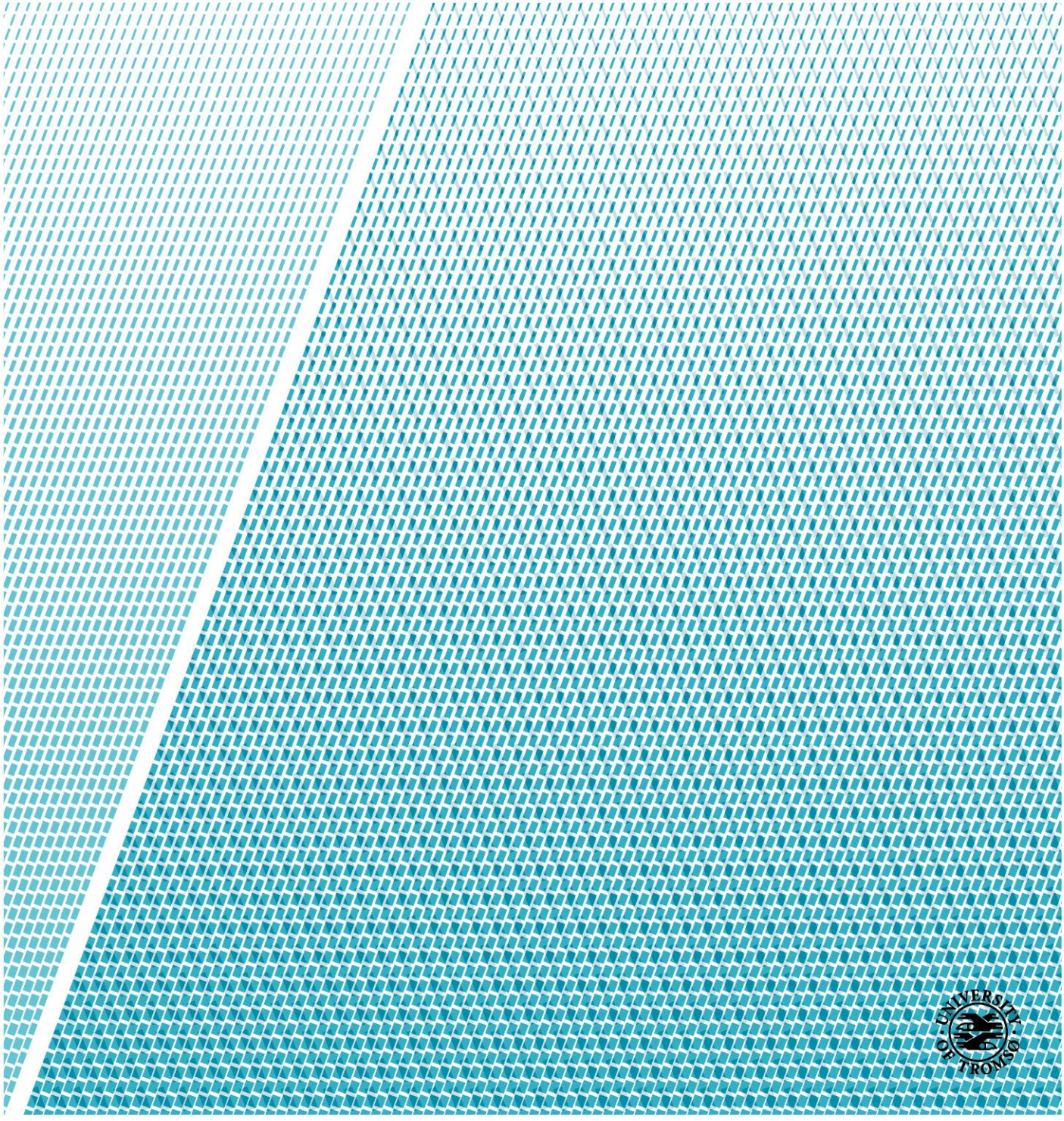
UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

Faculty of Health Sciences

A systematic review of association between organic environmental pollutants and testicular cancer

Sofie Jonassen Vibe

Master's thesis in Medicine, June 2020



Foreword

When planning to write this paper I wished to do a literature search on a topic of personal interest in Medicine. Two of my fields of interest within Medicine has been Environmental Health and Sexual Health and I hoped to combine these in a research. I contacted the Department of Public Health at the University of Tromsø and got linked with special advisor and scientist on environmental pollutants at the University Hospital of North Norway, Department of Laboratory Medicine, Sandra Huber. She further suggested to team up with Maria Averina. Averina is scientist at the Department of Community Medicine at the university, and chief physician at the Department of Laboratory Medicine at the University Hospital of North Norway. She suggested to do a systematic review on the association between environmental pollutants and testicular cancer, which I agreed was a good item for research. The purpose of this research has been to enlighten about environmental pollutants and what possible effects they may have on humans and our health.

The work started in the Spring of 2019 and continued throughout the year until the end of Spring 2020, when the work was completed. Throughout the process I had continuous support from my supervisors.

I would like to express my gratitude to my supervisor, Maria Averina, and assistant supervisor, Sandra Huber. They both have contributed with their knowledge on the field of environmental pollutants and on how to structure and write a paper like this. Whenever I have had questions or been in need of guidance, they have assisted me and always responded quickly. Your help has been indispensable!

Sofie Jonassen Vibe
Ørnes, 7th of June 2020

Table of Contents

List of Tables

List of Figures

Abstract

Objective

There is an increasing incidence of testicular cancer in the world, which cannot be explained by factors as increasing life expectancy as this type of cancer typically affects young men. Particularly in certain countries that also score high on the Human Development Index (HDI) the incidence of testicular cancer is high, and as countries increase the HDI so does the incidence of testicular cancer. It is hypothesized that something introduced in the environment may induce with testicular cancer, like organic environmental pollutants. The research question for this review was articulated as: "A systematic review of association between organic environmental pollutants and testicular cancer"

Methods

A systematic literature in PubMed search was conducted on the 23rd of October 2019. The search involved articles published the last ten years. Inclusion criteria were articles reviewing the effects organic environmental pollutants have on cell mechanisms that could be linked to the development of testicular cancer, or incidents that could be linked to exposure. Articles involving cancer treatment, or other effects organic environmental pollutants have on human health were excluded. After sorting out the relevant articles there were a total of 28 articles to be used in this systematic review.

Results

After a selection process the literature search yielded 20 articles that were included in this research, most of which were reviews. The articles presented conflicting conclusions, while some supported the hypothesis of an association between organic environmental pollutants and testicular cancer, others did not imply association.

Conclusion

There has been done too little research on this field to conclude whether organic environmental pollutants have a causal effect on the development of testicular cancer. However, some articles suggested that there could be an association. Especially cell studies demonstrated an effect of organic environmental pollutants on cell components. Further

research on the field of environmental pollutants and the development of testicular cancer is therefore needed.

Abbreviations

Below is a list of abbreviations used in this review.

