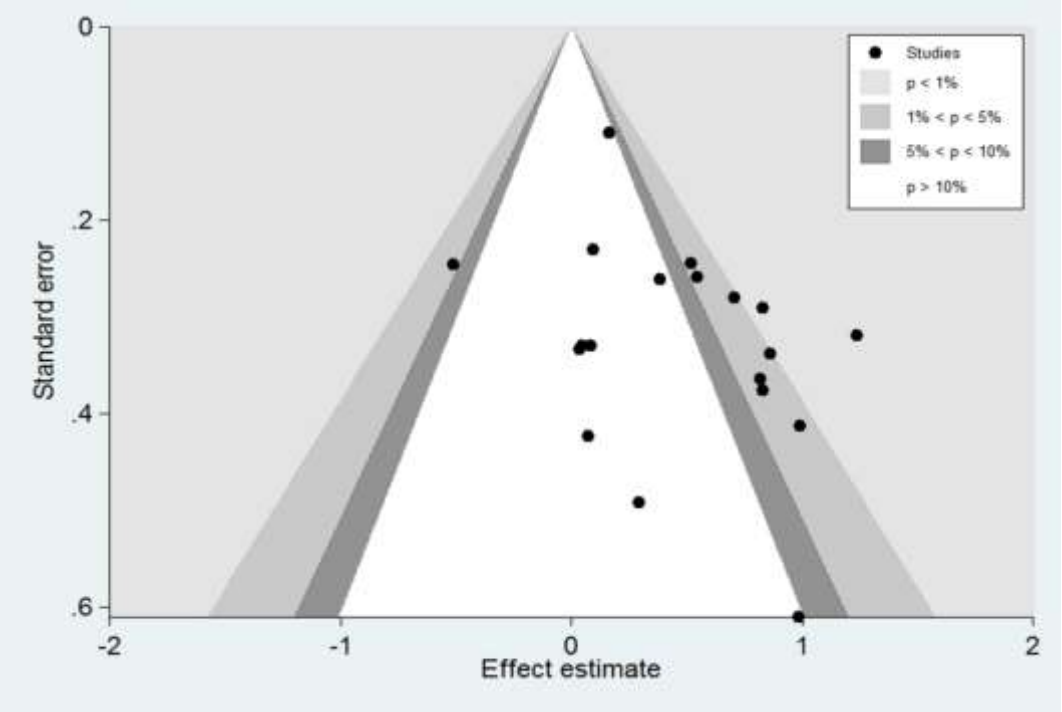


Figure 37. Contour enhanced funnel plots for the natural logarithm of prostate cancer risk estimates for high exposure to pesticides versus their standard errors

(Note the large number of scattered studies at the right part that tends to present the significant association while studies seem to be missing in areas of non-significance)

We have also been interested to examine if the detected publication bias was more apparnet for a group of studies more than another. Therefore, we repeated the contour enhanced funnel plot seperately by the overall quality of the studies as well as by the exposure assessment quality. As displayed in figures 38 and 39, we have noted that studies with lower quality seem to be the source of publication bias than the better quality studies.

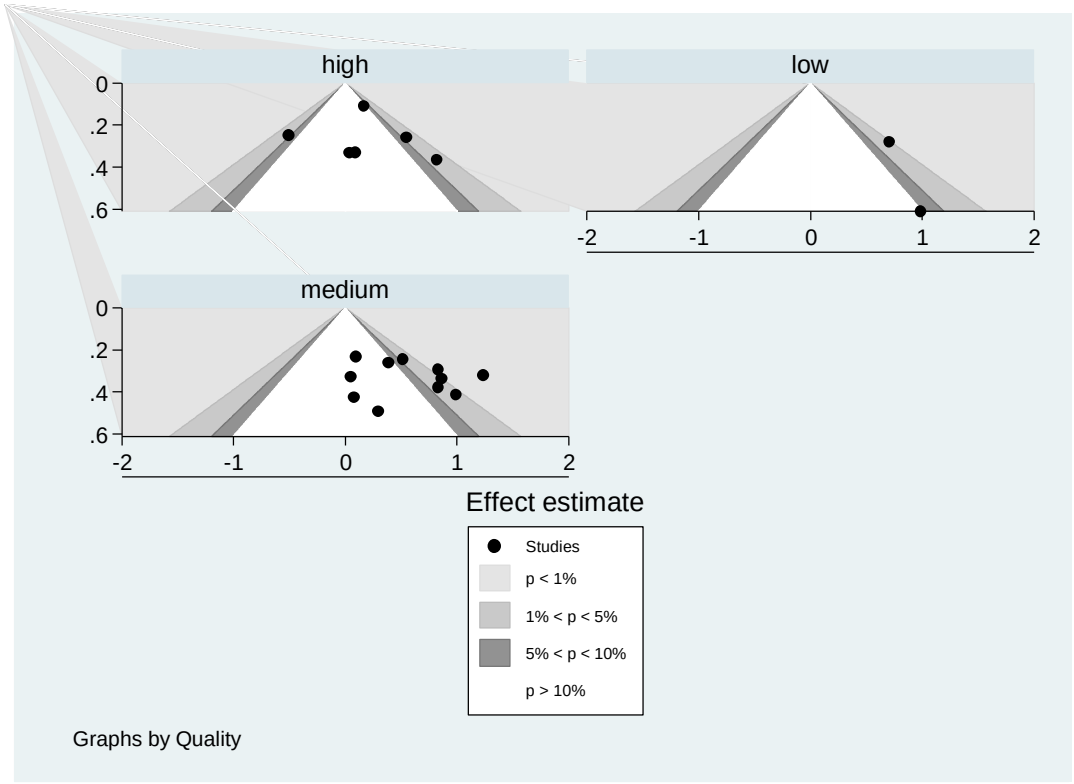


Figure 38. Contour enhanced funnel plots for the natural logarithm of prostate cancer risk estimates for high exposure to pesticides versus their standard errors, Sub-stratifying studies by quality (NOS)

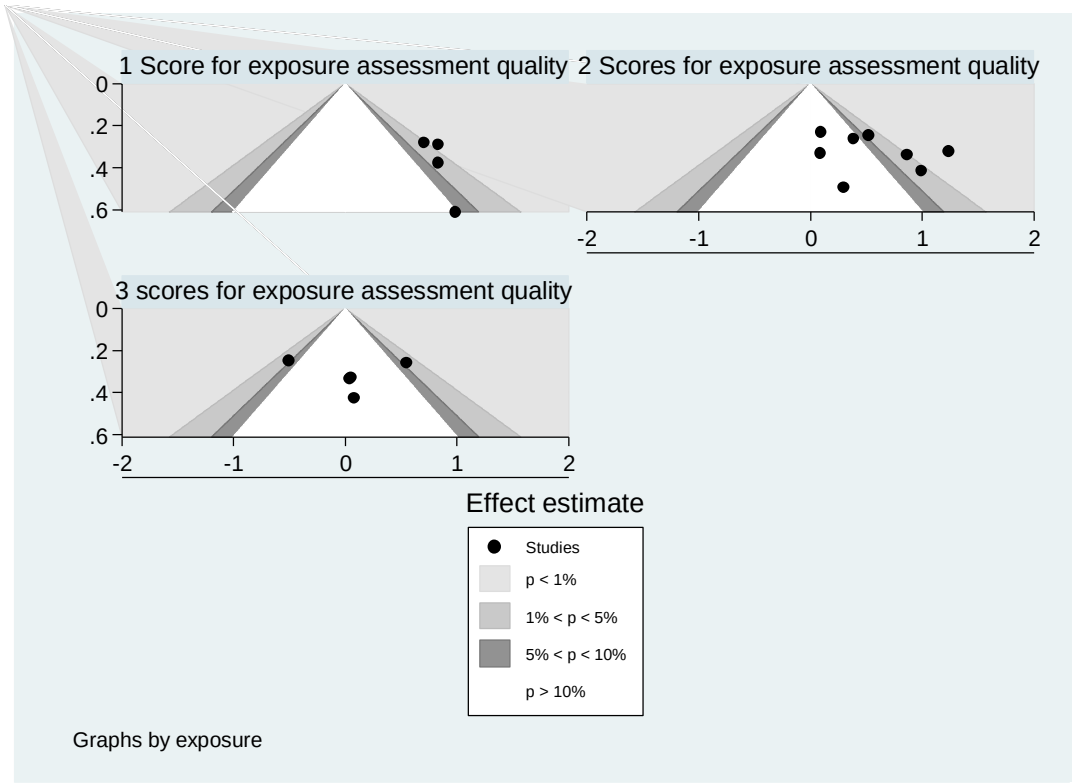


Figure 39. Contour enhanced funnel plots for the natural logarithm of prostate cancer risk estimates for high exposure to pesticides versus their standard errors, Sub-stratifying studies by exposure assessment quality

C) Organochlorine Pesticide exposure and Prostate cancer

1) Selection of the studies addressing specific organochlorine pesticides and prostate cancer

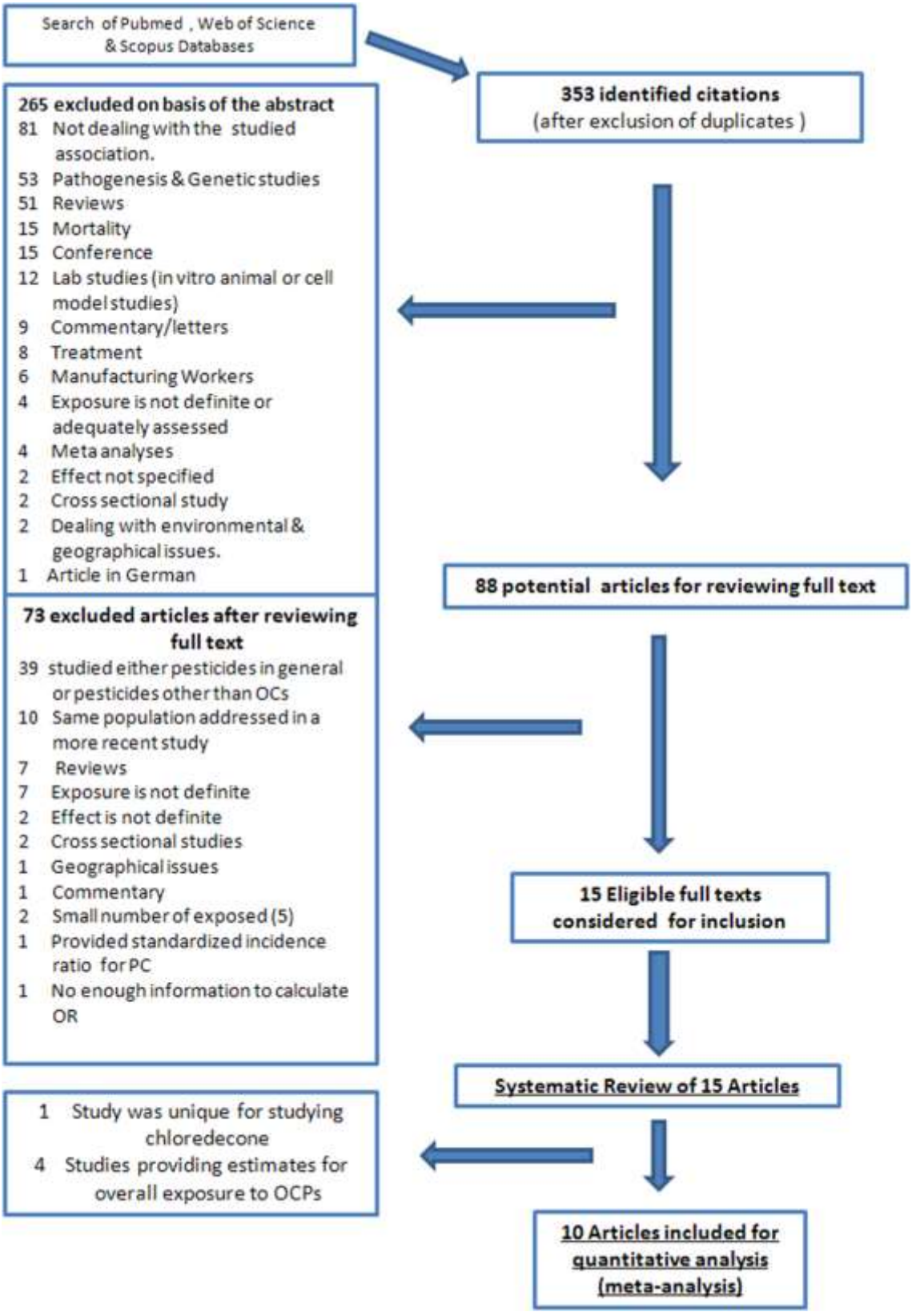
The search strategy that we applied to collect data about specific pesticide exposure and prostate cancer yielded a number of 353 articles after omitting duplicates. We eliminated 265 ineligible articles based on the abstracts. After reviewing full text of the 88 potentially eligible articles, 73 were excluded for various reasons as presented in the flowchart. Finally, we systematically reviewed fifteen studies, (Alavanja et al. 2003; Mills et al. 2003; Ritchie et al. 2003; Settimi et al. 2003; Hardell et al. 2006; Fritschi et al. 2007; Purdue et al. 2007; Aronson et al. 2010; Multigner et al. 2010; Sawada et al. 2010; Band et al. 2011; Cockburn et al. 2011; Koutros et al. 2013; Emeville et al. 2014; Koutros et al. 2015) while the estimates reported by ten articles were included in the meta-analysis. These include nine case-control studies (Mills et al. 2003; Ritchie et al. 2003; Settimi et al. 2003; Hardell et al. 2006; Aronson et al. 2010; Multigner et al. 2010; Sawada et al. 2010; Band et al. 2011; Emeville et al. 2014) and the most recent study belonging to the prospective cohort, the AHS. (Koutros et al. 2013) (Figure 39)

Published data were available for a number of OC pesticides including DDT and its metabolites (p,p´-DDE & o,p´-DDE), alachlor, chlordane, oxychlordane, chlordecone, dieldrin, endosulfan, heptahlor, hexachlorobenzene (HCB), methoxychlor, hexachlorocyclohexane congeners (α, β and γ), mirex, nonachlor and toxaphene. For the sake of pooling estimates, we considered for inclusion in the meta-analysis only pesticides for which there were estimates provided in at least two different studies. OC

pesticides that satisfied this condition were DDT, p,p´-DDE, trans-nonachlor, oxychlordane, HCB and lindane.

We could not include in the quantitative analysis one case-control study that was the only study evaluating chlordecone exposure, (Multigner et al. 2010) and another two studies that assessed overall use of OC pesticides. (Cockburn et al. 2011; Fritschi et al.2007)

Figure 40. Flow chart summarizing the selection process of the articles for the second meta-analysis (association between specific organochlorine pesticides & prostate cancer risk)



2) Characteristics of the studies addressing exposure to specific organochlorine pesticides

Geographical distribution

Study populations were from eight different countries that included USA and Canada (n= 5), European countries (n=4) and Japan (n=1).