- 1,2,3,4,7,8-HCDD, 1,2,3,4,7,8-heptachlorodibenzodioxin
- 3β-HSD, 3β-hydroxysteroid dehydrogenase
- β-HCH, β-hexachlorocyclohexane
- γ-HCH, γ-hexachlorocyclohexane
- BDE-47, 2,2',4,4'-yetrabromodiphenyl ether
- BPA, bisphenol A
- BPAF, bisphenol AF
- cAMP, cyclic adenosine monophosphate
- CXORF6, chromosome X open reading frame 6
- DBP, di-n-butyl-phthalate
- DDE, dichlorodiphenyldichloroethylene
- DDT, dichlorodiphenyltrichloroethane
- DEHP, diethylhexyl phthalate
- DMF, dimethylformamide
- DMSO, dimethyl sulfoxide
- EDC, endocrine disrupting chemicals
- HCB, hexachlorobenzene
- HDI, Human Development Index
- hCG, human chorionic gonadotropin
- LC540, Leydig Cells 540
- LHR, luteinizing hormone receptor
- *MAMLD1,* Mastermind-like domain-containing protein *1*
- MBP, mono-n-butyl phthalate
- MEHP, mono-(2-ethylhexyl) phthalate
- mLTC1, mouse Leydig tumor cell line
- MMP2, matrix metalloproteinase 2
- MMP9, matrix metalloproteinase 9
- MMP, matrix metallopeptidase
- $NaHCO_3$, sodium bicarbonate
- *o,p'*-DDD, 1,1-(dichlorodiphenyl)-2,2-dichloroethane
- *o,p'*-DDT, 1,1,1-tricholoro-2-(p-chlororphenyl)-2-(o-chlorophenyl)ethane
- OR, odds ratio
- P450scc, cytochrome P450
- PAH, polycyclic aromatic hydrocarbons
- PBDE, polybrominated diphenyl ethers
- PBR, peripheral benzodiazepine receptor
- PCB, polychlorinated biphenyls
- PVC, polyvinyl chloride

- PCB-156, 2,3,3',4,4',5-hexachlorobiphenyl
- PCB-157, 2,3,3',4,4',5'-hexachlorobiphenyl
- PCB-209, decachlorobiphenyl
- PFAS, perfluoroalkyl substances
- PFOA, perfluorooctanoic acid
- PFOS, perfluorooctane sulfonate
- POPs, persistent organic pollutants
- *p,p'*-DDD, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
- *p,p'*-DDE, 1,1'-dichloro-2,2'-bis(p-chlorophenyl)ethylene
- *p,p'*-DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
- PXR, pregane X receptor
- SR-B1, scavenger receptor class B type I
- StAR, steroidogenic acute regulatory protein
- TC, testicular cancer
- TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin
- TGCT, testicular germ cell tumours

Introduction

The increasing incidence in testicular cancer

Globally the incidence of testicular cancer is relatively low – 1:100 (1%) in all men. Although relatively rare, it is the most common type of cancer for men aged 15-44 (1-3). The top countries at the list with the highest testicular cancer in 2018 are Croatia, Norway, Hungary, the Netherlands along with other European countries as well as Chile, Australia and New Zealand (4). All these countries also have high Human Development Index (HDI). The HDI is a ranking system of countries based on factors as life expectancy, educational level, per capita income and so on. Countries that are at the lower scale of the HDI correspondingly have lower incidence of testicular cancer – although, the mortality rates are higher in these countries (1).

But tables are about to turn. While the incidence curve seems to start flattening out at the top in countries with already high incidence of testicular cancer, the rate is starting to increase in other countries with previous low incidence rates (1).

The causes of testicular cancer have so far remained somewhat of a mystery. Cryptorchidism and a family history of testicular cancer are the main risk factors described (5). This, however, does not explain the distinct global distribution of this type of cancer. Testicular cancer is also a type of cancer with a young usual onset, and therefore the increased rates cannot be explained by increased life expectancy.

With the dramatic increase in incidence during the last years, a rapidly changing incidence pattern and an incidence pattern that follows the one of the HDI, it seems reasonable to believe that the cause of testicular cancer has a link to something that is being introduced in the environment of the more prosperous countries.

One group of already extensively discussed chemicals are the Endocrine Disrupting Chemicals (EDCs). EDCs are a wide group of chemicals. These are chemicals that can mimic natural hormones and therefore disrupt their natural function, production, metabolism and elimination in the human body (6). In previous studies, EDCs have shown, as the name might imply, to interfere with the endocrine system in humans (7-9). With this in mind I wish to

further investigate whether there are reasons to believe that EDCs also are a driving force in the development of testicular cancer.

Testicular cancer

By testicular cancer, this review is referring to tumors in the testis arising from germ cells which are the most common cause of testicular cancer. That means excluding testicular lymphomas and sex cord–gonadal stromal tumors. Germ cell tumors can be divided into seminomas and non-seminomas, which each stand for half of the germ cell tumors in the testicle (10). Seminomas originates in the germinal epithelium of the seminiferous tubules with the cells resembling progenitor cells, while non-seminomas are usually a mix of two or more types of cells. While seminomas have a later onset, median age about 35 years, non-seminomas are usually diagnosed at around 28-29 years of age.

Symptoms of testicular cancer are usually sparse but may present as lower back pain or development of breasts. Some experience a feeling of heaviness or change of size of the testicle or may palpate a lump (11). Presenting with these symptoms medical examination will then be performed including blood tests, ultrasound and a CT (12). Confirmation of the disease and classification of tumor is done through biopsy.

A variety of testicular co-malignancies have been observed in patients that develop testicular cancer, such as low sperm count hypospadias and other genital abnormalities(13). And in line with an increase in testicular cancer, these health disorders also increase in incidence.

Environmental pollutants in the world

Environmental pollutants are basically everywhere in our environment. Persistent organic pollutants (POPs) are organic chemicals that are known to have a serious negative impact on human health and ecosystems (14). Dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCB), perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are POPs with EDCs effects (15). Due to their potential harmful effects, these chemicals have been regulated through the Stockholm Convention on Persistent Organic Pollutants(16). The POPs in the Stockholm Convention include 26 chemicals. The convention started out with twelve chemicals and has since added new ones through the years. The chemicals are sorted into three different annexes: annex A are chemicals that parties must aim to eliminate, annex B are chemicals that the parties must take measures to restrict the production of, and annex C are

chemicals reduce the unintentional release of. Although most of the world's countries have signed the convention and are regulating these chemicals, their ability to bioaccumulate in the food-chain enables the POPs to still persist in the environment.

Other than the POPs there are multiple other EDCs in the environment. Even though their endocrine effects have been scientifically proven there are still uncertainties whether the doses humans are exposed to in their daily life are high enough to produce any effects. Therefore, they have not yet been regulated in any convention. The most commonly known are probably phthalates, parabens, bisphenol A and triclosan(17, 18).

The possible link between testicular cancer and organic environmental pollutants

A recent study showed that environmental pollutants may have an impact on the sperm quality in dogs and humans and also increased incidence of cryptorchidism (19). The study highlights that chemicals such as diethylhexyl phthalate (DEHP) and di-n-butyl-phthalate (DBP), both are grouped as phthalates, have a direct negative effect on sperm motility and increase DNA defragmentation in sperm. This suggests that certain environmental pollutants do have an impact on testicular health problems. The question is whether they also may be responsible for the development of cancer.

As cryptorchidism and defects in testicular development are strongly associated with the development of testicular cancer, the prenatal exposure to harmful chemicals might be of significance. It was shown that PFOS and PFOA cross the placenta from the mother and over to the fetus. Studies suggest that these chemicals affect the fetus and decrease the birth weight in both, humans and rodents, when the pregnant mother is exposed to these (20).

In the embryonic life, after the testis have formed from the genital ridge, which is a hormone-independent process, the masculinization process begins. This is a completely hormone-dependent process (21). This in turn makes the process susceptible for endocrine disruption. The two processes are not totally separate occurrences and hormones produced during the masculinization process also have an effect on the evolving testicles. The foetal testicles are responsible for the hormone production with the most dominant ones being the anti-Müllerian hormone (AMH), testosterone and insulin-like factor 3 (Insl3).

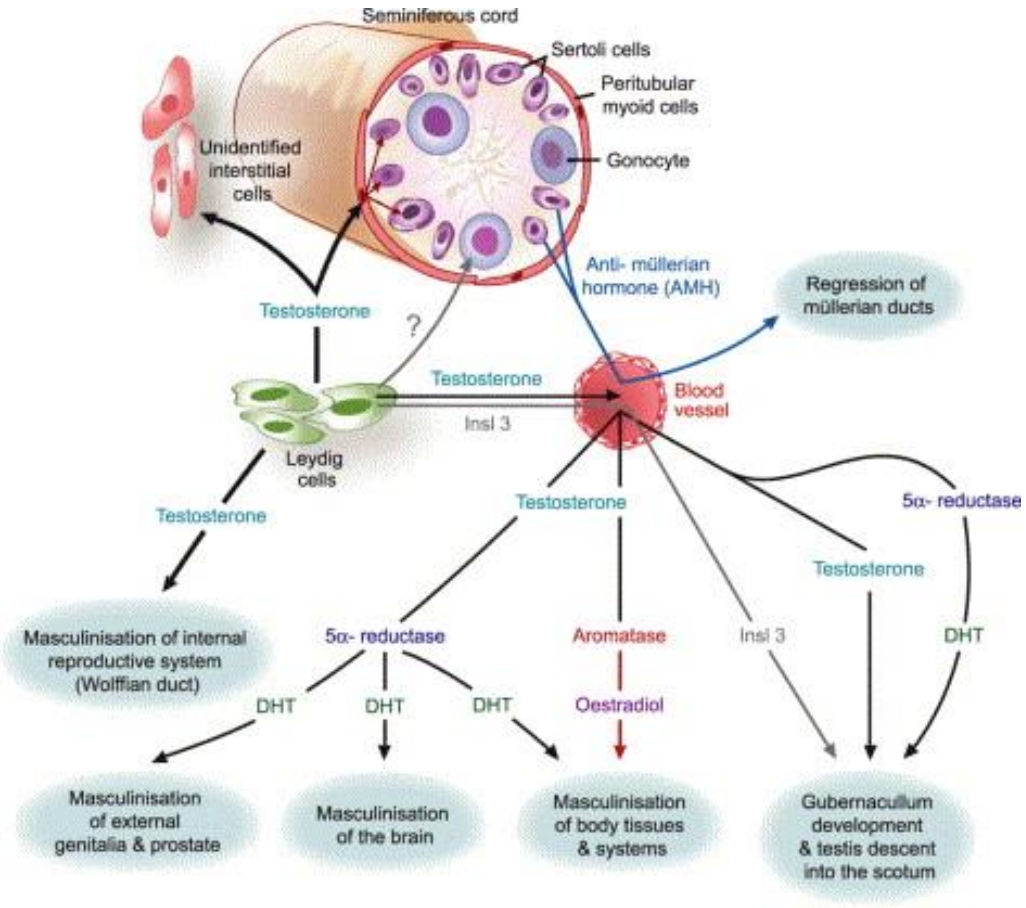


Figure 1: The Masculinization process. Source:(21)

The mechanism in testicular (germ cell) cancer is still not fully understood, however the occurrence of testicular dysgenesis is likely to give rise to abnormal hormone production in the fetal testis and trigger downstream aberrations of hormone-dependent processes, such as masculinization, testis descent and Sertoli cell proliferation (21). Failure of normal differentiation of foetal germ cells could in turn convert them to pre-malignant carcinoma-in-situ (CIS) cells.

Presentation of the research question

The research question of this paper is drafted to: “Is there an association between organic environmental pollutants and testicular cancer?”. Main object of the research will be to reveal whether certain chemicals in the environment may seem to cause non-seminoma and seminoma testicular cancer, if there is any relation at all. The purpose of this review is to highlight the possible effects of environmental pollutants and whether further caution should be taken concerning these substances with respect to men’s health in the future.

As the object of research are gonads which are highly susceptible for hormones, the review will mainly focus on EDCs.

Hill's criteria for causation

Low p-values gives a probability for association between the objective and the exposure but does not tell whether there is a true causality. Austin Bradford Hill therefore made a list of nine criteria that he argued should be taken in consideration when evaluating whether there is a true causality between exposure and disease. *Hill's criteria for causation* is widely recognized, and are as follows (22):

1. Strength of association: The supposed cause must be significantly present in the exposed compared to the non-exposed. Associations that cause significant statistical results may be due to: 1) a true causality 2) chance 3) random error, or 4) a systematic error.

2. Consistency: The result must reproducible in other studies. When the findings are consistent in different studies, with different object, at different times, at different places, this will strengthen the likelihood of causation.

3. Specificity: This suggests that it is more likely that an association is casual when the exposure only causes one disease. However nowadays it's a widely accepted fact that one exposure could cause or be a risk factor of many different conditions.

4. Temporality: The phenomenon has to occur *after* the exposure for there to be a causality.

5. Biological gradient: An increase in the exposure of the supposed cause would also cause an increased incidence or severity of the phenomenon.

6. Plausibility: Based on the up-to-date knowledge accessible about the disease, a causality would be probable, and may be explained through biological studies.

7. Coherence: When there is a coherent finding between epidemiological studies and the results in laboratory findings the likelihood of causality strengthens.

8. Experiment: Experiments in animals and cells, or epidemiological studies that apply preventing measures with the expected result.

9. Analogy: When there are phenomenon or exposure that resemble or behave in the similar way as the studied objects, this may explain a causality.

Through search in the literature relevant to the research question I will evaluate whether the findings in the studies are negative or positive considering the issue.

Methods and materials

This review is based on relevant literature for the research retrieved on PubMed. The results are only based on the articles retrieved and sorted from this search.

The literature search

The search for literature was structured in the following way: *exposures* were linked with *outcomes* with *AND,* as illustrated in **Figure 2.** The different *exposures* were linked with *OR*. The search was done without MeSH-terms for the exposures and with MeSH-terms for the outcome *testicular cancer*. Terms used in *exposures* were chosen from studying the different POPs and other EDCs, and with the guidance from my supervisors. The term *testicular cancer* used in *outcomes* were selected with regard to the main object of this review.