Methodologies applied for quantification of exposure

Half of the included studies (Aronson et al. 2010;Emeville et al. 2015;Koutros et al. 2015;Ritchie et al. 2003;Sawada et al. 2010) estimated OR for prostate cancer in relation to plasma levels of a number of OC pesticides, all of which were conducted among the general population. One study (Hardell et al. 2006) utilized adipose tissue biopsy that was taken from abdominal wall of cases and controls. In most of these studies, authors also used questionnaire to collect information about at job exposure to pesticides. (Aronson et al. 2010; Hardell et al. 2006; Ritchie et al. 2003; Sawada et al. 2010)

For studies that assessed occupational exposure to specific OC pesticides, one study utilized a JEM as well as self administered questionnaires including lifetime job descriptions. (Band et al 2011) On the other hand, for two included studies (Mills et al. 2003; Settimi et al. 2003) quantification approach was a form of partially ecological exposure assignment. The only cohort we encountered in our search that addressed specific pesticides was the AHS. We utilized data reported by the latest article that examined the association between a large number of specific OC pesticides and prostate cancer. (Koutros et al. 2013)

Studied population

Six studies assessed exposure among the general population (Aronson et al. 2010, Hardell et al. 2006, Ritchie et al. 2003, Sawada et al. 2010, Emeville et al. 2014, Koutros S et al. 2015), while four studies were concerned with occupational exposure that included farmers (Band et al. 2011; Mills et al. 2003, Settimi et al. 2003) and licensed pesticide applicators. (Koutros et al. 2013)

Study type

We included in the meta-analysis six population based case-control studies, (Band et al. 2011; Hardell et al. 2006; Mills et al. 2003; Sawada et al. 2010; Emeville et al. 2014; Koutros et al. 2015) while three were clinic based. (Aronson et al. 2010; Ritchie et al. 2003; Settimi et al. 2003)

Diagnosis of prostate cancer

It was based on cancer registries in case of four included case-control studies [Band et al. 2011, Mills et al. 2003; Sawada et al. 2010; Koutros et al. 2015) and for the cohort study, (Koutros et al. 2013) while the other five studies depended directly on histopathologically confirmed specimens. (Aronson et al. 2010; Hardell et al. 2006; Ritchie et al. 2003; Settimi et al. 2003; Emeville et al. 2014) Four out of the included studies provided separate estimates for those developing aggressive forms of prostate cancer (metastatic cases) (Koutros et al. 2013; Sawada et al. 2010; Emeville et al. 2014; (Koutros et al. 2015)

3) Assessment of the quality of the articles included in the meta-analysis

The majority of studies that assessed specific pesticides are of high quality. According to NOS, seven studies received from 7 to 9 points, while three received 6 points. (Table 11) Although we encountered only one cohort study that assessed OC pesticides, it used data of the large prospective high quality AHS. Other available cohort studies that addressed the association between pesticide exposure and prostate cancer were of lower quality and compared the incidence of prostate cancer among pesticides applicators to the general population, without any assessment of exposure to specific pesticide types. (Fleming et al. 1999; Dich and Wiklund 1998; Zhong and Rafnsson 1996)

Table 11. OR and 95% CI of prostate cancer for various levels of exposure to organochlorine pesticides and the NOS quality assessment of the studies.

Study, Year	Pesticide	OR, 95% CI	Intensity of exposure	P value	NOS Quality Assessment			
					Selection	Comparability	Exposure	Score
Cockburn M *et al* (2011)	OCPs	**1.64(1.02 − 2.63)** 1.25(0.75 – 2.08) **2.03(1.17 − 3.52)**	Ever exposed Low exposed High exposed	**0.037**	++	++	+	**5**
Band PR *et al* (2011)	DDT	1.24(0.71 – 2.16) **1.68(1.04 − 2.70)**	Low High	**0.03**	++	++	++	**6**
	Lindane	0.91(0.44 – 1.89) **2.02(1.15 − 3.55)**	Low High	**0.03**				
Mills PK & Gang R (2003)	Lindane	1.14(0.45 – 1.77) **1.86(1.10 – 3.17)** **2.37(1.22 – 4.61)**	Level 2 Level 3 Level 4	**0.003**	+++	++	+	**6**
	Heptachlor	1.13(0.73 –1.73) **2.07(1.21– 3.54)** **2.01(1.12– 3.60)**	Level 2 Level 3 Level 4	**0.003**				
	Dicofol	0.86(0.57–1.29) 1.04(0.64 –1.67) 1.09(0.65 –1.83)	Level 2 Level 3 Level 4	0.84				
Settimi L *et al* (2003)	OCPs	**2.50(1.40–4.20)** **2.50(1.10–5.30)** **2.70(1.20–6.30)**	Ever ≤ 15 years > 15 years	---	++	++	++	**6**
	DDT	**2.10(1.20–3.80)** 2.10(0.90–2.10) **2.20(1.10–4.80)**	Ever ≤ 15 years > 15 years					
	Dicofol	**2.80(1.50–5.00)** **2.40(1.20–5.30)** **3.00(1.30–7.00)**	Ever ≤ 15 years > 15 years					
Aronson KJ *et al* (2010)	DDT	1.19(0.63–2.26) 1.05(0.55–2.00)	T2 T3	0.90	++	++	+++	**7**
	P,p′-DDE	0.97(0.52–1.83) 0.73(0.38–1.40)	T2 T3	0.35				
	Oxychlordane	1.14(0.61–2.13) 0.95(0.49–1.85)	T2 T3	0.89				
	Hexachloro benzene	1.25(0.65–2.39) 1.27(0.66–2.43)	T2 T3	0.48				
	Trans-nonachlor	1.17(0.62–2.21) 0.83(0.42–1.65)	T2 T3	0.58				
Ritchie JM *et al* (2003)	P,p′-DDE	0.72(0.31−1.71) 1.08(0.47− 2.50)	T2 T3		+++	+	+++	**7**
	Oxychlordane	3.11(1.27−7.63) 1.23(0.42−3.55)	T2 T3					
	Trans-nonachlor	1.96(0.83−4.66) 1.18(0.45−3.08)	T2 T3					
	Heptachlor	0.58(0.21−1.64) 0.33(0.10−1.03)	T2 T3					
Hardell L *et al* (2006)	P,p′-DDE Oxychlordane HCB	2.30(0.77−6.85) 1.90(0.62−5.79) 2.39(0.81−7.09)	Adipose tissue levels higher than median concentrations	--	+++	++	++	**7**
Fritschi L *et al* (2007)	OCPs	0.76(0.33−1.75)	Low exposure	0.40	++++	+	+++	**8**

Sawada N *et al* (2010)	DDT	1.51(0.87−2.63)	Q2		+++	++	+++	**8**
		0.92(0.50−1.70)	Q3					
		1.00(0.52−1.92)	Q4	0.61				
	P,p´-DDE	1.00(0.60−1.66)	Q2					
		0.89(0.52−1.53)	Q3					
		0.90(0.52−1.54)	Q4	0.65				
	Oxychlordane	1.06(0.61−1.82)	Q2					
		0.74(0.40−1.36)	Q3					
		0.75(0.34−1.64)	Q4	0.40				
	HCB	0.78(0.38−1.57)	Q2					
		0.86(0.40−1.81)	Q3					
	Trans-nonachlor	0.52(0.21−1.25)	Q4	0.11				
		1.11(0.67−1.86)	Q2					
		0.92(0.49−1.69)	Q3					
		0.86(0.42−1.78)	Q4	0.61				
Koutros S *et al* (2013)	DDT	0.98(0.78−1.22)	Q1		+++	++	+++	**8**
		1.27(1.02−1.58)	Q2					
		1.27(1.02−1.58)	Q3					
		1.18(0.95−1.48)	Q4	0.14				
	Heptachlor	1.08(0.80−1.47)	Q1					
		1.05(0.77−1.44)	Q2					
		1.03(0.76−1.40)	Q3					
		1.05(0.78−1.44)	Q4	0.73				
	Lindane	0.88(0.63−1.23)	Q1					
		1.06(0.70−1.49)	Q2					
		1.06(0.76−1.48)	Q3					
		1.16(0.84−1.60)	Q4	0.33				
Purdue MP *et al* (2007)	OCPs [a]	1.10(0.80-1.50)	T1		+++	++	+++	**8**
		1.10(0.80-1.40)	T2					
		1.20(0.80-1.70)	T3					
		0.90(0.60-1.40)	T4	0.82				
Alavanja MC *et al* (2003)	Chlorinated pesticides [b]	**1.29(1.02-1.63)**	T2		+++	++	+++	**8**
		1.51(1.15-2.00)	T3L					
		1.39(0.99-1.97)	T3H	0.005				
Emeville E *et al* (2015)	DDE	0.96(0.66-1.42)	Qu2		++++	++	+++	**9**
		1.05(0.71-1.55)	Qu3					
		1.02(0.67-1.53)	Qu4					
		1.53(1.02-2.30)	Qu5	0.01				
Koutros S *et al* (2015) [c]	Heptachlor	1.01(0.50-2.02)	Q2		++++	++	+++	**9**
		1.19(0.60-2.36)	Q3					
		2.01(0.98-4.10)	Q4	0.05				
	DDE	0.80(0.42-1.51)	Q2					
		1.23(0.69-2.21)	Q3					
		0.90(0.47-1.73)	Q4	0.99				
	DDT	0.75(0.41-1.38)	Q2					
		0.62(0.33-1.19)	Q3					
		0.99(0.50-1.97)	Q4	0.58				

[a] Included aldrin, chlordane, DDT, dieldrin, heptachlor, toxaphene and lindane.
[b] Included aldrin, chlordane, DDT, dieldrin, heptachlor and toxaphene.
[c] This study provided ORs for aggressive forms of PC. Other Organochlorines as oxychlordane, trans-Nonachlor, Hexachlorobenzene and trans-Nonachlor were also assessed.
T3L= lower tertile 3, T3U= upper tertile 3, Q1, Q2, Q3, Q4 = Quartiles, Qu1, Qu2, Qu3, Qu4, Qu5 = Quintiles
Bold fonts indicate significant associations.

4) Summary of the results reported by the studies included in the meta-analysis:

4.1. Qualitative analysis of the results

As shown in Table 10, results reported for specific pesticides were inconsistent. However, we observed that positive significant associations were reported by three case-control studies that share in common being conducted on farmers occupationally exposed to pesticides as well as having lower exposure assessment quality (NOS quality scale for exposure was either 1 or 2 stars), than the studies that did not report an association. (Band et al. 2011; Mills et al. 2003; Settimi et al. 2003)

However, a high quality study conducted in Guadeloupe has also reported positive significant association for the highest group of exposed among the general population. Nevertheless, the particular nature of the studied population, being of African descent is to be noted. (Emeville et al. 2014)

4.2. Quantitative analysis of the results (Meta-analyses)

Table 12 displays pooled estimates for low and high exposure levels to different OC pesticides. Pooled estimate for low and high exposure to **DDT** was 1.38 and 1.14 respectively, but was insignificant in both cases. This estimate was obtained from pooling of two studies that applied serum measurements of DDT in the general population, (Aronson et al. 2010, Sawada et al. 2010) and three studies of farmers and pesticide applicators that assessed exposure by self reporting, employment records and JEM. (Band et al. 2011, Koutros et al 2013; Settimi et al. 2003)

On the other hand, pooled estimate obtained for **p,p´-DDE,** which is the main metabolite of DDT, was 0.90 and 1.02 for low and high exposure respectively and was

insignificant for both groups. Worth mentioning is that all the five studies included in this analysis were population based studies that applied measuring p,p'- DDE in biological samples, and the reference category was the lowest exposed category Aronson et al. 2010; Hardell et al. 2006; Ritchie et al 2003; Sawada et al. 2010; Emeville et al. 2014)

Table 12. Prostate cancer pooled estimates, 95% CI, I^2 and p value for exposure to organochlorine pesticides.

Organochlorine	Exposure	Number of studies	Pooled OR	95% CI	Test of heterogeneity I^2	p
DDT	Low	5	1.38	0.91-1.85	68.3%	0.013
	High	5	1.14	0.81-1.47	30.2%	0.220
DDE	Low	5	**0.90**	**0.63-1.16**	**0.00%**	*0.866*
	High	5	**1.02**	**0.69-1.35**	**12.7%**	*0.333*
Hepatachlor	Low	3	**1.03**	**0.77-1.29**	**0.00%**	0.415
	High	3	0.95	0.25-1.66	79.1%	0.008
Hexachloro Benzene	Low	3	**0.98**	**0.43-1.53**	**0.00%**	0.665
	High	3	0.88	0.18-1.57	36.0%	0.210
Oxychlordane	Low	4	**1.22**	**0.69-1.75**	**0.00%**	0.704
	High	4	**0.91**	**0.46-1.35**	**0.00%**	0.809
Trans nonachlor	Low	3	**1.23**	**0.71-1.76**	**0.00%**	0.742
	High	3	**0.88**	**0.45-1.31**	**0.00%**	0.892
Lindane (γ HCH)	Low	3	**0.92**	**0.67-1.18**	**0.00%**	0.781
	High	3	1.56	0.82-2.29	41.7%	0.180

HCH= Hexachlorcyclohexane
Bold figures indicate homogeneity

Although there appeared to be a positive association for high occupational exposure to **lindane**, it was insignificant and there was mild degree of heterogeneity among the included studies. Pooled estimate, 1.56, 95% CI, 0.82-2.29, I^2, 41.7%, p = 0.180.

Pooling estimates for low exposure to **trans-nonachlor** as well as **oxychlordane** produced a higher estimate than that obtained from pooling estimates for low exposure. However, the association was not statistically significant.

We have also pooled estimates reported by four studies for aggressive forms of prostate cancer. Pooling estimates for three studies that assessed high exposure to DDT showed a weak yet insignificant association, 1.20(0.85-1.56), I^2 = 0.00%, p = 0.733. (Koutros et al. 2013; Sawada et al. 2010; Koutros et al. 2015) On the other hand, there was no association when we pooled three ORs of aggressive forms of prostate cancer for high exposure to DDE, 0.96(0.46-1.46), I^2 = 0.00%, p = 0.675. (Sawada et al. 2010; Emeville et al. 2014; Koutros et al. 2015)

Exploring heterogeneity and Sensitivity analysis

In order to obtain reliable pooled estimates, they should represent consistent studies and reveal homogeneity. For that, we have grouped pooled estimates separately according to the studied population, as well as the methodology applied by the studies for quantification of exposure. Figure 40 presents forest plot displaying random-effects meta-analysis of the association between high exposure to several OC pesticide types among the general population and prostate cancer risk (all these studies applied measuring serum levels of the studied OC pesticides), while Figure 41 presents forest plot displaying random-effects meta-analysis of the association between high occupational exposure to several OC pesticide types and prostate cancer risk.

We observed that pooling estimates obtained from serum pesticides measurements among the general population demonstrated greater homogeneity and consistently lower pooled estimates. This was evident in case of four OC pestcides including p,p´-DDE, oxychlordane, trans-nonachlor and HCB. Pooled estimates for both low and high exposure to these OC pesticides revealed homogeneity and therefore there was no

need to elaborate determinants of heterogeneity. On the other hand, for pooled estimates of DDT, and high exposure to lindane, heterogeneity was observed.

Homogeneity was revealed for high exposure to DDT when we stratified studies by the methodology applied for exposure quantification (which also coincides with stratifying by the exposed population). Pooled estimate for the two studies that measured serum DDT level in the general population was 0.81(0.95-1.26), I^2= 0.00%, p = 0.400 for highest tertile vs. lowest tertile, and for the three studies that comprised occupational exposure (farmers applying pesticides), it remained positive but insignificant, 1.30(0.94-1.67), I^2= 13.4%, p = 0.315 for the highest exposed group vs. the non-exposed. On the other hand, heterogeneity observed when pooling estimates for low exposure to DDT could not be resolved by methodology adopted by the studies or by the exposed population. However, heterogeneity decreased when we eliminated the only cohort study [37]. Pooled OR for the four case-control studies was high and significant, 1.53(1.05-2.00), I^2= 38%, p=0.183. However, complete homogeneity was obtained when excluding the study that was unique in categorizing exposure only by duration [41]. Pooled OR for the remaining three studies was insignificant, 1.25(0.79-1.79), I^2= 0.00%, p = 0.967. (Aronson et al. 2010; Band et al. 2011; sawada et al. 2010)

For high exposure to lindane, homogeneity was revealed on excluding the only cohort study, (Koutros et al. 2013) and pooled estimate for the two case-control studies that addressed farmers was high and significant, 2.14 (1.16-3.12), I^2 = 0.00%, p = 0.741.

For high exposure to heptachlor, heterogeneity decreased when we eliminated the study that assessed exposure among the general population. (Ritchie et al. 2003) The

pooled estimate for the remaining two studies addressing farmers was positive but non-significant. (Mills et al. 2003; Koutros et al. 2013) (Figure 30).