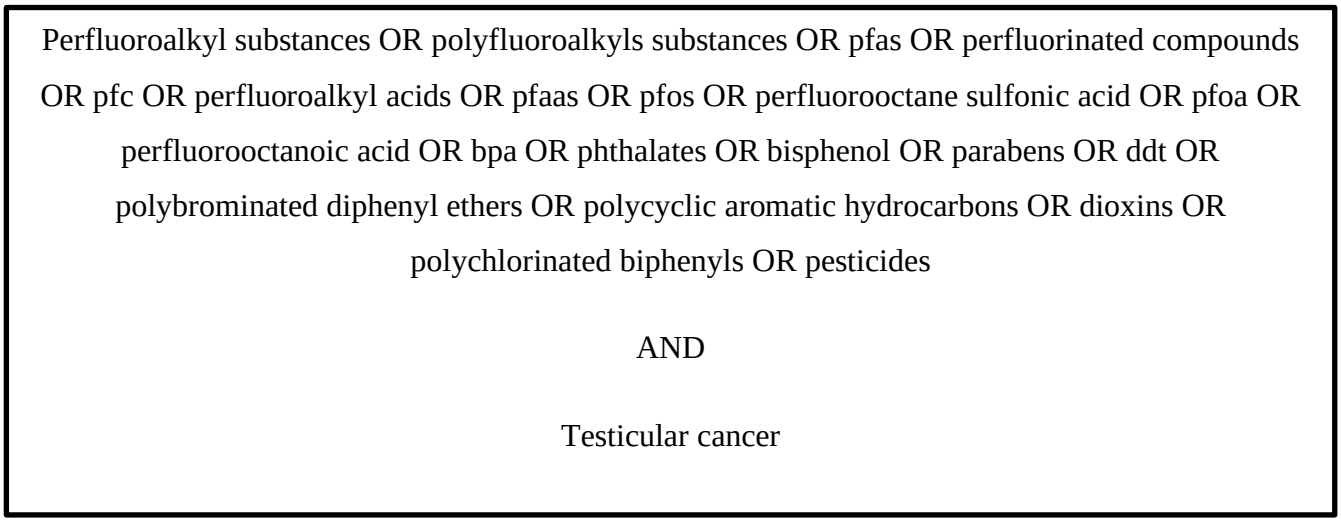


Figure 2: This shows how the research was drafted

The literature search was limited to articles that were published within the last ten years. It was conducted on the 23rd of October 2019, at 05.20 pm Norwegian time, and yielded 276

results. After going to the titles and abstracts to check for relevancy, the selected articles were downloaded to an EndNote library. The inclusion criterion for articles that were not excluded from the EndNote library was that they had to address mechanisms that could contribute to testicular cancer and be linked to environmental pollutants as a possible cause or present data that could link testicular cancer to exposure for endocrine pollutants. Articles based on mortality or cancer treatment were therefore considered inappropriate for this study. Some articles did address the theme but did not present a plausible link, and these were therefore excluded. This gave a total of 20 articles used in this systematic review. When reading the articles, some reviews studied multiple factors that could possibly be linked to testicular cancer. Only the parts that addressed environmental pollutants were included in this review.

Results

Below the articles are rendered in subchapters based on what pollutant that is evaluated in the study. At the end of each chapter the findings are summarized in tables. Findings that indicate that there is an association between the pollutant and testicular cancer have been grouped as *Positive findings. Negative findings* are findings that indicate no association. As some articles address multiple pollutants the same article may have been referred to multiple times. When an article referred to a finding that has already been cited under another article, this review will try to highlight that it has already been mentioned and that it is not an additional finding.

Pesticides

Out of the exposures matching the literature search, pesticides were one of the most investigated groups of EDCs. This subchapter evolves articles that investigated pesticides in general. Articles evolving the effect of chemicals that may also be used as components of pesticides are elaborated in the subchapters.

A 2013 review (Beranger, R. et. al) explored how the presumably most pesticide exposed workforces are affected. Namely the farmers, agricultural workers and pesticide applicators. None of the 11 studies presented and discussed in this review reported significant association between pesticide exposure and testicular cancer (23). The exceptions were one study on small subgroups and self-reported exposures showing a protective effect for seminoma tumors (24). And another study investigating agricultural occupations that found increased risk, but mainly due to a small subgroup of fish farmers (25). The review therefore concluded that

there were little to no correlation of pesticide exposure and testicular germ cell tumors in farmers.

The review also explored four registry-based studies focusing on licensed pesticide applicators. There were reported an increased risk for testicular germ cell tumors (TGCT) for a study conducted in Florida (US) (26) and a UK study (27), but not in a Swedish study (28) or for the US Agricultural Health study (29). The review depreciated these studies as they could be affected by misclassification bias, as those not registered as pesticide farmers, still could be using pesticides. One Norwegian study found a slight increased risk for workers in a fertilizer production plant, but no association to a particular product (30). It was suggested that the finding was the potential impact of life-long environmental exposures to other factors not investigated in the study, as the plant was situated in a rural area. No association were reported for workers in the pesticide industry in other studies from the review (23).

Parental exposure was also under investigation in this review (23). There was neither found increased risk of testicular cancer in children of farmers or pesticide applicators. One exception was a Norwegian study that reported increased risk of testicular cancer for sons of agricultural workers using nitrate fertilizer, especially with a high nitrate/phosphate ratio (31). A high nitrate/phosphate ratio is related to intensive farming. This study also showed a higher adjusted risk of testicular cancer for exposed men born between April and June or October and December. These findings could imply that there could be a link to specific related exposure patterns, including pesticides use, type of farming or farming practices. Another review (32) found studies indicating some association with parents' occupation, especially among fathers likely to have been exposed to organochlorine compounds, and also for mothers exposed to pesticides, although this was not statistically significant.

This review also explored further environmental pesticide exposure in rural areas (32). As some studies showed living in a rural area had protective properties or no association to TGCT, another showed increased risk of TGCT when living in a rural area (31).

Another large case-control study was a register-based study from the Nordic countries investigating TGCT and parental exposure to pesticides. This is the largest study of its kind and included 9,569 cases and 32,028 controls. The countries included were Denmark, Finland, Norway and Sweden and cases were born in 1960 or onwards and diagnosed

between 1978 and 2013. The conclusion was that there were no association between parental pesticide exposure and TGCT in male offspring (33).

In a cohort study from 2011 the British agricultural pesticide users were proven to have a lower mortality rate than the rest of the population of Great Britain (27). Even though the pesticide users show statistically significant reduction in certain cancers compared to the general population, there were also a statistically significant increase of incidence of non-melanoma skin cancer, multiple myeloma and testicular cancer. There were non-significant increase in mortality for the participants with testicular cancer. For the increase of incidence of testicular cancer the 95% CI was 1.04–1.53.

Another pesticide, Agent Orange (equal mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacentic acid as active ingredient and traces of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)), has been the subject of a review that explores its relation to different cancers in exposed individuals (34). During the Vietnam War this herbicide was spread widely to clear the way of crops and plants for the US. Army. TCDD has been linked to different types of cancers. Although not an herbicide/pesticide in itself, Agent Orange contains traces of TCDD, sometimes in higher concentrations. One of the articles had categorized the individuals in two groups of high exposed and low exposed to TCDD (35). When comparing individuals of high exposure to individuals of low exposure, there was no association. Among the subjects of research (85,809 categorized as high exposure and 94,442 as low exposure) there were only 5 incidences of testicular cancer. Two in the high exposure group and three in the low exposure group. There was no difference in the incidence of testicular cancer when comparing the study population to the general population of Korea.

Other articles in this review didn’t show a clear association between exposure to Agent Orange and testicular cancer incidence.

Table 6. Incidence of testicular cancer in those exposed to Agent Orange [4]

Incidence				
Population	**Exposed cases**	**Agent Orange chemical of interest**	**Relative risk (95% CI)**	**Study**
District of Columbia Vietnam Veterans diagnosed in 1976–June 30, 1981	31	All	2.3 (1.0–5.5)	Tarone et al. [37]
Massachusetts Vietnam-era veterans aged 35–65 years in 1993 diagnosed in 1988–1993 vs. gastrointestinal cancers	30	All	1.2 (0.4–3.3)	Clapp [25]
Australian Vietnam Veterans, all branches, 1982–2000	54	All	0.9 (0.6–1.1)	ADVA [18]
Australian Conscripted Army National Service, 1982–2000	17	All	0.7 (0.4–1.2)	ADVA [26]
Korean Vietnam Veterans, high exposure ($n = 2$) and low exposure ($n = 3$)	5	All	1.05 (0.42–2.63)	Yi and Ohrr [30]

Figure 3: Screen shot from the article: A review of Agent Orange and its associated oncologic risk of genitourinary cancers (35)

The table below sums up the findings in the literature search evolving pesticides in general.

Table 1: Summary for pesticides and the risk of TGCT

***Article*/Type of article**	**Positive findings**	**Negative findings**
Occupational and environmental exposures associated with testicular germ cell tumours: systematic review of prenatal	Increased risk in fish farmers (25)	11 studies reported no significant association between pesticide exposure and testicular cancer (23).

***and life-long exposures (23)*/Review**		One study reported a protective effect for seminoma tumors (24).
***Mortality and cancer incidence among British agricultural pesticide users (27)*/ Full length research report**	For the incidence of testicular cancer the CI was 1.04–1.53 for the studied population compared to the general population in Britain	
***A review of Agent Orange and its associated oncologic risk of genitourinary cancers (34)*/Review**		Articles in this review didn't show a clear association between exposure to Agent Orange and testicular cancer incidence
***Testicular germ cell tumours and parental occupational exposure to pesticides: a register-based case-control study in the Nordic countries (NORD-TEST study) (33)*/Case-control**		No association between parental pesticide exposure and TGCT in male offspring
***Occupational causes of testicular cancer in adults (36)*/Review**		Articles in this review did not show clear association for TGCT in men in occupations that involved exposure to agents that could possible cause TGCT.
***Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls (37)*/case-control**	Self-reported household insecticide use: OR adjusted = 3.23 Self-reported mixing pesticides for gardening: OR adjusted = 4.80	

Allethrins

Allethrins are synthetic pyrethroids of the chemical naturally found in the chrysanthemum flower. They are widely used in household insecticides and agricultural work and are toxic by

inhalation, consumption or skin absorption (38). Allethrins degrade rapidly in the environment and are therefore not listed as a POP.

In a study on testicular LC540 in rats, allethrins, were put to the test. At concentrations above 100 µM, allethrins induced stress, calcium release and apoptosis in the rat testicular cells LC540 (39). This supports the theory of toxicity to Leydig cells.

Table 2: Summary of allethrin and the risk of TGCT

***Article*/Type of article**	**Positive findings**	**Negative findings**
***Allethrin induces oxidative stress, apoptosis and calcium release in rat testicular carcinoma cells (LC540)* (39)/ Cell-line study**	- At concentrations above 100 µM, cell killing was observed in LC540. - Allethrin was found to be cytotoxic to primary Leydig cells in a dose-dependent manner with an IC_{50} value of 59 µM. - Cytotoxic mechanisms of allethrin were proven to alter the cell.	

Dichlorodiphenyltrichloroethane

Dichlorodiphenyltrichloroethane (DDT) was a commercial available pesticide until it was banned in many countries in the 70s/80s (40). Under the Stockholm Convention it is listed as a POP under Annex B – restriction, and use is accepted for the purpose of disease vector control (15). It is still used in insecticides products applied for malaria control. DDT as pesticide product consists mainly of the 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (*p,p'*-DDT) isomer as active chemical and impurities of 1,1,1-tricholoro-2-(p-chlororphenyl)-2-(o-chlorophenyl)ethane (*o,p'*-DDT), 1,1'-dichloro-2,2'-bis(p-chlorophenyl)ethylene *(p,p'*-DDE), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (*p,p'*-DDD) and 1,1-(dichlorodiphenyl)-2,2-dichloroethane (*o,p'*-DDD). The last 4 compounds are also known to be metabolites of its

corresponding DDT isomers (42). One of its more known effect is the thinning of eggshells in birds consuming DDT-contaminated fish, and the neurological effects on insects. DDT is an organochloride pesticide and hydrophobic, which enables an accumulation in the fatty tissue of whatever creature that consumes it (41).

A review from 2012 had a closer look at this agent (42). In a large study of Finnish men, exposure to insecticides was associated with an increased risk of seminoma (43). In a small Norwegian case-control study it was shown that increased plasma levels of *p,p'*-DDE, a metabolite of DDT, weakly increased the risk of developing TGCT (44). A larger case control study backed up these findings that men with seminoma also had higher plasma concentrations of *p,p'*-DDE and also chlordanes, while men with non-seminoma germ cell tumors only had elevated *p,p'*-DDE concentrations (45). Despite all these studies proving association, a case-control study in the Washington region compared serum levels of TGCT cases to controls, which showed no increase of organochlorine serum concentrations in men with testicular cancer (46).

The review by Beranger, R. et. al from 2012 also mentioned eight studies that investigated environmental exposure to organochlorines using blood samples or questionnaires (23). The review reported form the study, already mentioned in the review from 2012. This study proved elevated plasma *p,p'*-DDE to be associated with TGCT risk, especially with seminomas (45). Other studies did not report any association with trans-nonachlor, total chlordanes, and *p,p'*-DDE serum levels. (37, 44-48)

A nested case-control study of prenatal exposure to DDT and testicular cancer demonstrated that mothers of cases had a higher ratio of *p,p´*-DDT to *p,p´*-DDE ($p < 0.03$) and lower *o,p'*-DDT ($p < 0.25$) (49). *p,p'*-DDEs are metabolites of *p,p'*-DDT (42). This corresponds to early DDT exposure during pregnancy and a slower elimination of *p,p'*-DDT among mothers of cases. However, these findings were of low statistical power.

A systematic review and meta-analysis from 2016 (50) investigated different possible risks to the male reproductive system when exposed to different environmental pollutants. Among the risks investigated were testicular cancer and the most frequent examined compound was DDT. The eight articles provided 36 risk estimates, of which 26 were above unity and nine exceeded a relative risk of 2 (50). The results are shown in the figure below and the overall

OR was calculated to be 1.20 (95% CI 0.78–1.89), meaning a slightly elevated risk, but still not statistically significant.