Figure 41. Forest plot displaying random-effects meta-analysis of the association between high exposure to several organochlorine pesticides and prostate cancer risk among the general population

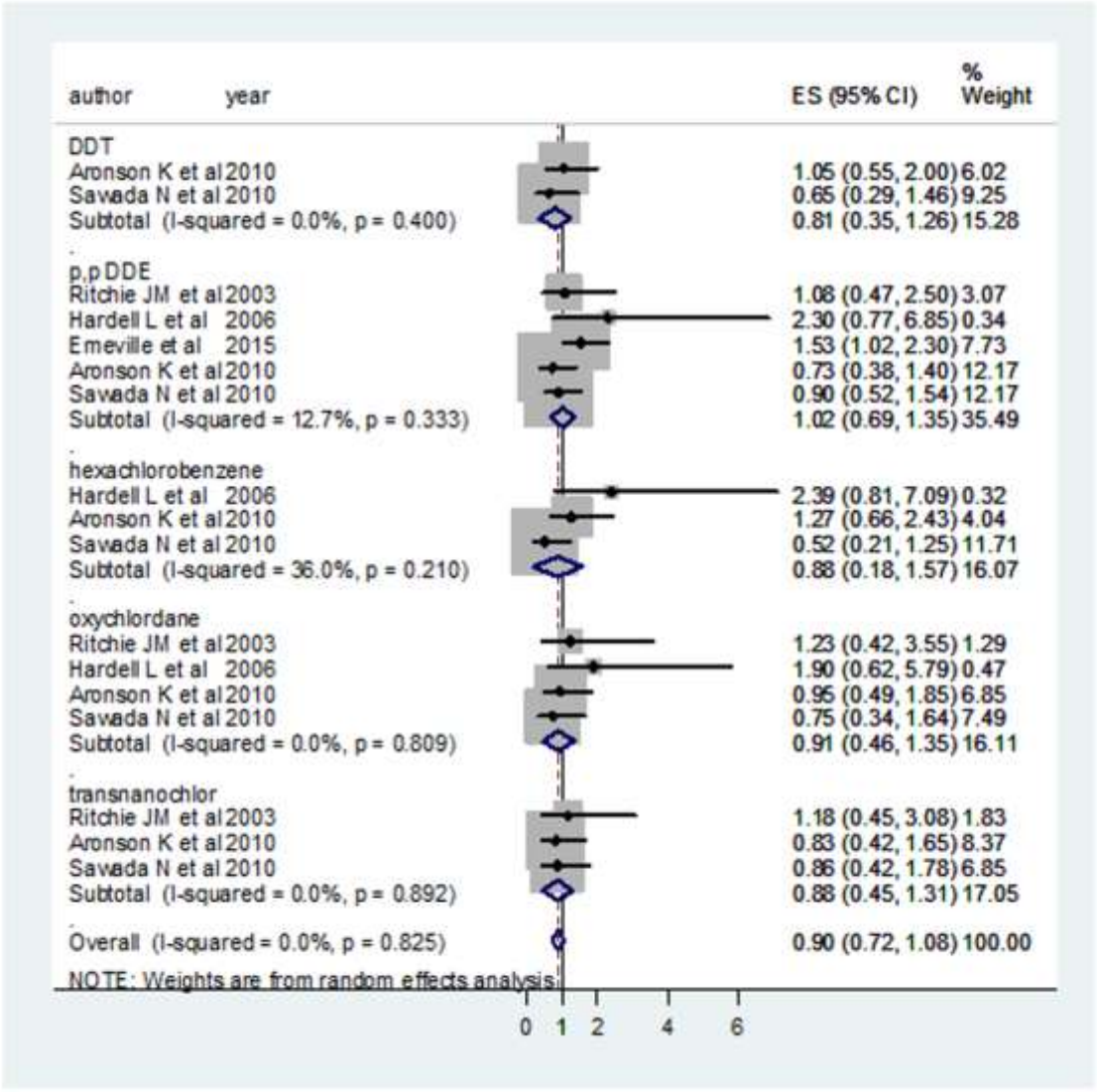
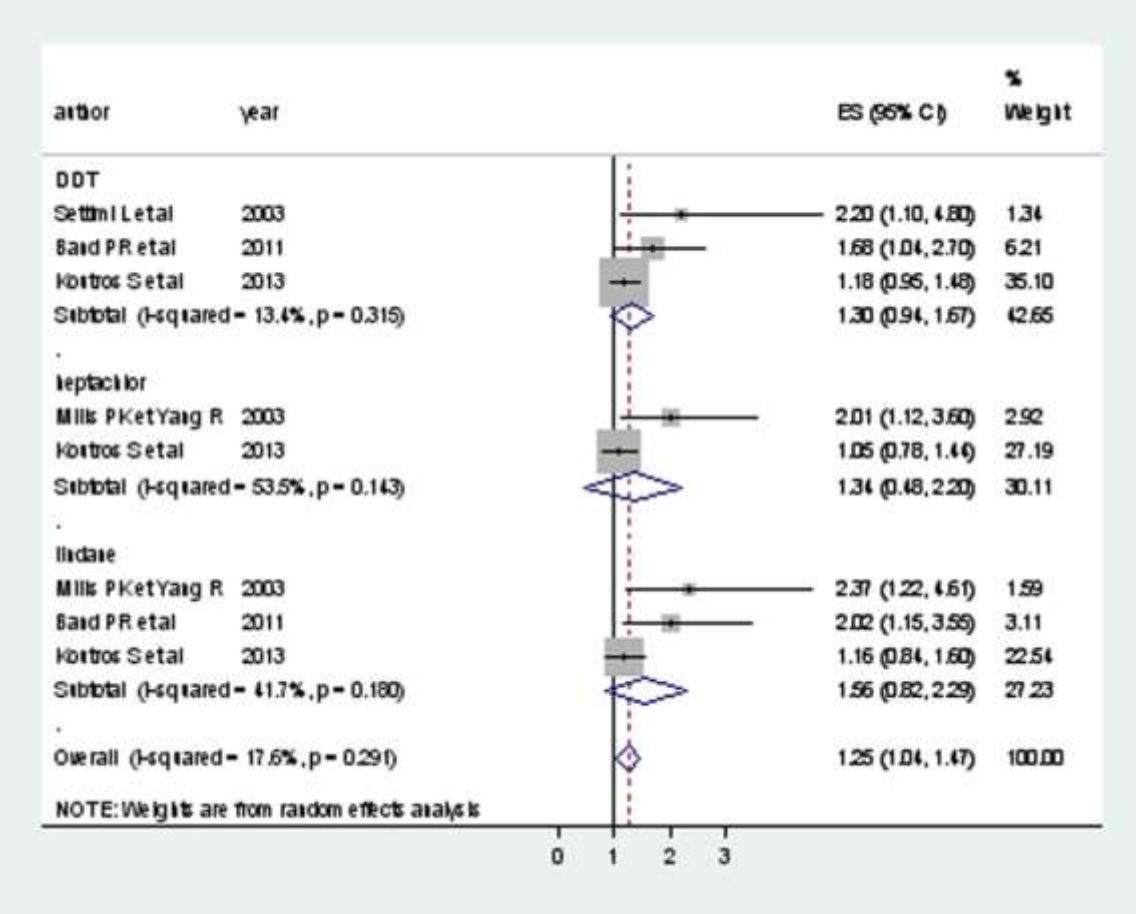


Figure 42. Forest plot displaying random-effects meta-analysis of the association between high occupational exposure to several organochlorine pesticides and prostate cancer risk



Discussion

Farming and prostate cancer

According to the results of our systematically reviewing available epidemiological data relating farming to prostate cancer, we consider that the strength of the evidence for an increased prostate cancer risk in farmers is weak. Studies relating prostate cancer to farming that depended solely on job title are only of a hypothesis generating potential. However, the consistent finding of a weak yet statistically significant association among this specific group of workers have directed us to up-date the previously published reviews. We have included more recent studies and conducted a meta-analysis concentrating on exploring as much as was possible of sources of heterogeneity between studies. The results we have obtained make us less supportive of an excess of prostate cancer risk in farmers than previous reviews.(Acquavella et al. 1998;Blair et al. 1985;Blair et al. 1992;Van Der Gulden and Vogelzang 1996)

According to the pooled estimates obtained from combining the results of case-control studies, we found a weak yet significant increased risk of prostate cancer among farmers. In spite of the consistency with previously conducted meta-analyses, (Acquavella et al. 1998;Van Maele-Fabry and Willems 2003;Van Maele-Fabry and Willems 2004) the potential sources of selection and information bias in a large portion of these studies call for a cautious interpretation of the modest pooled estimate obtained.

On the other hand, pooling the results obtained from cohort studies revealed no association. However, proper interpretation should also be in context with several limitations and observations. First, cohort studies calculated SIR, which involves comparison of the farmers with the general population. Using an external comparison

group inherently introduces bias due to the healthy worker effect.(Checkoway H. et al. 2004) Second, the three large cohort studies that comprised the main weight in the meta-analysis have provided negative results.(Laakkonen and Pukkala 2008;Pukkala et al. 2009;Zeegers et al. 2004) It has to be taken in consideration that they share in common being conducted in Scandinavian countries, which might involve distinct working conditions. Also, in two out of the three studies, authors adopted the same design where incidence of prostate cancer was compared for different occupations including farming.(Pukkala et al. 2009;Zeegers et al. 2004) Since prostate cancer commonly presents in older age groups and farmers often work well beyond the conventional retirement age, differentiating groups by occupation codes may have missed older age-groups beyond traditional working-age parameters, with the possibility of missing prostate cancer cases. (Depczynski and Lower 2014)Thus, in spite of the advantage of low potential of publication bias when comparing prostate cancer incidence among different occupations, this design might render detecting differences in prostate cancer incidence between farmers and other job titles more difficult.

Third, SIR of the majority of other types of cancer among farmers has consistently shown low values. (Alavanja et al. 2003;Blair et al. 1985;Blair et al. 1992;Fleming et al. 1999) In one of the recent studies belonging to the large prospective cohort of the AHS, authors also calculated relative SIR that puts into account the low incidence of all cancers combined among the farmers/pesticide applicators and higher significant values have been reported.(Koutros et al. 2010a)

Another important point that should be put into account is the difference in PSA screening rates. In spite of the fact that PSA testing has been widely used in recent

years and this resulted in an increased incidence of prostate cancer, its use has been uneven across countries and social classes. Access to health services in rural areas has been found to be less among farmers than in the general population. (Coory and Baade 2005;Jemal et al. 2005;Koutros et al. 2013a) This fact might bias the results towards the null as it has rarely been taken into account in the available epidemiological studies. All these drawbacks might make interpretation of the results quite challenging.

However, in spite of the fact that there has been no profession for which an association with prostate cancer has been decisively established, (Zeegers et al. 2004) farming has been the most consistently studied. Concerning our main objective which is examining the potential association between pesticide exposure and prostate cancer, an obvious drawback of previous reviews and meta-analyses is that farming was consistently considered a surrogate of pesticide exposure. Authors have pooled results without any quantification of exposures, yet they have attributed the increased prostate cancer risk among farmers to be most likely due to exposure to pesticides. Other authors have also suggested that farmers are exposed for longer durations and to higher intensities of pesticides than other pesticide applicators.(Buranatrevedh and Roy 2001) Considering all farmers as pesticide exposed has been lately criticized and was suggested to entail the possibility of biased results. (Macfarlane et al. 2009)

The potential exposure of farmers to a number of different exposures, not only pesticides, that might include engine exhausts, fuels and solvents, welding fumes and others makes it difficult to assign a specific exposure as causal to prostate cancer, especially that most of these exposures have been suggested to be of carcinogenic

potential.(Blair and Zahm 1995; Parent and Siemiatyaki 2001) Therefore, for all these reasons, we consider that the hypothesis that farmers might be at an increased risk for prostate cancer merits further investigation.

Pesticide exposure and prostate cancer

Our systematically reviewing epidemiological studies that provided estimates specific for pesticide exposure in relation to prostate cancer have contributed to drawing a clearer picture than studies of farmers that did not account for specific exposures. We observed that the magnitude of the association between high pesticide exposure and prostate cancer was related to the methodology adopted by the included studies for assessing pesticide exposure. Interestingly, we have obtained consistent findings that might indicate an impact of the quality of the articles on the pooled estimates, as a stronger association was detected when less accurate methodologies for exposure assessment was applied.

A positive significant association was observed when combining the results of studies that assessed occupational pesticide exposure in agricultural settings among farmers. These studies applied grouped assignment of exposure that depended mainly on similarities between groups of farmers as regards types, dates, patterns or locations of crops grown, or information indicating similarities in workplace conditions collected from employment records. Authors have used these indirect approaches for evaluation of duration, probability and/or levels of pesticide exposure. Applying these aggregate measures of exposure assignment impose uniform exposure of individuals within a group and imply also fixed exposure overtime. Accordingly, we expect that this simplifying assumption may mask individual variation and introduce more sources of

bias than when applying individualized exposure assessment. (Loomis and Kromhout 2004)

On the other hand, we have observed that studies that applied biological monitoring of pesticides have consistently found no increased prostate cancer risk among those who had the highest serum levels of several studied pesticides types compared with the group that had the lowest detectable levels.

Methodologies applied for assessment of pesticide exposure: Strengths & Limitations

We have considered that biological monitoring of pesticides levels may provide a potentially optimal index of exposure that reflects the internal dose of pesticides.(Barr et al. 2008) Measuring the serum level of pesticides may be more relevant than other subjective measures, as individual differences in metabolizing these chemicals might be considered. Also, it offers the advantage of integrating aggregate pesticide intake across multiple exposure routes.(Fenske 2005; Ott 2005; Checkoway et al. 2004; Barr and Needham 2002)

Nevertheless, whether serum levels of pesticides reflect cumulative exposure is a matter of the type of pesticide studied. Moreover, the feasibility and cost of biological monitoring have restricted its use in large epidemiological studies. (Nieuwenhuijsen 2015) It has to be noted that most of the studies that we encountered that applied this methodology were conducted among the general population and not a specific occupationally exposed group. They also shared in common being conducted on smaller numbers of individuals compared to other studies that applied less sophisticated methodologies.

Many studies that were conducted in occupational settings have used self-reporting of exposure. Although the potential for recall bias is a well known drawback of collecting exposure information retrospectively, several authors have compared and validated self-reporting and found it to be exceptionally reliable in case of chemicals that are regularly used by workers. In case of pesticides, farmers and pesticide applicators have been reported to be very knowledgeable about pesticides they use. (Blair and Zahm 1995) We have obtained a positive yet insignificant association when pooling studies that applied self-reporting.

In recent years, other methodologies have been recommended for quantification of occupational exposure, but have not yet been rigorously applied for assessment of pesticide exposure. Expert judgment for assignment and quantification of occupational exposure has been considered to be a promising way of high validity,(Fritschi et al. 2003) but still was applied minimally by the available studies. We have noted that the pooled estimate obtained from studies that applied expert assessment of pesticide exposure revealed no association with prostate cancer.

Furthermore, job exposure matrices have been developed to provide a ranking within the study population that can be used as an ‘‘exposure score’’ with which to compare groups on a continuous scale. It is important to note that although it is not an absolute exposure measurement, it does offer a substantial advance over dichotomous categories based on self report, particularly when subjects are unlikely to recall specific pesticide names and dates of use. (Wood et al. 2002;Young et al. 2004) However, the validity of Job exposure matrices is not yet estimated. (Pukkala et al. 2005;Teschke et

al. 2002) Job exposure matrices were minimally applied by the studies that we encountered for our meta-analyses.

Overall, we have observed deficient methodologies for quantification of exposure to pesticides, especially in occupational settings. Future studies should put more effort in validating the quantification methods used, in order to obtain more reliable results. The ideal exposure assessment is defined as estimation of the concentration of an agent in a specific medium during a specific time period.(Vlaanderen et al. 2008b) However, this might not be feasible for pesticide. Also, the main goal of exposure assessment for epidemiological studies is to identify the variability of exposure in the study population and then classify study subjects with respect to their variability in exposure. (Nieuwenhuiijsen 2010) Therefore, combining biological monitoring with other methods as questionnaires, JEMs and experts assessment of exposure may provide a possible way for considering the variability of host factors that determine the amount of internal dose and allow for the validation of external exposure information.

Exposure misclassification in occupational epidemiological studies

In spite of the fact that exposure misclassifications are almost inevitable in occupational epidemiology studies, direction of this effect has not been rigorously assessed. (Blair et al. 2007) Many authors have recommended the need for more critical evaluation of inaccuracies in exposure measurements. (Brenner and Loomis 1994)We have consistently obtained results suggesting that less accuracy of pesticide exposure assessment might exaggerate the magnitude of the association with prostate cancer. The magnitude of the association for the ever versus never exposed, which was almost equal to that retrieved for the highest exposed groups, might be explained

from the same perspective, where collapsing exposure scales into only two categories might have biases the results away from the null. We have also observed that other sources of bias as non adjustment of important confounders as family history, and selection bias (using cancer patients as controls) have biased the results away from the null. (Checkoway H. et al. 2004;Jurek et al. 2005)

Organochlorine pesticide exposure and prostate cancer

Given the fact that pesticides are a very heterogeneous group of chemicals, associating overall use of pesticides to an increased prostate cancer risk should be interpreted cautiously. For this reason, we have put much effort to pool results provided for specific pesticides. However, we have observed that there is limited epidemiological data about specific pesticides. The only pesticide group for which we have found some data sufficient for pooling was a number of OC pesticides.

Based on the available studies that applied measuring serum levels of OC pesticides, no increased risk of prostate cancer have been observed among the groups who had the highest serum OC levels compared to those with the lowest detectable levels. Interestingly, pooled estimates have consistently shown homogeneity for the majority of the examined OC pesticides. Studying specific pesticides may have decreased the burden of the heterogeneity when combining results of exposure to different pesticides types.

However, to draw factual conclusions, several points should be considered. Firstly, all of these studies were conducted in developed countries, where most OC pesticides have been banned since decades. Concentrations of these pesticides were found to be

of higher levels in adipose tissue and serum of people from Africa, Asia and Latin America.(Jaga and Dharmani 2003) Worth mentioning that a recent case-control study conducted in India reported significantly higher levels of β-HCH, γ- HCH and p,p´-DDE in serum of prostate cancer cases compared to controls.(Kumar et al. 2010)

Second, although measuring serum concentrations of studied pesticides may reflect biologically relevant exposures and consider individual variation in absorption and metabolism, a single sample may not be a true reflection of the life-long cumulative burden. (Nieuwenhuijsen 2015)

Third, available studies that measured serum levels of OC pesticides were conducted among the general population, and not confined to a highly exposed occupational group. On the other hand, available epidemiological studies concerned with relating prostate cancer to occupational exposure to specific OC pesticides shared in common applying less precise exposure assessment methodologies (Band et al. 2011;Mills and Yang 2003;Settimi et al. 2003) than studies that aimed to assess the association for the general population.

Fourth, toxicological evidence indicated that timing of exposure to endocrine disruptors may be critical.(Huang et al. 2004;Lopez-Cervantes et al. 2004) Earlier exposure during developmental stages is expected to aggravate the association. (Boberg et al. 2009; Prins 2008) Our included articles assessed exposure at later stage in life so potentially relevant time periods were not involved.(Martin et al. 2007)

Fifth, in spite of the fact that pooling estimates of studies that measured serum levels of the studied pesticides had consistently showed homogeneity for all of the examined

OC pesticides, some differences between the studies have been observed. These include the limits of detection chosen, the statistical handling of non-detectable levels and the time periods of collection of samples. However, these differences may be considered inevitable as standardizing a unique method for all the studies would not be feasible.

In spite of these issues, measuring serum level of studied pesticides may overcome methodological problems as recall bias of self-reporting or inaccuracies and exposure misclassification of grouped assignment of exposure. Moreover, the validity of biological monitoring is related to the type of studied pesticide. For OC pesticides, they are very slowly eliminated which makes biological monitoring a relatively accurate mean of assessing past exposure. Serum levels of DDT and DDE have also been closely correlated with levels in adipose tissue.(Beard 2006)

Regarding studies that examined the association between specific pesticides and prostate cancer in occupational settings, sporadic positive findings have been reported for a very concise number of pesticides. (Band et al. 2011;Bonner et al. 2010;Lynch et al. 2009;Mills and Yang 2003;Settimi et al. 2003). It has to be noted that all the encountered studies applied methodologies other than biological monitoring of the pesticides, and some of which were of lower accuracy as previously discussed. (Mills and Yang 2003;Settimi et al. 2003) The observed marked deficiency concerning quantification of occupational exposure to OC pesticides makes it hard to reach a firm conclusion regarding higher levels of at job exposure.

Difficulty in pesticide exposure quantification

In spite of the fact that a weak association has been detected from pooling the results of studies of farmers, the quality of exposure assessment may not allow us to draw firm conclusions. The consistency of our results with previous meta-analyses in finding a weak yet significant association between farming and prostate cancer, as well as farmers applying specific types of pesticides might reflect the carcinogenic impact of high levels of pesticide exposure, but it could also reflect exposure misclassification that likely occurs from the complexity of exposures in agricultural settings. Moreover, it is expected that exposures among different farmers may vary considerably, but trials to stratify farmers would result in a very heterogeneous mixture of exposure situations. (Blair and Freeman 2009)

Providing an adequate characterization of the intensity, durations and frequency of such exposures is quite challenging in epidemiological studies. Direct causality between exposures to different agents at the workplace and increased risk of cancer prostate continues to be very difficult to prove especially when investigating the carcinogenic effects of long term exposure.

Moreover, pesticide as a specific exposure entails its particular challenges of quantification. Issues related to epidemiological evidence concerning pesticide exposure may include (1) the diversity of pesticide types; (2) the need to obtain information on exposure to specific individual pesticides; (3) variability in the duration and intensity of exposures among different occupationally exposed groups; (4) there are marked changes in pesticide use pattern overtime which is difficult to track. This might explain the different methodologies that the authors have applied in the

available epidemiological studies relating pesticide exposure to prostate cancer.(Fenske 2005)

Dose response association

Deficiency of quantification of exposure continues to limit interpretation of results, and does not allow sufficient research on dose-response association. Therefore, commenting on a dose-response association would only be relevant for the several specific OC pesticides for which studies available have adopted high quality exposure quantification methods.

For the majority of the examined OC pesticides, there was no difference between low and high exposure levels. However, a higher pooled estimate was obtained for low exposure to DDT when compared to high exposure. This might be explained by the specific nature of DDT as an endocrine disruptor where a nontraditional dose-response dynamics has been proposed. Low doses were observed to exert more potent effects than higher doses, as well as exerting nontraditional dose-response curves, such as inverted-U or U-shaped curves.(Diamanti-Kandarakis et al. 2009) This has also been suggested for HCB, as in vitro and in vivo data suggested that HCB agonizes androgen action at low levels but not at higher levels. (Ralph et al. 2003)

Toxicological and mechanistic studies addressing the association between prostate cancer and pesticide exposure (Biological Plausibility)

A huge body of literature including mechanistic and toxicological studies has been published examining the carcinogenic potential of pesticides (Vakonaki et al. 2013; Mrema et al. 2013) Moreover, most of the data associating exposure to endocrine

disruptors to hormone dependant cancers arise from toxicological studies. (Prins 2008; McKinlay et al. 2008; Mnif et al. 2011) Mainly on the basis of animal data, a number of OC pesticides have been classified as of Group 2B (possible carcinogens) (Rogan and Chen 2005) According to the IARC monographs on the evaluation of carcinogens to humans, *possible* carcinogenicity is used for "agents for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals, or when there is inadequate evidence of carcinogenicity in humans but there is sufficient evidence of carcinogenicity in experimental animals". Our results support this classification as available epidemiological evidence does not support a concrete association while mechanistic and animal studies seem to provide a basis of biological plausibility.

However, it is important to note that only few studies have directly examined the carcinogenic potential of specific pesticides on prostate cell lines. (Hu et al. 2012;Tessier and Matsumura 2001) The association revealed from laboratory studies was usually demonstrated for other cancers as breast cancer. Nevertheless, results from a recent meta-analysis provided strong evidence to discard the putative relationship between p,p'-DDE and breast cancer risk, in spite of the fact that DDT appear to possess the strongest evidence of biological plausibility as an endocrine disruptor. (Lopez-Cervantes et al. 2004) After a substantial amount of research on organochlorines and breast cancer, the overall conclusion is that the evidence does not support the previously indicated positive associations. (Alavanja et al. 2013)

Even if evidence of carcinogenic and endocrine disrupting effects generated under experimental conditions supports the biological plausibility, interspecies extrapolation

is a source of uncertainty. In experimental studies on animals, the conditions of exposure and sometimes the genetic makeup of the animals are controlled by the researcher and because of these conditions; the results of animal studies may not be easily extrapolated to humans. (Clapp et al. 2008)The validation of toxicological testing methods for endocrine disruptors end points is well underway and much controversy remains over the lack of reproducibility of observed low effects. (Martin 2007)

Many authors have also declared that an important uncertainty for occupational risk assessment is the disparity between animal studies and worker exposures in terms of route, frequency, and duration of exposure. (Acquavella et al. 2003;Ross et al. 2001) In spite of the fact that in recent years rapid advances of biochemical sciences have resulted in the development of bioassay techniques that contribute invaluable information regarding toxicity mechanisms at the cellular and molecular level, the extrapolation of such information to predict effects in an intact organism for the purpose of risk assessment is still in its infancy. (Gundert-Remy et al. 2005)

Furthermore, in spite of the large body of literature suggesting different mechanisms of actions of endocrine disruptors including many pesticides in the pathogenesis of hormone dependent cancers, it seems that the variability of suggested mechanisms of actions may make the net effect difficult to determine. Different pesticides have been suggested to act as agonists or antagonists to estrogen or androgen receptors, as well as exerting effects on synthesis or metabolism of enzymes, thus implying discrete and sometimes conflicting ways that varies too with exposure levels. Moreover, many authors have also stressed on the complexity of the role of estrogen in the

development of prostate cancer that further complicates the suggested role of the hormonally active substances. (Soronen et al. 2004; Carruba 2006)

Despite a compelling role of evidence as well as decades of observational research of suspected modifiable risk factors, no conclusive modifiable environmental or lifestyle risk factors have been identified to date. Some authors have proposed that a possible reason for this is the predominant focus of prostate cancer research on mid or late life exposures long after the prostate has developed. Whereas earlier life exposures, such as those that occur during childhood and adolescence when the prostate is still maturing, may be as or more important for prostate cancer risk.(Sutcliffe and Colditz 2013) Experimental toxicological evidence with rodents indicates that timing of exposure to endocrine disruptors may be critical for prostate cancer development.(Boberg et al. 2015)

Gene environment interaction involved in the association between prostate cancer and pesticide exposure

The role of genetic susceptibility has long been established for prostate cancer. In recent years, with the advance of genotoxicity and molecular biology mechanistic studies, more research has been directed to gene-environment interaction. Some authors have demonstrated a significant interaction between certain genetic susceptibility loci, exposure to specific pesticide and prostate cancer risk. (Koutros et al. 2010b;Koutros et al. 2011;Koutros et al. 2013b) Others have even reported a dose response association between deficiencies in nucleotide excision repair capacity (which plays a critical role in repairing DNA damage caused by exposure to pesticides) and elevated prostate cancer risk. (Hu et al. 2004) Recent studies have also postulated

very specific roles of genetic variations as vitamin D pathways genes where authors demonstrated specific genetic variants to be linked to modifying pesticide associations with prostate cancer risk. (Karami et al. 2013) Furthermore, variation in lipid metabolism genes was found to modify pesticide associations with prostate cancer; in spite of the authors commenting on the need of further replication of their results. (Andreotti et al. 2012)

Some evidence has also been displayed from epidemiological studies. The consistent findings of a high incidence of prostate cancer among the inhabitants of French West Indies might be a reflection of gene environment interaction. This is because 90% of the population is black Africans and exposure has been particularly high to several organochlorine pesticides that polluted the rivers due to plantations of banana trees. (Belpomme et al. 2009;Belpomme and Irigaray 2011;Emeville et al. 2015;Landau-Ossondo et al. 2009;Mallick et al. 2005)

Suggestions for future exposure assessment procedures involving the evaluation of gene-environment interaction

There is a manifest need to consider the variability of genetic susceptibility factors that eventually determine the internal and biologically effective dose that might be related to the carcinogenic risk of pesticide exposure.

Recently, a method of estimating the biologically effective dose has been adopted by integrating the levels of external exposure with the protective ability of genetic susceptibility markers. In this process, the level of external exposure may either be reduced or increased depending on the capacity of Phase I (activation) and Phase II

(detoxification), and DNA repair enzymes. In this approach, genetic susceptibility markers, e.g. CYP1A1, GSTM1, NAT1, NAT2, or DNA repair capacity are used as if they were internal personal protective equipment.

This approach might allow us to evaluate the association between an unlimited number of genetic susceptibility markers and exposure to specific carcinogens including different types of pesticides. However, the challenge is in finding the appropropriate biological markers that interact with specific pesticides in the carcinogenic process of prostate cancer. (Nieuwenhuijsen 2010)

Challenges faced when studying etiology of prostate cancer

Quantification of pesticide exposure is not the unique challenge when investigating its role in prostate cancer development. Prostate cancer also has a specific nature and characteristics that make studying it epidemiologically problematic. Therefore, results should be interpreted in light of the existing sources of bias.

The high proportion of latent prostate cancer is expected to create particular difficulty for epidemiological studies. Autopsies have revealed that the prevalence of latent prostate cancer is very high. More than 20% of men who have reached the age of 50 have prostatic carcinoma that meets the histopathological criteria for malignancy. A major question is whether the apparently indolent disease merely represents an earlier stage that would eventually become aggressive, or if it will remain indolent and whether the two represent etiologically distinct entities. (Jahn et al. 2014)

Furthermore, the high prevalence of indolent cases is expected to produce inevitable selection bias. Using general population as controls in case-control studies, which is

generally recommended, would include a high percentage of un-diagnosed cases. For that, some authors have explained that using clinic controls that demonstrate normal PSA levels or free biopsy may exceptionally provide a better selection of the controls. (Aronson et al. 2010)

On the other hand, the emergence of PSA testing in the late eighties has completely altered the approach to prostate cancer diagnosis. Prostate cancer once diagnosed at an advanced stage in older men, is now often detected at an early stage in younger men as a consequence of more widespread screening of the disease. This trend toward earlier diagnosis of prostate cancer has most likely changed the definition of a "case" of cancer, since many men who would have qualified as controls in previous epidemiological studies are known to have prostate cancer as a result of prostate cancer screening. (Nelson et al. 2003) Prostate cancer cases diagnosed in the PSA era are more likely to have early lesions, which may differ in etiology from advanced lesions and more aggressive tumors. Therefore, risk estimates from the newer studies that include a large number of early-stage cases may differ substantially from older studies including mostly clinically relevant tumors. It is therefore of marked importance that future studies include prostate tumor sub-classification, such as methods of detection and markers of biological aggressiveness, in order to provide more accurate and comparable risk estimates for specific risk factors. This was applied only minimally in recent epidemiologic investigations. In a recent study, authors reported significant increased risk of aggressive prostate cancer associated with a number of pesticides.(Koutros et al. 2013a)

Another important issue that might be of close relevance to studies on farmers is the w*ide variation in PSA test frequency.* The impact of PSA screening on clinical practice is well-recognized, but its effect on epidemiologic research is less appreciated. (Jahn et al. 2014) It should be clear that any factor that affects diagnostic intensity and particularly PSA testing will inevitably affect observed prostate cancer incidence, making it difficult to distinguish between factors that influence disease development and those that influence disease detection. This is of special relevance to our study due to the consistently reported lower PSA screening rates among farmers than among the general population on account of variability in the availability of health insurance or access to care in rural areas which applies to different countries. (Baade et al. 2011;Coory and Baade 2005;Jemal et al. 2005;Obertova et al. 2012) It has also been reported that farmers over 50 years were less likely to have had a digital rectal examination of the prostate. (Muldoon et al. 1996) If it is subsequently found that farmers do have lower PSA screening rates, it would suggest a bias towards the null for any comparative incidence ratios not adjusted for this effect which implies masking of any increased prostate cancer incidence that exists among farmers.(Depczynski and Lower 2014) PSA testing variability was very rarely handled in the available epidemiological literature. In one study, the authors mentioned that an increased risk of prostate cancer was observed after adjusting for prostate cancer screening as a negative confounding factor, but results were not reported. (Multigner L. et al 2010)

Another crucial aspect in investigating the etiology of prostate cancer is the so far ambiguous role of other potential risk factors. This makes eliminating residual confounding very difficult, as many determinants of prostate cancer are still

unknown.(Zeegers et al. 2004) In spite of the numerous studies addressing possible etiologies of prostate cancer, there are no established risk factors other than age, family history and ethnicity. Consequently, authors chose to adjust for diverse factors discordantly due to lack of concrete evidence about confounding factors.