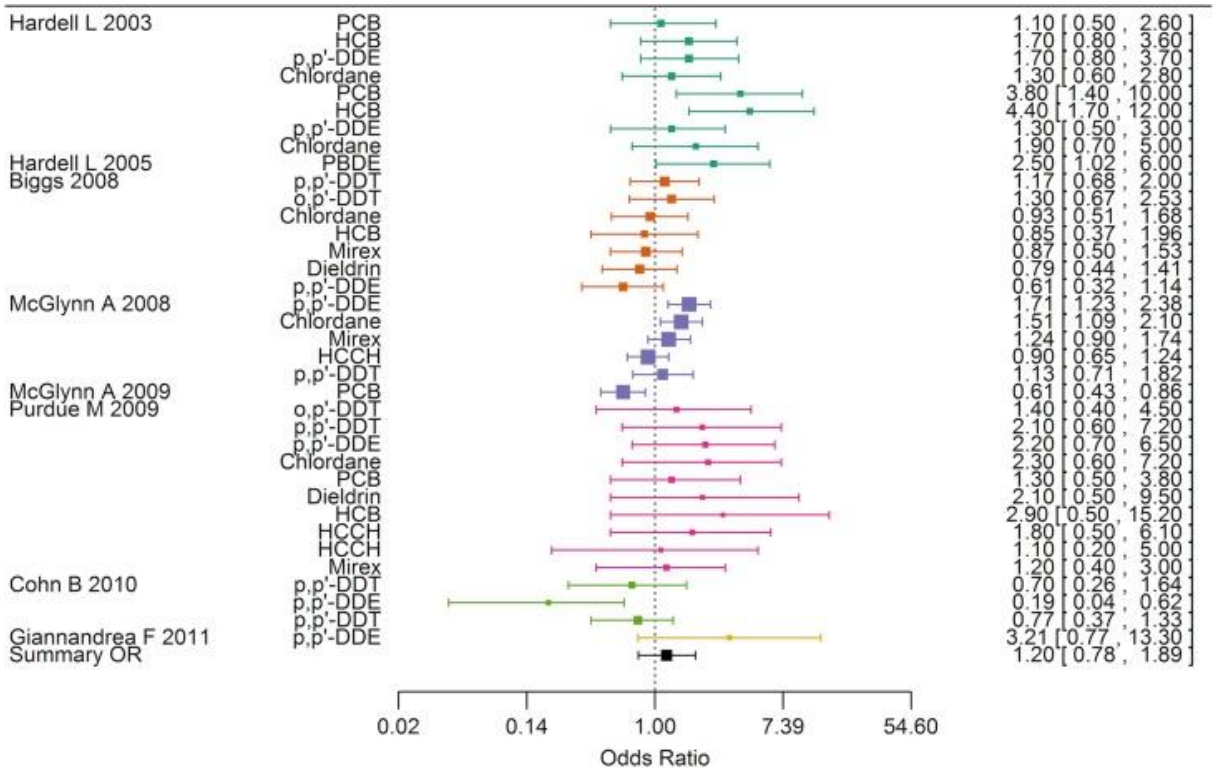


Figure 4: Results from the review article "The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis" (50).

The review *Adolescent and adult risk factors for testicular cancer* (51) also investigated reports that examined whether serum levels of organochlorine pesticides and their metabolites, with DDT and it's breakdown product DDE among them, are associated with testicular cancer. Eight reports examined whether serum levels of organochlorine pesticides and its metabolites are associated with testicular cancer. Molecular studies are likely to be accurate to evaluate exposure than self-reported based studies due to recall bias. Out of the eight reports six reports included men who had been diagnosed with testicular cancer, while three studies included mothers of sons who developed testicular cancer. All four studies that investigated DDT and DDE reported no association with DDT and testicular cancer (44-46, 48). However, three of these four studies found that DDE had positive association with testicular cancer (51). A later study also reported the positive association found in the previous studies to DDE and cancer (37).

Table 3: Summary for DDT and the risk of TGCT

***Article*/Type of article**	**Positive findings**	**Negative findings**
***Environmental toxicology of testicular cancer (42)/* Review**	- exposure to insecticides was associated with an increased risk of seminoma (43) - increased plasma levels of *p,p*'-DDE weakly increased the risk of developing TGCT (44) - Men with seminoma had higher plasma levels of *p,p*'-DDE and chlordanes, while men with non-seminoma germ cell tumors only had elevated *p,p*'-DDE (45)	
***Occupational and environmental exposures associated with testicular germ cell tumours: systematic review of prenatal and life-long exposures (23)/* Review**	A study found that higher *p,p'*-DDE serum concentrations are associated with TGCT risk (mentioned in the findings in the article above) (45).	Four studies did not show any association with TGCT and *p,p'*-DDE (37, 44, 46, 47), *p,p*'-DDT (44-46) or *o,p'*-DDT (44, 46).
***Prenatal DDT exposure and testicular cancer: a nested case-control study (49)/* Full length research report**	Mothers of cases had a higher ratio of *p,p´*-DDT to *p,p´*-DDE and lower o,*p*'-DDT than mothers of controls. Low statistical power.	
The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male	OR was calculated to be 1.20 (95% CI 0.78–1.89). Meaning a slightly	

reproductive disorders: a systematic review and meta-analysis (50)/ **Review**	elevated risk for TGCT, although not statistically significant.	
Adolescent and adult risk factors for testicular cancer (51)/ **Review**	Four studies reported positive association with DDE serum levels and testicular cancer (37, 44, 48, 52).	4/4 studies reported no association with DDT serum levels and testicular cancer (44-46, 48) (Three of these articles have already been mentioned above).
Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls (37)/ **case-control**	Serum levels of *p,p'*-DDT ≥0.2 ng/mL gave an OR adjusted at 3.21 for having TC. Together with HCB the OR adjusted was 3.34.	

Hexachlorobenzene

Hexachlorobenzene (HCB) is an organochlorine and is listed as a POP under Annex A and Annex C, chemicals for elimination and chemicals for reduction of unintentional release respectively, in the Stockholm Convention (15). It was introduced as a fungicide and extensively used on wheat crops in Eastern Turkey 1954-59 resulting in a variety of symptoms in consumers like neurological abnormalities, hirsutism, weakness and porphyrinuria (53). It was also proven that mothers transferred HCB concentrations over to their offspring through the placenta and breast milk (54).

The Finnish-Danish study from 2010 collected breast milk samples from 68 mothers of healthy boys to investigate the amount of chemicals in the breast milk (54). The chemicals that were found in significant larger amounts in the Danish breast milk compared to the Finnish were 1,2,3,4,7,8-heptachlorodibenzodioxin (1,2,3,4,7,8-HCDD), dieldrin and HCB.

A previously mentioned case-control study from 2015 also found association of consumption of dairy products and TGCT (32). This corresponds to the fact that consumption of dairy products are the highest in the Scandinavian countries which also have the highest incidence of TGCT. The authors argue that dairy, meat and fish are the highest contributors to

organochlorines in the diet, through plants contaminated by pesticides being consumed by the animals which further accumulates in the food chain. However, there were no significant association between TGCT and consumption of meat, fish or fruit and vegetables. There was non-statistically significant risk for TGCT for individuals with increased HCB serum concentrations. There were statistically significant differences between cases and controls regarding total PCB and the sum of HCB + PCB concentrations.

HCB serum concentrations were measured in a case-control study (37). When comparing the measured serum HCB concentrations for cases and controls only 3 out of 50 cases had a serum concentration ≥ 0.2 ng/mL. Although none of the controls measured serum concentrations at 0.2 ng/mL or above, this finding was not significant. When the serum levels of HCB and *p,p`*-DDE this gave an OR *adjusted* = 3.34.