Adjusting for confounders

In spite of the documented increased risk of prostate cancer among those with a positive family history, (Frank et al. 2014) very few studies adjusted for this important confounder. We obtained a high and significant pooled OR from pooling the results of three studies (Koutros et al. 2013; Multigner et al. 2010b; Strom et al. 2008) that estimated prostate cancer risk among the highest pesticide exposed groups that have a positive family history. An important genetic component contributing to prostate cancer risk associated with exposure to specific pesticides has been suggested in many recent studies, which may point to gene-environment interaction. (Koutros et al. 2010b;Koutros et al. 2011;Koutros et al. 2013b)

Owing to the importance of adjustment for confounders, we have chosen to use the adjusted rather than the unadjusted ORs. However, in spite of our attempts to examine the impact of adjustments on the pooled estimates, we could not obtain relevant results due to the obvious discrepancy in the numbers of studies adjusting for different types of variables to those not adjusting. For example, our trials to stratify studies by adjustment for ethnicity have failed, due to the small proportion of studies that adjusted for it. However, the role of ethnicity should be taken into account when interpreting the results provided by different studies.

General quality of the available epidemiological data

Available epidemiological data, although not quantitatively deficient, may be qualitatively questioned. Previous meta-analyses, addressing other exposures and risks of different types of cancer have also reported the need for improving the quality of exposure assessment component of epidemiological studies. (Lenters et al. 2011) For this reason, we have attempted to display potential sources of bias to be able to interpret results more precisely.

There is an obvious scarcity in cohort studies that address the association between pesticide exposure and prostate cancer risk. Except for the AHS, the quality of the other cohort studies was observed to be low. This is due to marked deficiencies in assessment of pesticide exposure that depended mainly on crude non specific data serving as surrogate measures of exposure. For that reason, the majority of the analyses we conducted in our second meta-analysis (pesticide exposure and prostate cancer) were exclusive to case-control studies.

We have also observed that available cohort studies were published in the nineties while collecting information dates back to the seventies. (Fleming et al. 1999;Wiklund and Dich 1995;Zhong and Rafnsson 1996) The new data is exclusively provided by articles utilizing data of the AHS. In contrast to the other cohort studies, this large prospective cohort study was unique in using determinants of subject-specific and pesticide-specific exposure assessment, thus an expected reduction of exposure misclassifications by considering the between-individual variability. Apart from this study, there is an obvious deficiency that calls for conducting more rigorously designed

prospective cohort studies with improved detail on specific pesticide exposure and potential confounders.

Publication bias

In spite of the several sources of publication bias, available tests to detect it are deficient (Ioannidis 2008) and confined only to those for small study effect.(Egger et al. 1997) Small study effect implies that authors tend to publish small sized studies more commonly when they report positive results. According to Egger's test, our results from pooling studies of the association between prostate cancer and high pesticide exposure have shown that small study effect might exist. What was interesting is that this was only observed when we pooled estimates for high, but not for low exposure levels. There was also observed asymmetry in the funnel plot representing estimates for high exposure to pesticides and not for low exposure might which might further indicate the existence of publication bias.

Furthermore, other sources of publication bias as location as well as language bias are almost inevitable. In spite of our including studies that were published in diverse geographical locations, it is quite evident that the majority of studies were conducted in USA and European countries. We have tried also to extend our search strategy regarding languages sought, but a domination of English language studies was observed.

Moreover, the role of data irregularities cannot be ruled out, especially that we have observed heterogeneity when pooling estimates of high exposure to pesticides.(Sterne

AC 2009) Our results have explained, to an extent, several sources of these heterogeneities.

The role of meta-analysis in cancer epidemiological studies

Some authors have recommended meta-analyses as an essential tool for interpretation of the vast number of studies in cancer epidemiology. (Morris 1994) Also, a well conducted meta-analysis has been considered an important technique for causal inference that provides precise estimates of the overall strength of association of epidemiological evidence. Well conducted meta-analyses have also been considered to provide, when possible, an improved technique for determining the extent to which the evidence is consistent.(Weed 2002) However, some authors have also criticized meta-analyses especially of observational studies for combining results that may represent different measures and therefore mixing of data to obtain a single estimate that might lack relevance.

We have managed to avoid the limitations previously criticized and followed the recommendations set specifically for meta-analyses of occupational and cancer epidemiology. (McElvenny et al. 2004;Morris 1994) First of all, we have explored as much as was possible of sources of heterogeneity between studies, as relying on pooled estimates that does not represent homogenous studies would be inapplicable.(Egger et al. 1998) We have applied sensitivity analysis and sub-stratification rigorously, as we believe that detection of sources of variability between studies and identification of the direction of its effect might be even of more value than reporting a single pooled OR.

We have also put much effort in assessing the quality of the articles as well as stratifying studies by various measures that might indicate the quality of the articles. This is because meta-analysis does not put more weight to studies that use more valid exposure assessment or have better quality.

Moreover, owing to the relatively small studies especially about exposure to specific OC pesticides, we have used I^2 test which does not depend on the number of studies Thus we have avoided solely depending on Q test that has been criticized for being poor at detecting true heterogeneity among studies as significant and of having low power in case of pooling small number of studies.(Higgins et al. 2003)

Comparison to previous reviews and meta-analyses

A number of previously published reviews summarized the association between pesticides and multiple cancer sites. However, we observed a tendency to emphasize outcomes of borderline statistical significance and to display positive findings or high risk estimates without including CI that, in most cases, demonstrated a statistically insignificant association.(Bassil et al. 2007;Clapp et al. 2008)

More meticulous meta-analyses provided a modest risk estimate that could not be definitely attributed to a specific pesticide group.(Van Maele-Fabry and Willems 2003;Van Maele-Fabry and Willems 2004) This is because in most of the included studies, methodologies applied suffered from inadequate quantification techniques and non specificity of the pesticides studied.

It is important to note that previous published meta-analyses concerned with the association between pesticide exposure or farming and prostate cancer did not assess

the quality of the included articles. Some authors have even questioned the accuracy of the results of these meta-analyses due to a possible impact of the quality of the articles on the obtained results that was not examined. (Parent and Siemiatycki 2001)

Results obtained from pooling estimates for high exposure to pesticides are to a high degree in concordance with previous meta-analyses, which also presented a weak yet significant positive association. (Van Maele-Fabry and Willems 2003;Van Maele-Fabry and Willems 2004)However, given the heterogeneity detected between studies, considering results obtained from further stratifications would be more accurate than relying on the overall pooled estimate. (McElvenny et al. 2004)

Keller Burne and colleagues (1997) examined the association between farming and prostate cancer, based on articles published from 1988 to 1994. The pooled estimate provided for thirteen retrospective studies was 1.29 (1.10-1.51). Although there was no assessment of exposure to pesticides, authors proposed hormonally active agricultural chemicals to be responsible for the obtained association. (Keller-Byrne et al. 1997) One year later, Acquavella reported similar results and explained heterogeneity by design and geographical location of the studies. (Acquavella et al. 1998) Van Maele Fabry and colleagues included studies published till 2001. The pooled estimates were 1.13(1.04-1.22) and 1.24(1.06-1.45) for two consecutive meta-analyses. (Van Maele-Fabry and Willems 2003;Van Maele-Fabry and Willems 2004) Our finding higher pooled estimates for USA and Canada than for European countries is consistent with the results noted by previous meta-analyses. (Acquavella et al. 1998;Van Maele-Fabry and Willems 2003;Van Maele-Fabry and Willems 2004) Also, the higher pooled OR we noted for case-control studies where controls were cancer

patients is in concordance with previous findings. (Van Maele-Fabry and Willems 2003;Van Maele-Fabry and Willems 2004)

For studies assessing specific OC pesticides, we have observed that significant associations were only reported occasionally by studies that assessed occupational exposure using solely geography based usage and employment records (grouped assignment of exposure). (Band et al. 2011;Mills and Yang 2003;Settimi et al. 2003) This is in concordance to results of a critical review that assessed all available epidemiological data relating exposure to Agent Orange to an increased prostate cancer risk. Authors demonstrated that positive findings have been confined to studies that relied on geography based and self report, whereas no association was reported by studies measuring serum levels of Agent Orange. (Chang et al. 2014)

Strengths of our study

We consider that the points of strength of our meta-analysis of pesticide exposure and prostate cancer include the following. First, we have put much energy on making use of specific estimates provided in the included articles according to degrees of exposure. We have combined data in a precise manner and obtained pooled estimates for three specific categories of exposure that was not the case for the previous meta-analyses.

We have also included more recent studies than previous meta-analyses and explored sources of heterogeneity not previously tackled. Since our main objective was to examine the potential association between exposure to pesticides and prostate cancer risk, we have pooled results of studies providing specific estimates for pesticide

exposure. This was to avoid the inadequacy of exposure information that was highlighted by previous meta-analyses as a limitation for drawing firm conclusions.(Acquavella et al. 1998;Blair et al. 1992;Van Maele-Fabry and Willems 2004) In another analysis, we have pooled results of studies about farming and prostate cancer that did not provide direct measurements of pesticide exposure. Therefore, we did not mix data about specific pesticide exposure with those that assume pesticide exposure by job title.

Also, in spite of broadening our inclusion criteria, we chose not to include studies depending merely on mortality rates. This is because we consider them to be a poor indicator of prostate cancer risk, given the usual chronic nature of prostate cancer as well as its high survival rate.(Ilic et al. 2013) Thus, our relying on incidence rates may have served more our etiological hypothesis.

A major strength of the results obtained from the pooling estimates for specific OC pesticides is the originality of the analysis. To our knowledge, no previous meta-analysis has been conducted to examine the association between exposure to specific types of OC pesticides and prostate cancer risk. Moreover, the majority of the included studies were of high quality which may add to the value of the obtained estimates. This is in addition to the importance of the number of OC pesticides examined that share in common being highly persistent in nature, classified by IARC as Class 2B, as well as possessing endocrine disrupting potential. Homogeneity has been consistently observed for almost all of the pooled estimates which might add to the robustness and reliability of the results obtained.

We have also explored as much as was possible of the potential sources of heterogeneity, and stressed on pooled estimates for homogenous studies. Thus, we consider that we have combined the rigor of the purely quantitative meta-analysis with the concern for variations in study design exhibited in review articles.

Limitations of the meta-analyses

Although we have tried to manage the data as accurately as possible, there might still be some sort of non-differential misclassification. We utilized risk estimates presented for quartiles 4, tertiles 3 and the higher exposed (in studies presenting low and higher exposed categories) and those with the longest duration of exposure, to present the highest exposed groups. However, this grouping was inevitable, as stratifying into more narrow categories has led to the production of un-informative strata.

For studies that reported a number of studied pesticides, we utilized estimates for OC pesticides. This was the only way to avoid losing data especially that we have chosen not to be hindered by different methodologies adopted by studies, but to assess their impact on obtained results. OC pesticides were thought to be the best option as it was the common pesticide assessed by the included studies, as well as its studied endocrine disruptor and carcinogenic potential which was also in favor of our choice.

Regarding pooling estimates for specific OC pesticides, the main drawback was our depending on a relatively small number of studies, which is attributed to the limited epidemiological data that focuses on specific OC pesticides, especially for occupational exposure. Also, in spite of the higher chance of detecting association with more specificity when pesticides are treated as separate types, possible effects related to

mixtures could be missed. However, data on simultaneous co-exposures and interactions between chemicals are limited.(Diamanti-Kandarakis et al. 2009)

On the other hand, pooling estimates provided by studies associating prostate cancer to farming that lacked pesticide exposure assessment was only of a hypothesis generating potential due to the lack of exposure data. However, we meant to explore the hypothesis that farmers are more at risk of developing prostate cancer compared to other jobs which has been previously highlighted by many authors.

CONCLUSIONS

1. Our systematic review summarizes the currently available epidemiological evidence on the association between pesticide exposure and prostate cancer. The results obtained do not support an association between pesticide exposure and prostate cancer among the general population. As for occupational exposure to pesticides, despite sporadic findings, existing evidence does not point to any pesticide as satisfying widely used guidelines for establishing a concrete association with prostate cancer.

2. Heterogeneity between studies was explained by the methodology applied for quantification of exposure as well as the quality of the articles. We observed that positive findings were mostly confined to farmers exposed to high levels of specific groups of pesticides, but an impact of the exposure assessment methodologies on the pooled results was also observed. Higher magnitudes of associations were observed when pooling results of studies with poorer quality of exposure assessment.

3. An increased prostate cancer risk was observed for high occupational exposure to a number of pesticides among individuals with a positive family history, which may point out to gene-environment interaction. In spite of that, only a small number of studies adjusted for family history of prostate cancer.

4. For studies concerned with specific organochlorine pesticides, according to the currently available epidemiological data, we cannot confirm the existence of a concrete association between exposure to specific organochlorine pesticides and prostate cancer. Our results are consistent with classifying many types of organochlorine pesticides by IARC as possible carcinogens. Evidence was suggested

from experimental studies, due to the challenge faced when assessing exposure to pesticides in case of epidemiological studies.

5. We have noticed deficient methodologies applied to quantify exposure to pesticides in occupational settings, which make it difficult to relate specific organochlorine pesticides to prostate cancer risk. Studies that quantified exposure more precisely were conducted among the general population, where prostate cancer risk was compared according to pesticide serum levels. The need for further research is evident to reveal if exposure to specific pesticides types is related to prostate cancer, especially in occupational settings.

6. Farmers might have a slightly increased risk of developing prostate cancer than the general population. However, testing the hypothesis that this might be directly linked to pesticide exposure is not possible, given the deficiency in assessment of specific exposures in the available epidemiological literature. Available studies need to be balanced by a greater number of more rigorously designed cohort studies, with improved detail on farm exposures and potential confounders.

7. There are still gaps in the available research that makes the association between occupational pesticide exposure and prostate cancer unclear. Future research should put more effort on: (1) validating quantification methods applied for assessment of occupational exposure to specific pesticide categories to detect the magnitude of the potential association with prostate cancer more precisely; (2) conducting more studies, especially in the developing world, where pesticides that have been banned in USA and Europe are still in use; (3) designing new studies taking into account well known sources of biases as differences in PSA testing rates, selection of population

controls and confounding factors as family history of prostate cancer that should be adjusted for more rigorously; (4) conducting more research on aggressive forms of prostate cancer which may better contribute to targeted prevention strategies in occupational setting, given the high incidence of latent prostate cancer.

8. Overall, epidemiological research offers no convincing evidence of causal association between exposure to pesticides and prostate cancer. More accurate exposure assessment is needed in large epidemiological studies to rule out a probable association more conclusively. Rigorous studies that accurately and objectively estimate pesticide exposures and consider gene-environment interactions are still needed to determine if there is an association between pesticides and prostate cancer.

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GLOSSARY

AHS= Agricultural Health Study

BPH = Benign Prostatic Hyperplasia

CI = confidence interval

DDT = dichlorodiphenyltrichloroethane

EPA = Environmental Protection Agency

FH = Family history

HCB = hexachlorobenzene

HCH = hexachlorocyclohexane

IARC = International Agency of Research on Cancer

IWLD= Intensity weighted lifetime days of exposure

JEM = job exposure matrix

LED= Lifetime exposure days

NOS= Newcastle-Ottawa Scale

OC= Organochlorine pesticides

OR = Odds ratio

PC = Prostate cancer

PPE = personal protective equipment

P,p´-DDE = p,p -dichlorodiphenyldichloroethylene

RR = Relative Risk

SE = standard error

SEER = the surveillance, epidemiology and end results program (Program of the national cancer institute that works to provide information on cancer statistics in effort to reduce the burden of cancer among US population).