Table 4: Summary for HCB and the risk of TGCC

***Article*/Type of article**	**Positive findings**	**Negative findings**
***Adolescent and adult risk factors for testicular cancer (51)*/Review**	One study suggested a possible association with high serum concentrations of HCB and testicular cancer (37).	Three studies did not show any association between HCB and testicular cancer (44, 46, 48).
***Country-specific chemical signatures of persistent environmental compounds in breast milk (54)*/ Full length research study with including an ecological study**	The breast milk of Danish mothers generally had higher concentrations of measured HCB than the breast milk of Finnish mothers. Danish men have a higher incidence of testicular cancer compared to Finnish.	
***Exposure to polychlorinated biphenyls and hexachlorobenzene, semen quality and testicular cancer risk (32)*/ Case-control**	PCB + HCB serum concentration had a statistically significant increase in testicular cancer risk (14.4 %) compared to controls (1.0 %) ($p < 0.001$).	HCB was detected in five cases and one control; this difference was not statistically significant (OR 4.25; 95 % CI 0.49–36.97; $p = 0.22$).

***Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls (37)/* Case-control**	Only cases (three individuals) had a serum concentration of HCB ≥0.2 ng/ml (p Fisher <0.01). *p,p'*-DDE +HCB were significantly associated with higher risk of testicular cancer (OR =3.15. 95 % CI: 1.00-9.91; $OR_{adjusted}$ =3.34, 95 % CI: 1.09-10.17).	When measuring only HCB the finding was not significant.

Hexachlorocyclohexanes

Hexachlorocyclohexanes (HCH) is a group with many isomers for this molecule structure and belong to the group of organochlorine pesticides. The Stockholm Convention listed the isomeres α- and β-HCH as POPs under Annex A, meaning that parties must take measures to eliminate the chemicals listed. β-HCH is highly persistent, also in colder waters, and can therefore accumulate in the food web in the arctic waters (15).

The already mentioned review *Adolescent and adult risk factors for testicular cancer (51)* investigated a few other organic compounds. Two molecular studies have been performed on β-HCH and γ-HCH. There was no association between TGCT and γ-HCH observed, and only one study showed increased risk with TGCT and β-HCH (51).

Table 5: Summary for HCH and risk of TGCT

***Article*/Type of article**	**Positive findings**	**Negative findings**
***Adolescent and adult risk factors for testicular cancer (51)/* Review**	One study found evidence of an increased risk of TGCT with β-HCH concentrations in serum (46).	No evidence of an association with TGCT and γ-HCH concentrations in serum (44, 55).

Other pollutants, pesticides and fungicides

The review by Beranger, R. et. al (23) found an excess TGCT for men with hobbies that potentially involved endocrine disrupting chemicals during adolescence (like the involvement of paints, glues, or solvent), but a different outcome was described for occupational exposure to the same chemicals (chlordane, DDT, dieldrin, Mirex) (50). An excess risk with self-reported domestic insecticide use (without specifying what chemicals the insecticides contained) was reported by Giannandrea, F et. al (37), but blood samples of *p,p'*-DDE and HCB could not confirm the findings. HCB has been assessed in four studies, and only one study suggested a weak association (51).

One study found an excess risk for TGCT in sons born to mothers with high serum concentrations of insecticides and fungicides (HCB and chlordanes) in maternal serum, as well as high PCBs and polybrominated diphenyl ethers (PBDE) concentrations. The maternal blood serums were however collected at time of the diagnosis, and there is likely to have been some selection bias.

In four molecular epidemiological studies, chlordane and its derivates (oxychlordane, *cis*-nonachlor, *trans*-nonachlor and MC6) have been under the loop. Three of these studies supported increased risk for TGCT with association of *cis*-nonachlor and *trans*-nonachlor(51). Little evidence indicated increased risk with MC6 and oxychlordane or other derivates of hexachlorocyclopentadiene (e.g. heptachlor, dieldrin and mirex).

Table 6: Summary of other pesticides and fungicides and the risk of TGCT

Studied pollutant(s)	***Article*/Type of article**	**Positive findings**	**Negative findings**
	Occupational and environmental exposures associated with testicular germ cell tumours:	An excess risk with self-reported domestic insecticide use (37).	

	***systematic review of prenatal and life-long exposures (23)/* Review**		
1,2,3,4,7,8-HCDD, dieldrin, and many more	***Country-specific chemical signatures of persistent environmental compounds in breast milk (54)/* Full length research study including an ecological study**	The breast milk in Danish mothers generally had higher levels of measured persistent bio accumulative chemicals than the breast milk of Finnish mothers. Danish men also have a higher incidence of testicular cancer.	
Chlordane and its derivatives (oxychlordane, *cis*-nonachlor, *trans*-nonachlor and MC6)	***Adolescent and adult risk factors for testicular cancer (51)/* Review**	Three studies supported associations between both *cis*-nonachlor and *trans*-nonachlor and increased risk of testicular cancer (44, 45, 48).	One study did not support association between the chemicals and testicular cancer (46).

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a group of compounds that are listed as POPs under Annex A with specific exemptions and under Annex C in the Stockholm Convention. They are used in a wide variety of areas for example as heat exchange fluids, in electric transformers and as additives in paint and in plastics. Mass poisoning of rice fields in Japan in 1968 and Taiwan in 1979 caused consumers a variety of symptoms like nausea, fatigue, pigmentation of skin, nails and mucous membranes and increased secretion from eyes (56). Severity of symptoms were closely linked to the amount of consumed rice oil and PCB concentration measured in blood serum. PCB exposure has also been linked as the cause for reproductive failure in seals (57).

In Beranger, R. et. al's review (23) a Swedish registered-based cohort study suggested a non-significant increased risk for TGCT in men living in environments contaminated by PCBs and heavy metals (58).

Another review (42) also found articles with inconsistent conclusions concerning our research question. In a case-control study in 2015, PCB serum concentrations were higher in the mothers of the 125 TCGT cases than in the mothers of the 103 controls (OR 3,8) (32). The long half-life of PCBs was taken into account, the levels could also have been elevated during pregnancy (32).

The review *Adolescent and adult risk factors for testicular cancer* (51) presented Swedish (59) and Norwegian (44) case-control studies which proved PCBs to have positive association with TGCT. The review discussed the main weakness of the studies was having only small sample sizes and its strength that the Norwegian study also included prediagnostic samples.

All the three reviews (23, 42, 51) were discussing a larger US case-control study that showed possible inverse association between PCBs and testicular cancer (24, 52). After analyzing prediagnostic samples of cases and controls they concluded that the findings of this study did not support the hypothesis of any association between PCBs and TGCT. This study is also already mentioned in this present review in the *Pesticide* subchapter.

Table 7: Summary for PCBs and the risk of TGCT

***Article*/Type of article**	**Positive findings**	**Negative findings**
Occupational and environmental exposures associated with testicular germ cell tumours: systematic review of prenatal and life-long exposures (23)/ **Review**	A cohort study found a non-significant increased risk of TC for men living in an area contaminated by PCBs and metals (58).	

***Environmental toxicology of testicular cancer (42)/ R*eview**	Higher serum concentration of PCBs in the mothers of 125 TGCT cases than in the mothers of 103 controls (32).	Inverse effects of PCB serum concentrations to TGCT (24, 52).
***Adolescent and adult risk factors for testicular cancer (51)/* Review**	Two studies proved PCBs to have positive association with TGCT (44, 59).	
***Country-specific chemical signatures of persistent environmental compounds in breast milk (54)/* A full length research and ecological study**	Danish mothers had generally higher measured concentrations of PCB-156, PCB-157 and PCB-209 in the breast milk than Finnish mothers. Danish men also have a higher incidence of testicular cancer.	
***Exposure to polychlorinated biphenyls and hexachlorobenzene, semen quality and testicular cancer risk (32)/* Case-control**	PCB serum concentrations were measured to be higher in the mothers of the TCGT cases than in the mothers of the controls (OR 3,8). PCB + HCB serum concentration had a statistically significant increase in testicular cancer risk (14.4 %) compared to controls (1.0 %) ($p < 0.001$).	