SIR = Standardized Incidence Ratio

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APPENDIX

Medical Subject Headings (MeSH) Terms

("prostate"[MeSH Terms] OR "prostate"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "neoplasm"[All Fields]) OR ("carcinoma"[MeSH Terms] OR "carcinoma"[All Fields]) OR ("adenocarcinoma"[MeSH Terms] OR "adenocarcinoma"[All Fields]) OR ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND ("pesticides"[Pharmacological Action] OR "pesticides"[MeSH Terms] OR "pesticides"[All Fields]) OR ("hydrocarbons, chlorinated"[MeSH Terms] OR ("hydrocarbons"[All Fields] AND "chlorinated"[All Fields]) OR "chlorinated hydrocarbons"[All Fields] OR "organochlorines"[All Fields]) OR ("organophosphates"[MeSH Terms] OR "organophosphates"[All Fields]) OR ("ddt"[MeSH Terms] OR "ddt"[All Fields]) OR DDE[All Fields] AND (("pesticides"[Pharmacological Action] OR "pesticides"[MeSH Terms] OR "pesticides"[All Fields] OR "pesticide"[All Fields]) AND exposure[All Fields]) AND occupational[All Fields] AND farming[All Fields] OR farmers[All Fields] OR (farm[All Fields] AND ("manpower"[Subheading] OR "manpower"[All Fields] OR "workers"[All Fields])) OR (farm[All Fields] AND laborers[All Fields]) AND case-control[All Fields] OR cohort[All Fields] AND ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "incidence"[All Fields] OR "incidence"[MeSH Terms])

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

I CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
a) yes, with independent validation *
b) yes, eg record linkage or based on self reports
c) no description

2) Representativeness of the cases
a) consecutive or obviously representative series of cases *
b) potential for selection biases or not stated

3) Selection of Controls
a) community controls *
b) hospital controls
c) no description

4) Definition of Controls
a) no history of disease (endpoint) *
b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
a) study controls for _____ (Select the most important factor.) *
b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
a) secure record (eg surgical records) *
b) structured interview where blind to case/control status *
c) interview not blinded to case/control status
d) written self report or medical record only
e) no description

2) Same method of ascertainment for cases and controls
a) yes *
b) no

3) Non-Response rate
a) same rate for both groups *
b) non respondents described
c) rate different and no designation

II COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and
Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
a) truly representative of the average _____ (describe) in the community *
b) somewhat representative of the average _____ in the community *
c) selected group of users eg nurses, volunteers
d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
a) drawn from the same community as the exposed cohort *
b) drawn from a different source
c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
a) secure record (eg surgical records) *
b) structured interview *
c) written self report
d) no description

4) Demonstration that outcome of interest was not present at start of study
a) yes *
b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
a) study controls for _____ (select the most important factor) *
b) study controls for any additional factor ¯ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
a) independent blind assessment *
b) record linkage *
c) self report
d) no description

2) Was follow-up long enough for outcomes to occur
a) yes (select an adequate follow up period for outcome of interest) *
b) no

3) Adequacy of follow up of cohorts
a) complete follow up - all subjects accounted for *
b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) *
c) follow up rate < ____% (select an adequate %) and no description of those lost
d) no statement

TABLE A. Case-control studies examining the potential association between farming and prostate cancer

Study, Year, Country	Design	Cases	Control Group	Category of farming	Odds ratio	Source of data on occupation	Adjusted for	NOS quality score
Talamini R, 1986, Italy	Comparing different risk factors for PC including occupation among cases and controls	166 PC cases admitted to the hospital, diagnosed within the previous year (1980-1983)	202 controls admitted to the hospital for other diseases (not cancer nor hormonal nor urological diseases)	Agricultural workers	Relative risk 1.68(0.83-2.83)	Questionnaire	Age, marital status, occupation, BMI & diet	6
Pearce N, 1987, New Zealand	Comparing incidence of PC among different jobs	617 cases from New Zealand cancer registry (1979)	1234 controls from cancer registry (other cancer types)	Farmers/ farm managers	1.14(0.90-1.46) 90% CI, *1.14(0.85-1.53) 95% CI*	Recorded occupation in cancer registry	Age & year of registration	3
Brownson RC, 1989, USA	Evaluating different types of cancers among farmers	432 PC cases of farmers from Missouri cancer registry (1984-1988)	All other cancer sites	Farmers or farm workers	1.33(1.18-1.51)	Hospital medical record at time of diagnosis	Age	3
Reif J, 1989, New Zealand	Evaluating different types of cancers among farmers	2435 PC cases registered in New Zealand cancer registry (1980-1984)	All other cancer sites (17,469)	Farmers	1.26(1.13-1.41)	Recorded occupation in cancer registry	Age	3
Fincham SM, 1992, Canada	Evaluating different types of cancers among farmers	1130 male farmers identified from Alberta cancer registry (1983-1989)	Non farmer (3563 men with other occupations)	Farmers	1.31(1.11-1.55)[¥]	Self reported occupational history (SAQ) for major jobs	Age & smoking	3

Franchesi S, 1993, Italy	Evaluating different types of cancers among farmers	161 PC case from all hospitals located in North east Italy (1985-1991)	Patients admitted to the hospitals for acute, non-neoplastic conditions	Farmers	0.9(0.6−1.4)	Self reported occupational history (SAQ) for lifetime occupations	Age, smoking & alcohol drinking	6
Keller JE, 1994, USA	Evaluating different types of cancers among farmers	Farmers from Illinois state cancer registry (1986-1988)	All other cancer sites	Farmers, using codes of US census occupational classification	1.15(0.99-1.35)	United states census of Agriculture	Age & smoking	3
Aronson KJ 1996 Canada	Evaluating the association between different jobs + occupational exposures & PC	449 pathologically confirmed PC cases from a population based cohort (1979-1986)	533 population controls&207 cancer controls (by random digital dialing)	Farmers & horti-culturalists	1.18(0.77-1.81)	SAQ then a team of hygienists analyzed each job into exposures	Age, race, socio-economic status & respondent status	8
Van der Gulden JWJ 1995 Netherlands	Evaluating association between work environment & PC risk	345 PC cases from Cancer registry of cancer center IKO	1346 controls with benign prostatic hyperplasia	Agriculture & farm workers	Agricultural 0.85(0.57-1.25) Farm workers 2.74(0.94-7.98)	Self reported occupational history and exposures (SAQ)	Age	6
Ewing P &Bowei C 1996 UK	Prospective case-control study evaluating possible risk factors for PC including farming	Hospital based, 159 newly diagnosed PC cases (1989-1991)	161 men diagnosed with BPH & 164 non-urological hospital controls	Farming	0.74(0.46-1.18)	IAQ	Age	5
Krestev S, 1998, USA	Comparing OR of PC among different jobs	Hospital based, 981 newly diagnosed pathologically confirmed PC cases	1315 Population controls residing in area covered by cancer registries	Farmers/ Agricultural workers (based on broad 2 digit codes)	Agriculture 1.03(0.82-1.29) General farmers 2.17(1.18-3.98)[α]	IAQ (including detailed work histories)	Age , race & study area	7

Band PR 1999 Canada	Population based case –control study, comparing OR of PC among different jobs	1516 PC cases from British Columbia Cancer registry (1983-1990)	4994 controls with other types of cancer	Farmers (based on broad 2 digit and 3 digit occupational codes)	Ever working as a farmer, 1.22(1.07-1.40) Usual occupation as a farmer 1.38(1.14-1.67) 90% CI *1.38(1.09-1.73) 95% CI*	Questionnaire for life time job descriptions	Age, Smoking, education, alcohol & person filling the questionn-aire	6
Settimi L 2003 Italy	Hospital based case-control study, comparing OR of PC among different jobs	124 newly confirmed cases of PC from hospitals covering 5 rural areas	659 hospital controls; other types of cancer	Agricultural workers	Ever been employed in agriculture 1.4(0.9-2.0)	Employment records&IAQ	Age, family history & interview (direct/ Indirect)	6
Meyer TE 2007 USA	Population based case-control study evaluating the risk of PC among farmers by different activities	405 PC cases from South California Central cancer registries (1999-2001)	392 healthy men matched by age, race & region	Farmers	Ever working as a farmer 1.4(1.1-1.9)	Telephone interviews	Age, race & region	7

IAQ = Interviewer administered questionnaire, OR = odds ratio, RR = relative risk, SAQ = self-administered questionnaire, SIR = standardized incidence ratio
¥ Relative risk of PC among farmers for insecticides use was 0.73(0.54-0.98)
α OR for PC differed substantially when the authors stratified farmers by duration of employment. OR was 3.02, 95% CI (1.05-8.69) for being employed for < 5 years versus 0.90(0.24-3.42) for those employed ≥ 20 years.
For two case-control studies that provided 90% CI (Pearce N et al &Band PR et al), we calculated 95% CI and used it in the meta-analysis.

TABLE B. Cohort and Linkage studies providing an incidence rate for prostate cancer among farmers.

Study, Year, Country	Design	Collecting information about PC	Source of data about occupation	Category of farming	Estimate	Comments	Variables adjusted for	NOS quality score
Olsen JH, ™ 1987, Denmark	Linking different types of cancers to job titles using records	Danish cancer registry (1970-1979)	Two computer based national registries	Agricultural workers (job held longest)	PIR 1.13(0.93-1.38)	Record linkage, cancer cases only are included in the study	Age & calendar period	2
Gunnarsdottir H, 1991, Iceland	A retrospective cohort study evaluating cancer risks among 5922 farmers registered at the farmers' pension (compared to general population of Iceland)	Icelandic cancer registry (1977-1987)	Register of the farmers' pension fund	Farmers	SIR 0.71(0.53-0.93) 90% CI 0.71(0.51-0.98) 95% CI	No available data on duration of employment	Age	2
Wiklund & Dich, 1995, Sweden	A retrospective cohort study evaluating cancer risks among 140,208 farmers (compared with the general male population of Sweden)	Population based cancer environment registry (1971-1987)	Swedish population & housing census	Farmers	SIR 0.93(0.90-0.96)	--	Age	4
Kristensen P, 1996, Norway	A retrospective cohort study evaluating cancer risks among 136,463 farmers (compared with the total rural population of Norway)	Norwegian cancer registry (1969-1999)	Agriculture census & central population registries	Farm owners	SIR ɣ 0.96(0.85-1.08)	Depending on census information of activities on the farm, Rate ratio of PC for greenhouse workers was 1.45(1.01-2.09)	Age	5

Parker AS, 1999, USA	A prospective cohort study, with 9 years follow up of 1177 cancer free cohort. 103 PC cases were identified by linking to Iowa state cancer registry and compared to PC free cohort.	Iowa cancer registry (1986-1989) followed till 1995	Mailed interview (90%) & telephone interview (10%)	Farmers as usual occupation/ occupation held longest	Relative risk 1.5 (0.90-2.50)	Relatively small size of the cohort	Age, family history, smoking, alcohol, diet	6
Sharma Wagner, ™ 2000, Sweden	Record linkage, comparing 36,269 PC cases with employment information	Swedish National cancer registry (1961-1979)	Swedish cancer-environment registry	Farmers, agriculture, forest, garden and park workers	SIR 1.08 (1.05-1.11)	No information on duration of employment, nor other data that might be related to cancer	Age & region	3
Bouchardy C, 2002, Switzerland	Retrospective cohort/ case-referent study, comparing incidence of different cancer types in different occupations. For each cancer site, ORs by occupation were evaluated in reference to all other occupations.	Swiss cancer registry (1980-1999)	Swiss cancer registry	Farmers (at the time of registration)	OR 1.2(1.0-1.4)	Case referent study, using job codification of cancer registry (present or last job held), excluding males > 65 years due to lack of data on occupation	Age, registry, urbanity, nationality, marital status & calendar period	3
Zeegers MPA 2004, Netherlands	Prospective population based cohort study including 58, 279 men. Compared incidence of PC among different occupations. 830 PC cases were identified & compared to1525 sub-cohort (follow up from 1986 till 1993)	Self administered questionnaire	Cancer registries & Dutch National database of pathology reports	Farmers	Rate ratio for ever being a farmer 0.86(0.53-1.40)	No information on duration of employment	Age, family history, smoking, alcohol diet, education	7

Laakkonen A, 2008, Finland	Retrospective cohort study, 87,534 male farmers compared to the general population of Finland for different cancers	Finnish cancer registry (1995-2005)	Finnish farm registry and Statistics Finland	Farmers	SIR 0.98(0.93-1.03)	---	Comparing 5 years age groups	6
Pukkala E, 2009, (5 Nordic countries)	Retrospective cohort study (15 million individuals). Incidence ratios of different cancer types compared by occupation to the corresponding populations of the included 5 Nordic countries	Cancer registries of the 5 included Nordic countries (1961-2005)	Self administered questionnaire recorded in the census data of each country	Farmers	SIR 0.99(0.98-1.00)	---	Age	6
Koutros S, 2010, USA	Prospective cohort study, 52394 male farmers compared to general population of Iowa & North Carolina for incidence of different cancer types	Cancer registries of Iowa & North Carolina (1993-2006)	Self administered questionnaire	Pesticide applicators (mostly farmers)	SIR¶ 1.19(1.14-1.25)		Age	7
Frost G, 2011, UK	Retrospective cohort study, incidence of different types of cancers among British pesticide applicators was compared to the general population of UK	National Health service central (1987-2004)	Pesticides users health study	Agricultural pesticide applicators	SIR 1.07(0.93-1.22)	Lack of information on potential confounding variables	Age	4
Mills PK & Shah P, 2014, USA	Proportionate incidence of different types of cancers was compared between the united work farmers and the Hispanic population of California	California cancer registry	Membership of the united farm workers of America.	Farm workers	PIR 1.00(0.93-1.07)	Proportionate incidence rates, and no adjustment for confounders	Age	3

™ Linkage studies, ɣ Rate ratio for farmers working in orchards and greenhouses1.45(1.01-2.09), ¶ This is the SIR calculated for private applicators (1719 PC cases), for commercial applicators, number of cases was only 73 cases, the SIR was1.28 (1.00 − 1.61), we utilized the former due to the obviously larger number of cases.

TABLE C. Characteristics of the case-control studies included in the meta-analysis

First Author (year)	Country/ Location of the study	Cases	Sources of controls	Number of *cases/ controls*	Sources of exposure information	Factors adjusted for	Definition/Assessment of pesticide exposure	OR(95%CI)/ reference category	Conclusion/*Comments*
Forastiere F et al (1993)	Central Italy	Licensed farmers, cancer cases identified from records	Hospital controls, men with other types of cancer, non farmers	1579/*462*	Employment records	Age	Assessment was non-individualized (Grouped exposure assignment) Carried out in different ways; according to the type of crops grown and duration of farming.	≤ 10 year licensed pesticide application: 1.53(0.5-4.14) >10 years: 2.68(0.81-8.23) Ref = Non farmers	An increased but insignificant risk for PC among farmers applying pesticides for > 10 years. Also for those growing wheat; OR= 3.45(1.78-6.88) PC was one among a group of other types of cancer assessed. *Expected selection & information bias.*
Van der Gulden JWJ et al (1995)	Netherlands	Histologically confirmed PC cases identified from cancer registries	Men diagnosed with Benign Prostatic Hyperplasia (BPH)	345/1346	SAQ	Age	A case referent study. Exposure was self-reported by participants. Pesticides were studied as one among a large group of different at job exposures.	Sometimes exposed: 0.84 (0.63-1.13) Frequently exposed: 1.47 (0.88-2.46) Ref = Non exposed	Elevated OR was found for farm laborers but not for agriculture in general. *Expected selection bias (Controls with BPH).*
Aronson KJ et al (1996)	Canada, Montreal	PC cases from a population based cohort. Age (35-70)	533 population controls & 207 cancer control	449/740	Expert assessment of exposure (Detailed job history information translated into potential exposures) & IAQ	Age, race, socio-economic status & respondent status.*	Pesticides exposure among occupationally exposed personnel was assessed by experts. A model was applied for defining unexposed and 2 groups of exposed, included latency & duration in years, concentration x frequency of exposure	Non substantial exposure: 0.93 (0.48-1.83) Substantial exposure: 1.09 (0.57-2.08) Ref = Non exposed	OR was compared as regards substantial versus non substantial exposure. Elevated risk that was found for substantial exposure to pesticides disappeared in the fully adjusted model.
Ewings P & Bowie C (1996)	Great Britain, Somerset & east Devon	159 newly diagnosed PC cases at 3 hospitals (May 1989 till May 1991)	161 men diagnosed with BPH and 164 non-urological hospital controls (2 controls/case)	159/325	IAQ	Age	Dichotomous response (exposed/unexposed) to pesticides obtained for those who reported working as farmers.	OR for PC risk associated with pesticides exposure 0.68 (0.44-1.04) Ref = Non exposed	The study found no association between PC and farming or applying pesticides. *Expected selection bias (Controls had BPH and other urological problems; hospital controls)*

TABLE C. Characteristics of the case-control studies included in the meta-analysis

Krstev S et al (1998)	USA (Atlanta, Detroit, New Jersey)	981 new pathologically confirmed PC cases (479 blacks & 502 whites)	1315 Population controls (594 blacks & 721 whites) residing in area covered by cancer registries	981/1315	IAQ (including detailed work histories)	Age , race (blacks/whites) & study area	Authors studied farming as one among the many jobs correlated to PC. Ref = Non farmers	Working in crop farms for <5 years: 1.62 (1.04-2.53) 5-19 years: 1.27 (0.84-1.92) ≥ 20 years: 1.34 (0.51-3.51)	There was a significant association between farming and PC, but risk was restricted to short term workers. *Pesticide exposure was indirectly assessment (crop/livestock farming).*
Sharpe CR et al (2001)	Canada, Montreal	A subset from a population based case-control study. histologically confirmed incident cases(Age= 47-70)	Population Controls (selected randomly by digit dialing), matched by age & area of residence	*400/476*	IAQ	Age, race, smoking, alcohol intake, BMI, income & response rate	Exposed population were defined as those who were exposed once a week or more for ≥6 months to each of the studied substances (Among which are pesticides). Ref = Non exposed	OR for high exposure during leisure activities: 2.3 (1.3-4.2) Joint exposure at work and at leisure: 1.2 (0.6-2.8)	Exposure during leisure to pesticides and garden sprays has been associated with increased risk of PC but no association with occupational exposure
Mills PK et Yang R (2003)	USA	New cases of PC among a cohort of predominantly Hispanic labor union farm workers identified from cancer registries	Cancer free cohort (the study design was a nested case-control study)	*222/1110*	Employment records	Age & race (an entirely Hispanic population)	A large number of pesticides was assessed; Approach was a form of partially ecological exposure assignment (linking information about employment dates & location to records of pesticides kept by department of pesticide regulation)	For Lindane Low exposure 1.14 (0.45-1.77) Higher exposure 1.86 (1.10-3.17) Highest exposure 2.37 (1.22-4.61) Ref = lowest exposed	Risk was increased with lindane & heptachlor (OC pesticides). Suggestive increase for dichlorvos and methyl bromide, but no increased risk for other pesticides. A dose response association was detected for lindane & heptachlor exposure. *Expected information bias*

TABLE C. Characteristics of the case-control studies included in the meta-analysis

Ritchie J et al (2003)	USA	Pathologically confirmed new cases of PC enrolled in 2 clinics serving the area	Patients with no previous history of PC during annual check-up at family care clinics	*58/99*	Blood Samples + SAQ	Age, BMI & history of prostatitis	Serum was tested for a number of OC pesticides + Self reporting of various exposures using chemical checklists	OR for p, p' DDE exposure T2: 0.72 (0.31-1.71) T3: 1.08 (0.47-2.50) Ref= T1	There is suggestion that long term exposure to specific OC pesticides in the general population may contribute to an increased risk of PC
Settimi L et al (2003)	Italy, Including 5 rural areas	Hospital based study. New cases of PC, covering 5 rural areas (5 local & 3 university hospitals)	Hospital controls; other types of cancer.	*124/659*	Employment records & IAQ	Age, FH & interview (direct/indirect)	Exposure to different types of pesticides was assessed assuming that in areas under study, crop infestations were treated according to established protocols.	Ever been exposed; 1.4(0.9-2.0) OR for exposure to organochlorines for < 15 years: 2.5 (1.1-5.3) For > 15 years: 2.7 (1.2-6.3) Ref = Non exposed	Positive significant association was observed for exposure to OCs including DDT, dicofol & tetradifon. For the last 2 pesticides, there was tendency for the estimate to increase with duration of exposure. *Expected information & selection bias*
Boers D et al (2005)	Nether-lands	Population based, cases identified from cancer registries	Sub cohort from the Netherlands cohort study (NLCS) that included 58,279 men aged 55-69 years	*1386/2335*	Expert evaluation of at job exposure using SAQ & employment records	Age, FH & education	IRR was calculated for different occupational exposures reported to be associated with PC. For exposure quantification, cumulative model was utilized, combining probability & duration of exposure. Experts blind to cases & controls	IRR for cumulative pesticides exposure T1: 0.85 (0.53-1.36) T2:0.72 (0.45-1.14) T3:0.60 (0.37-0.95) Ref = Non exposed	Negative association between pesticide exposure and risk of localized or advanced PC.

TABLE C. Characteristics of the case-control studies included in the meta-analysis

Hardell L et al (2006)	Sweden	All men living in Orebro, between 1997 & 1999 referred to university hospital & diagnosed as PC (histopathologically)	Patients undergoing transurethral resection for Benign Prostatic Hyperplasia	*58/20*	Adipose tissue level of a number of OCs & Questionnaire	Age, FH & BMI	Adipose tissue biopsy was taken from abdominal wall of cases and controls and analyzed for a number of persistent organic pollutants with endocrine disrupting potential. Investigators were blind to cases & controls.	A greater than median concentration of p, p' DDE yielded an OR= 2.30 (0.77-6.85)	PC may be related to a certain types of persistent organic pollutants including a number of specific pesticides. *Results of this study were based on small numbers especially of the controls.*
Fritschi L et al (2007)	Perth, Western Australia (WA)	Population based study. 606 PC patients (Age= 40-75) identified from WA cancer registry	471 healthy men randomly selected from general population	*606/471*	Detailed expert assessment of exposure & IAQ	Age	Case by case expert exposure assessment to different substances including OCs &OPs, blind to cases &controls). Model utilized included probability, frequency, intensity & determinants of exposure as utilizing PPE	OR for non-substantial exposure to OCs (Over entire working time): 0.76 (0.33-1.75) Ref = Non exposed	No evidence that occupational exposure to pesticides increase the risk of PC. *All cases and controls had either no or non substantial exposure to pesticides.*
Meyer TE et al (2007)	USA, South California	Population based, cases obtained from South California Central cancer registries, (Age= 65-79)	Matched healthy controls identified from Health Care Financing Administration	*405/392*	Computer assisted telephone Interviews.	Age, race (Caucasian/ African Americans) & region	Information on farming related activities were assessed. OR was re- calculated for Caucasians & for African Americans.	Farmers vs non-farmers 1.4 (1.1-1.9) Farmers that applied pesticides vs non-farmers 1.6 (1.2-2.2)	Increased risk of PC was found among farmers who were Caucasians but not among African Americans.1.8(1.3-2.7) Farming for shorter durations was associated with increased PC risk

TABLE C. Characteristics of the case-control studies included in the meta-analysis

Strom SS et al (2008)	USA, Texas, Greater Housten Area	Population based, 176 Hispanic men of Mexican descent, with histologically confirmed PC.	174 age matched men of Mexican origin identified through random digital dialing	*176/174*	JEM & IAQ	Age, FH, ethnicity, education & physical activity	Lifetime occupational history included job title, major duties & period of employment. Exposure to pesticides was estimated using a validated JEM for each job title held for at least 1 year. Intensity estimated as low, medium, high.	Low exposure 1.01 (0.53-1.93) High exposure 3.44 (1.84-6.44) Ref = non-exposed	Occupational exposure to pesticides was associated with increased risk to PC among Hispanic men of Mexican descent.
Parent ME et al (2009)	Montreal, Canada	Part of a population based large study including 49 case series who had ever been farmers	183controls who have ever been farmers (Combining 56 population controls & 127 cancer patients).	*49/183*	Detailed expert assessment of exposure & IAQ	Age, ethnicity, education level & respondent status	OR estimated for at work exposure to 10 agricultural chemicals including pesticides. Substantial exposure defined as having a medium or high frequency of exposure, a medium or high concentration and > 5 years exposure.	Any exposure 1.4 (0.7-2.7) Substantial exposure 2.3 (1.1-5.1) Ref= non exposed farm workers.	Though results were based on a limited sample, exposure was assessed individually by experts, based on detailed description of each job held by each subject. *Expected selection bias of both cases and controls*
Subahir MN et al (2009)	Malaysia	PC patients treated at the main university Hospital	Matched hospital controls	*112/112*	IAQ	Age	Exposure to pesticides was based on the respondent report as exposed or not exposed. Ref = Non exposed	OR of PC for being ever exposed to pesticides 5.57 (1.74-17.8)	Cases were more likely to have been exposed to pesticides. *Expected selection & information bias*
Aronson KJ et al (2010)	Ontario, Canada	Patients scheduled for prostate core biopsy or visiting urologist. Age = 50-80	194 controls with other urological problems & 135 undergoing biopsy (PC free)	*79/329*	Blood Samples + IAQ	Age, alcohol intake, smoking & physical activity	Different types of OCs measured in blood samples.	For p ,p'- DDT as an example: T2:1.19 (0.63-2.26) T3:1.05 (0.55-2.00)	Plasma OCs levels were not associated with PC risk. For most OCs tested, OR was very close to the null.

TABLE C. Characteristics of the case-control studies included in the meta-analysis

Multigner L et al (2010)	Guadeluope French West Indies Carribean Islands.	Consecutive histologically confirmed incident cases attending urology clinic	Men participating in a systematic health screening program open to the general population	*623/671*	Blood Samples + IAQ	Age, FH, race, & history of PC screening.	Exposure level was stratified into quartiles by cumulative exposure index.	OR for chlordecone exposure: Q2:1.35 (0.80-2.26) Q3:1.13 (0.66-1.95) Q4:1.27 (0.76-2.13) Ref = Q1	Exposure to chlordecone increases the risk of PC. Stronger association was observed for those with a family history. OR for the highest exposed with family history, 3(1.12-8.07). Exposure was correlated with aggressive PC, 2.16(1.33-3.51)
Sawada N et al (2010)	Japan	New PC cases participant in a population based prospective cohort.	2 matched controls for each case were selected from the cohort	*201/402*	Blood Samples + SAQ	Age, race (Japanese), smoking, alcohol, BMI & marital status.	OR was estimated in relation to plasma levels of 9 organochlorines including DDT, HCB, by quartile levels.	OR by quartiles of exposure to DDT : Q2: 1.39 (0.79-2.44) Q3:1.29 (0.71-2.34) Q4:1.04 (0.54-2.03) Ref = Q1	No overall association between OCs and PC among the studied Japanese men from the general population. No significant association was detected when they further stratified the data according to PC stage.
Cockburn M et al (2011)	California USA	All patients aged 60-74 diagnosed from 2005 to2006 (Obtained from Cancer registries)	Healthy general population	173/162	Pesticides use records + SAQ	Age, race & occupation	Ambient pesticide exposure, based on California pesticide use report. Ref= Non exposed	Risk of overall exposure to OCs: 1.64 (1.02-2.63) Low exposure: 1.25(0.75-2.08) High exposed: 2.03 (1.17-3.52)	Significant increased risk was observed for organochlorines & methyl bromide, but not for pesticides used as control (maneb, paraquat,simazine)

TABLE C. Characteristics of the case-control studies included in the meta-analysis

Band PR et al (2011)	British Columbia, Canada	Population based male cancer patients from registries.	Internal controls with all other types of cancer.	1153/3999	JEM & SAQ	Age, ethnicity, smoking, alcohol intake & education	180 specific active compounds assessed. Quantitative exposure assessment depended on tasks (mixing, loading and spraying). These estimates were derived from the North American Pesticide handlers' database.	DDT as an example Ever applied: 1.47 (1.02-2.12) Low exposure: 1.24(0.71-2.16) High exposure: 1.68 (1.04-2.7) Ref = Non exposed	Exposure was assessed as no, low or high exposure. Exposure to a number of pesticides like DDT, lindane, simazine, 2,4 D, dichlone, malathion & endosulfan had been significantly associated with increased risk of PC. *Expected selection bias of the cancer controls.*

FH= family history of prostate cancer, **HCB** = Hexachlorobenzene, **IAQ**= Interviewer administered questionnaire, **IRR**= Incidence Rate Ratio, **JEM**= Job exposure matrix, **OCs** = Organochlorine pesticides, **OPs** = Organophosphate pesticides, **OR**= Odds Ratio, **PC**= Prostate Cancer, **Q1, Q2, Q3, Q4**= First, second, third & fourth quartiles, **Ref**= Reference Category, **RR**= Relative Risk, **SAQ**= Self administered questionnaire, **T1, T2, T3=** First, Second & Third tertiles,**vs =** versus.

* = Respondent status refers to self/proxy status of the respondent.

TABLE D. Characteristics of the cohort studies assessing prostate cancer among pesticide applicators

Author (Year)	Country	Exposed population	Name of Pesticide studied	Number of exposed/ non exposed	Exposure Assessment method	Factors adjusted for	Estimate(CI), reference category	Comments/ Conclusion
Zhong Y et Rafnsson V (1996)	Iceland	Several subgroups including licensed pesticide applicators	Pesticides in general	2,449 (whole cohort)	Registries of licensed pesticide applicators & gardeners' associations	No adjustment	SIR = 0.7(0.33-1.29) Compared to the general population of Sweden	No increased incidence of PC among pesticide applicators compared to expected values for the general population, on the basis of cancer registries in Iceland.
Dich J Et Wiklund k (1998)	Sweden	Pesticide applicators in agriculture (licensed between 1965 & 1976)	Pesticides in general	20,025 (whole cohort)	Employment records	No adjustment	SIR = 1.13(1.02-1.24) Compared to the general population of Sweden	Weak but statistically significant increased risk of PC among studied pesticide applicators
Fleming LE *et al* (1999)	USA, Florida	Licensed pesticide applicators	Pesticides in general	33,658 (whole cohort)	Data Linkage	Age	SIR =1.91(1.72-2.20) Compared to the general population of Florida	PC was significantly elevated among the studied cohort (exposed to pesticides) compared to the general population.
Koutros S * *et al* (2013)	USA, Iowa & North Carolina	Pesticide applicators of the AHS	Life time use of 48 Different Pesticides	54,412 (whole cohort)	SAQ of AHS	Age, FH , race, smoking & use of other pesticides shown to be associated with PC	For DDT, Rate ratio (Quartiles of exposure by IWLD) Q1=0.98(0.78-1.22) Q2=1.27(1.02-1.58) Q3=1.27(1.02-1.58) Q4=1.18(0.95-1.48) Ref = Non exposed	A limited number of pesticides were significantly associated with increased PC risk. 3 OPs that were associated with aggressive PC: fonofos, malathion & terbufos as well as OC aldrin with high rate ratios for the higher quartiles of exposures.

* We have chosen the most recent article belonging to AHS that fit our selection criteria to be included in our meta-analysis (Koutros S *et al* 2013), **AHS** = Agricultural Health Study, a large prospective cohort study of licensed pesticide applicators in Iowa & North Carolina, **FH** = Family History, **IWLD**= Intensity weighted lifetime exposure days, **OC**= Organochlorine Pesticides, **OPs**= Organophosphate Pesticides, **PC**= Prostate cancer, **Ref** = Reference category, **SAQ of AHS**= Self administered questionnaire of the Agricultural Health Study, included general information on exposure to 50 different types of pesticides, application methods, use of personal protective equipment, pesticide mixing, other basic demographic characteristics including family history of cancer, **SIR=** Standardized Incidence Ratio**, Q1, Q2, Q3, Q4**= Quartiles of exposure.

TABLE E. Characteristics of articles belonging to the Agricultural Health Study

First Author (Year)	Name of Pesticide studied	Number of exposed/ non exposed	Follow up period (average in years)	Factors adjusted for	Estimate (CI)/ Reference category	Comments/Conclusion
Alavanja MC (2003)	Many types of pesticides	20,381/2,042	1993-1999 (4.3 years)	Age& FH	For Chlorinated Pesticides T2= 1.29(1.02-1.63) T3L=1.51(1.15-2.0) T3H=1.39(0.99-1.97) Ref = lowest exposed.	OR was calculated for a large number of pesticide (45 commonly applied pesticides) Family history was significantly associated with increased PC risk Use of chlorinated pesticides among applicators aged > 50 years as well as methyl bromide was associated with PC risk.
Lee WJ (2004)	Alachlor (Herbicide)	26,510/23,470	1993-2000 (5.5 years)	Age & FH	SIR for non exposed 1.13(0.99-1.28) SIR for the exposed 1.16(1.04-1.30)	Authors studied association between alachlor exposure and many types of cancer. For PC, a small but similar excess was seen in exposed as well as the non exposed groups.
Rusiecki JA (2004)	Atrazine (Tiazine herbicide)	36,943/ 17,430	1993-2001 (6.5 years)	Age, FH, smoking & alcohol intake	RR (Quartiles of exposure by IWLD) Q2=1.03(0.76-1.41) Q3=0.86(0.62-1.20) Q4=0.89(0.63-1.25) Ref = Q1	No clear association was found between atrazine exposure and any type of cancer analyzed including PC.
Beane Freeman LE (2005)	Diazinon (Insecticide)	4,961 /18,145	1993-1997	Age, FH, smoking, education & total days of any pesticide application	For IWLD of exposure: T1= 1.44(1.04-1.98) T2= 1.27(0.91-1.78) T3= 1.25(0.85-1.83) Ref = non exposed	The association between a number of cancers and diazinon exposure were studied. There was no association between exposure to diazinon & PC risk.
De Roos AJ (2005)	Glyphosate (Broad spectrum Herbicide)	41,035/13,280	1993-2001 (8.0 years)	Age, FH, smoking & alcohol intake	RR (For IWLD of exposure) T2= 1.0(0.8-1.2) T3= 1.1(0.9-1.3) Ref = T1	There was no association between exposure to glyphosate and PC risk.
Hou L (2006)	Pendimethalin (Herbicide)	9,089/15,285	1993-2002 (7.5 years)	Age, FH, & smoking	T1=1.1(0.8-1.6) T2= 0.9(0.5-1.5) T3L=1.0(0.5-1.6) T3H= 1.0 (0.5-2.1) Ref = non exposed	RRs were calculated using LED for a number of cancers. No significantly increased risk of PC among pesticide applicators exposed to pendimethalin.
Lynch SM (2006)	Cyanazine (Triazine Herbicide)	20,341/30,459	1993-2002 (7.5 years)	Age, FH & race	RR for tertiles of exposure by IWLD: T2=1.39(1.03-1.88) T3=1.15(0.83-1.58) Ref = T1	No clear association was found between exposure to cyanazine and incidence of PC.

Mahajan R (2006)a	Phorate (OP)	5,903/15,113	1993-2002 (7.5 years)	Age, FH, smoking, state of residence, education & use of 5 most correlated pesticides	LED for Lowest exposed 0.89(0.65-1.21) Highest exposed 0.93(0.55-1.57) Ref=non exposed	PC was not significantly related to phorate use overall or among those without a family history, but the risk increased among applicators with a family history of PC. Interaction RR=1.53(0.99-2.37)
Mahajan R (2006)b	Fonofos (OP)	9,059/36,313	1993-2002 (7.5 years)	Age, smoking, state of residence & use of the 4 most correlated pesticides	RR(tertiles of exposure by IWLD) T1= 0.99(0.74-1.32), T2= 1.1(0.83-1.46), T3= 1.14(0.86-1.53) Ref= non exposed	Although PC risk was unrelated to fonofos use overall, a significant dose response trend was observed for lifetime exposure-days. RR for highest tertile versus unexposed for those with a family history=1.77(1.03-3.05) versus 1.28(1.07-1.54). Association was observed for other cancer types.
Rusiecki JA (2006)	Metachlor (Herbicide)	23,395/26,798	1993-2002 (7.3 years)	Age, FH, race, smoking, alcohol intake, applicator status state of residence & use of the most highly correlated pesticides	RR(tertiles of exposure by IWLD) T2= 0.91(0.69-1.21) T3= 0.67(0.44-1.01) Ref=T1	No significant association between exposure to metachlor and PC. A decreased RR for PC in the highest category of lifetime exposure.
Samanic C (2006)	Dicamba (Herbicide)	22,036/19,933	1993-2002 (9.0 years)	Age & FH	RR(tertiles of exposure by IWLD) T1=0. 97(0.77-1.21) T2= 1.03(0.77-1. 37) T3= 1.11(0.83-1.50) Ref= non exposed	Exposure to dicamba was not associated with risk of PC.
Bonner MR[66] (2007)	Malathion (Insecticide)	19,717 (whole cohort)	1993-2002 (7.5 years)	Age, FH, smoking, education & use of the 5 pesticides highly correlated to Malathion.	RR(tertiles of exposure by IWLDs) T1= 1.20(0.92-1.56) T2= 0.98(0.75-1.28) T3= 1.06(0.80-1.39) Ref = non exposed	No conclusive evidence that occupational exposure to malathion is associated with increased risk of 9 types of cancers studied including PC.
Mahajan R (2007)	Carbaryl (Carbamate Insecticide)	8,810/12,606	1993-2003 (8.2 years)	Age, smoking & use of other correlated pesticides	RR (tertiles of exposure by LED) T1= 1.07(0.85-1.36) T2=0.89(0.68-1.18) T3L=0.87(0.59-1.27) T3U=0.71(0.46-1.11) Ref= non exposed	No significant association between exposure to carbaryl & PC.
Purdue MP (2007)	Organochlorines	24,384/26,627	1993-2002 (7.3 years)	Age, FH, state, education, smoking, alcohol use, & lifetime days of total pesticides application	RR(tertiles of exposure by IWLD) T1= 1.10(0.80-1.50) T2= 1.20(0.80-1.40) T3= 1.20(0.80-1.70) Ref = non exposed	No clear association observed between PC risk and organochlorine insecticides.
Greenburg DL (2008)	Captan (fungicide)	4,383/44,603	1993-2004 (9.1 years)	Age, FH, smoking, alcohol intake, education & applicator type	Relative Risk (tertiles of exposure by IWLD) T1=1.13(0.79-1.63) T2= 0.82(0.57-1.19) T3=1.02(0.73-1.44) Ref = non exposed	PC is the most commonly observed cancer among the cohort, yet no significantly increased risk of PC among pesticide applicators exposed to captan.

Kang D (2008)	Trifluralin (Herbicide)	25,712/24,415	1993-2002 (7.4 years)	Age, FH, smoking & alcohol intake	RR(tertiles of exposure by IWLD) T1= 0.81(0.66-0.99) T2=1.01(0.82-1.23) T3L=1.01(0.78-1.31) T3U=0.98(0.76-1.28) Ref = non exposed	No significant association was found between trifluralin exposure and PC risk.
Koutros S (2008)	Dichlorvos (Organophosphate Insecticide)	4,613/45,149	1993-2004 (9.4 years)	Age, FH & applicator type	RR for lower, higher & highest exposure Categories 1.13(0.82-1.56) 0.85(0.62-1.18) 0.99(0.71-1.37) Ref=non exposed	Exposure to dichlorvos (assessed by IWLD) was not associated with increased risk of PC but Small excess risk was found for those with a family history of PC. RR=1.18(0.73-1.82)
Mozzachio AM (2008)	Chlorothalonil (Broad spectrum fungicide)	3,657/43,968	1993-2004 (9.2 years)	Age, FH & smoking	RR for lowest exposure = 1.21(0.85-1.74) Highest exposure= 0.79(0.52-1.21) Ref= non exposed	Chlorothalonil exposure was not associated with incidence of PC in the studies cohort.
Van Bemmel DM (2008)	EPTC (Thiocarbamate Herbicide)	9,878/38,500	1993- 2004 (11.0 years)	Age, FH & race	RR(tertiles of exposure by IWLD) T1=1.02(0.77-1.36) T2= 1.61(1.24-2.11) T3=1.05(0.83-1.33) Ref=non exposed	The results suggest no increased risk of PC among the exposed cohort.
Delancey JOL (2009)	Metribuzin (A selective Trizinone Herbicide)	8,504/14,568	1993-2004 (11.0 years)	Age, FH, smoking & alcohol intake	RR(tertiles of exposure by IWLD) T2= 1.12(0.82-1.53), T3= 1.17(0.84-1.63) Ref= low exposed(T1)	Using IWLD, there was no association between exposure to metribuzin and PC risk.
Koutros S (2009)	Imazethapyr (Heterocyclic Aromatic Amine, Herbicide)	20,646/28,752	1993-2004 (9.2 years)	Age, FH, race, applicator type & use of correlated pesticides	RR(tertiles of exposure by IWLD) T1= 1.04(0.87-1.25) T3= 1.06(0.81-1.4) Ref= non exposed	No association was observed between exposure to imazethapyr & risk of PC.
Lynch SM (2009)	Butylate (Thiocarbamate Herbicide)	5,297/14,358	1993-004 (9.0 years)	Age, FH & race	RR for highest two levels of exposure 1.26(0.75-2.14) 2.09(1.27-3.44) Ref= low exposed	PC risk was significantly elevated among applicators in the highest LED category. A significantly elevated joint effect of prostate cancer family history and high butylate usage.
Rusiecki JA (2009)	Permethrin (Synthetic Pyrethroid)	11,623/37,470	1993-2004 (9.1 years)	Age, FH & race	RR(tertiles of exposure by IWLD) 0.89(0.68-1.16) 1.20(0.94-1.53) 0.87(0.65-1.16) Ref= non exposed	No association between exposure to metachlor and risk for PC.

Bonner MR (2010)	Terbufos (OP)	16,489/28,135	1993-2005 (10.6 years)	Age, FH, Smoking & Alcohol intake.	Hazard Ratio (tertiles of exposure by IWLD) T1=1.19(0.99-1.44) T2= 1.28(1.06-1.55) T3=1.21(0.99-1.47) Ref= non exposed	Suggestive association was observed between Terbufos use & PC.
Christensen CH (2010)	Coumaphos (OP)	3,689/44,133	1993-2005 (12.0 years)	Age, FH, Smoking & education.	Incidence RR (tertiles of exposure by LED) T1= 1.17(0.88-1.56) T2= 0.84(0.60-1.26) T3=0.93(0.62-1.41) Ref= non exposed	There was an association between PC and Coumaphos among those with a positive family history. RR for PC among those with FH comparing ever versus never users = 1.65(1.13-2.38) and 0.87(0.68-1.10) for those without FH.
Koutros S (2010)	Pesticides in general	52,394 (whole cohort)	1993-2006 (11.0 years)	Age	SIR 1.28(1.00 -1.61)	Both private and commercial applicators showed excess risk of PC compared to the general population, although SIR for all cancers combined was low.
Barry KH (2012)	Methyl Bromide	7,814/45,774	1993-2007 (12.3 years)	Age, FH & race	RR for lower exposed= 0.97(0.76-1.23) & higher exposed= 1.01(0.72-1.41) Ref=non exposed	IWLD calculated and correlated to different types of cancer studied. A non significant elevated risk of PC for methyl bromide use among those with a FH of PC.

Exposure assessment in all the studies belonging to the AHS was through Self administered Questionnaire that included general information on exposure to 50 different types of pesticides, application methods, use of personal protective equipment, pesticide mixing, other basic demographic characteristics including family history of cancer.

Exposed population in all the studies belonging to the AHS = Licensed Pesticide applicators in Iowa and North Carolina. They include two groups; private applicators (farmers & nursery workers) and commercial applicators (employees of pest control companies).

FH = Family History, **IWLD**= Intensity Weighted Lifetime Exposure Days, **LED** = lifetime Exposure-Days, **OCs**= Organochlorine Pesticides, **OPs**= Organophosphate Pesticides, **OR**= Odds ratio, **PC**= Prostate cancer, **Q1, Q2, Q3, Q4**= Quartiles of exposure, **RR**= Rate ratio, **Ref** = Reference category, **SIR=** Standardized Incidence Ratio, **T1, T2, T3**= Tertile 1, 2, 3. **T3L**=lower half of the tertile 3, **T3U**= upper half of tertile 3.

Table F. Study quality rating as assessed by NOS in comparison to risk estimates reported by the studies:

Study, publication year	NOS Criteria#			Odds ratio/ Relative risk	
	Selection	Comparability (adjustment for confounders)	Exposure/ Outcome	Ever exposed	High exposed
Forastiere F , 1993	+	+	+		2.68(0.81–8.23)
Van der Gulden, 1995	+++	+	+++	0.93(0.41-2.12)	1.47(0.88–2.46)
Aronson K, 1996	++++	++	++		1.09(0.57–2.08)
Ewings P , 1996	+++	+	++	0.68(0.44–1.04)	
Zhong Y, 1996	+		++	0.70(0.33 –1.29)	
Krstev S, 1998	++++	++	+		1.34(0.51–3.51)
Dich J,1998	+		++	1.13(1.02–1.24)	
Fleming LE, 1999	++	+	++	1.91(1.72–2.20)	
Sharpe CR, 2001	+++	++	+	1.20(0.60-2.80)	2.30(1.30–4.20)
Mills PK, 2003*	+++	++	+		2.37(1.22–4.61)
Settimi L , 2003	++	++	++	2.50(1.40-4.20)	2.70(1.20–6.30)
Ritchie JM , 2003*	+++	+	+++		1.08(0.47–2.50)
Boers D, 2005	++++	++	+++		0.60(0.37– 0.95)
Hardell L , 2006	+++	++	++	2.30(0.77–6.85)	
Fritschi L , 2007	++++	+	+++	0.76(0.33–1.75)	
Meyer E , 2007	+++	++	++	1.60(1.20-2.20)	1.10(0.70–1.90)
Strom SS , 2008	+++	++	++		3.44(1.84–6.44)
Parent ME , 2009	+++	++	++	1.40(0.70-2.70)	2.30(1.10–5.10)
Subahir MN , 2009	++	+	+	5.57(1.74–17.8)	
Aronson K , 2010*	++	++	+++		1.05(0.55–2.00)
Multigner L , 2010*	++++	++	+++		1.73(1.04–2.88)
Sawada N , 2010*	+++	++	+++		1.04(0.54–2.03)
Band PR, 2011	++	++	++	1.47(1.02-2.12)	1.68(1.04–2.70)
Cockburn M , 2011	++	++	+	1.64(1.02-2.63)	2.03(1.17–3.52)
Koutros S *et al*, 2013	+++	++	+++		1.18(1.06–1.60)

#NOS appraises quality according to assessing 4 sources of selection bias, 3 sources of information bias as well as adjustment for confounders (comparability between cases and controls or exposed and unexposed cohort).

"+" refers to stars given for fulfilling the criteria, more pluses indicate higher quality of the study and less sources of information or selection bias.

* In only these studies, reference is the low exposed (first tertile or first quartile), while in the other unmarked studies, reference category is the unexposed.

Table G: Rating of included case-control studies on pesticide exposure and prostate cancer according to New-castle Ottawa Scale

	Variable rating quality of the studies	Number of studies	%
Determination of selection Bias	**Case Definition**		
	Adequate with independent validation	16	76.20%
	Adequate with record linkage or based on self report	5	23.80%
	Representativeness of cases		
	Consecutive or obviously representative series of case	14	66.70%
	Potential for selection bias or not stated	7	33.30%
	Selection of Controls		
	Community Controls	12	57.00%
	Hospital Controls	9	43.00%
	Definition of Controls		
	No history of disease	16	76.20%
	No description of source	5	23.80%
Comparability	**Study adjusts for…**		
	Adjustment for age + family history &/or ethnicity or other potential confounders	14	66.70%
	Adjustment for age	21	100%
Determination of Information bias	**Ascertainment of exposure**		
	Secure record	5	23.81%
	Structured interview, blind to case control status	4	19.05%
	Interview not blinded to case-control status	9	42.86%
	written self report or medical record only	3	14.29%
	Method for ascertainment of cases & controls		
	Same method	21	100%
	Different method	0	0.00%
	Non response Rate		
	Same rate for both groups	14	66.67%
	Non respondents described	6	28.57%
	Rate different and no designation	1	4.76%

Table H: Grouping of included case-control studies on pesticide exposure and prostate cancer by different quality aspects according to Newcastle-Ottawa scale (NOS)

Criteria of quality assessment	Potential Sources of bias	Number of studies	%
Potential sources of selection biases *	No potential sources of selection bias	5	23.8%
	One potential source of selection bias	10	47.6%
	Two potential sources of selection bias	5	23.8%
	Three potential sources of selection bias	1	4.70%
Comparability between cases and controls	Adjustment for age & family history or age & race or 3 variables	15	71.4%
	Adjustment for age only or age & other less important variables	6	28.6%
Information bias ** (assessment of exposure to pesticides)	No potential sources of information bias (High quality)	7	33.4%
	One source of information bias (medium quality)	8	38.0%
	Two sources of information bias (Lower quality)	6	28.6%
Overall assessment of the 3 items (Selection, comparability & exposure assessment)	High overall quality articles (8 or 9 points)	5	23.8%
	Medium overall quality articles (6 or 7 points)	13	61.9%
	Lower overall quality articles (≤ 5 points)	3	14.3%

* Potential sources of selection bias include: Inadequacy of case definition, non-representativeness of cases, hospital controls, no description of source of controls.

**Potential sources of information bias include: exposure not well ascertained, method of exposure assessment is not the same for cases & controls, non-response is not described or no designation of difference in response rate between cases & controls.