Bisphenols

Bisphenols are a group of chemicals used as additives in production of plastics, epoxy resins and other products. They show a short elimination half-life in organisms and are not regulated under the Stockholm Convention.

A cell-study from 2018 produced data that indicates that bisphenol-AF (BPAF) exposure inhibits progesterone in hCG-stimulated mLTC1-cells (60). BPAF is a fluorinated compound

related to bisphenol-A (BPA). The difference of the two is that two methyl groups on BPA have been replaced with trifluormethyl groups in the BPAF (61). It's progesterone inhibition properties is through reducing the expression of SR-B1 and P450scc when inducing a disruption in cellular cAMP, and thereby decreasing cAMP. The cells were cultured in 6-well plates. The progesterone secretion by mLTC1-cells wasn't affected until the BPAF exceeded 30 µM BPAF compared to the control group. cAMP were dramatically affected at 30 µM BPAF and upwards. mRNA expression levels of luthenizing hormone receptor, PBR and 3β-HSD were not affected. PBR and 3β-HSD transport cholesterol to mitochondria in Leydig cells and convert pregnenolone to progesterone. SR-B1 and StAR, both related to cholesterol transportation was significantly effected at 70 µM BPAF exposure.

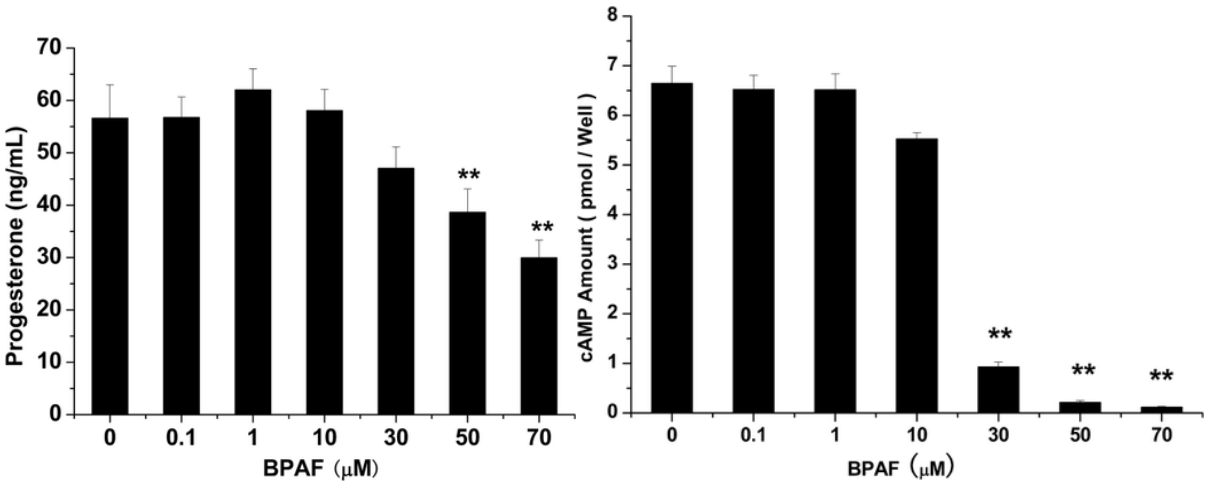


Figure 5: Progesterone and cAMP levels under influence BPAF(60)

BPA is an active compound by itself and has a short half-life, less than 6 hours. It is widely used in plastic. In the mouse, rat and human fetal testis cells the testosterone production was reduced at high doses BPA (60). The human fetal testis cells was also sensitive to lower doses (10^{-8}M). This was also a finding in a study (62) mentioned in the review *Effects of endocrine disruptors on the human fetal testis (63).*

The review *Occupational causes of testicular cancer in adults* (36) reported of findings from a cell study that proved that BPA stimulates seminoma cell proliferation through a membrane receptor belonging to the G-protein-coupled non-classical membrane estrogen receptor family (64). Another study on humans and occupational exposure showed an excess risk for

testicular cancer when working in occupations with exposure to polyvinyl chloride (65). However, in a later case-control study by the same authors there was no longer an excess risk of testicular cancer for occupational polyvinyl chloride exposure demonstrated (66).

Table 8: Summary for bisphenols and the risk of TGCT

***Article*/Type of article**	**Positive findings**	**Negative findings**
***Mechanism of bisphenol AF-induced progesterone inhibition in human chorionic gonadotrophin-stimulated mouse Leydig tumor cell line (mLTC-1) cells (60)/* Cell line study**	- Progesterone levels decreased at concentrations > 30 µM. - Decreased cAMP production. - Levels of SR-B1 at 70 µM and P450scc protein expression were reduced in cells exposed to 50 and 70 µM BPAF. StAR was markedly decreased at 70 µM.	Levels of mRNA of LHR, PBR or 3β-HSD was not altered.
Effects of endocrine disruptors on the human fetal testis (63)/ Review	At high doses BPA reduced the testosterone production of rat, mouse and human fetal testis. Human fetal testis was also sensitive to lower doses (10^{-8}M) (62).	
Occupational causes of testicular cancer in adults (36)/ Review	A study showed an excess risk of testicular cancer for occupational exposure to polyvinyl chloride (65). BPA stimulates seminoma cell proliferation triggered through a	From a case-control study: No excess risk of testicular cancer for occupational polyvinyl chloride exposure demonstrated (66).

	membrane receptor belonging to the G-protein-coupled non-classical membrane estrogen receptor family (64).	

Polybrominated diphenyl ethers & Perfluoroalkyl substances

Polybrominated diphenyl ethers

Polybrominated diphenyl ethers (PBDEs) are organobromine chemicals used as flame retardants in a broad application field and belong to the group of POPs regulated in the Stockholm Convention (15).

One individual PBDE, BDE-47, was under investigation in a 2012 cell culture study. The research found that mLTC-1 produced less progesterone, reduced the activity in P450scc and reduce intracellular cAMP under exposure to BDE-47 compared with the control groups (67). In other words, BDE-47 was found to have much of the same effect on mLTC-1 as BPAF, as mentioned earlier in the present review.

Perfluoroalkyl substances

Perfluoroalkyl substances (PFAS) have been used in very broad applications due to their unique physico-chemical properties. PFAS have been used in paper- and food packaging materials, as impregnation agent for furniture and outdoor equipment, in creams and cosmetics, as non-stick coatings metal coatings for frying pans, fire fighting foams, pesticides, pharmaceuticals and in the paint, photography and chrome plating industry. Perfluorooctane sulfonate (PFOS) and perfluorooctanate (PFOA) are the most known PFASs and are declared as POPs in the Stockholm Convention.

In a cell study (68) PFOA was found to have a biphasic effect on testosterone and progesterone production in mLTC-1 cells when the cells were subjected to 0, 50, 100 or 200 μM PFOA for 48 h. It altered the fatty acid transportation into mitochondria and disturbed cholesterol synthesis(68). The research concluded that PXR, SR-B1 and LHR are sensitive to PFOA. These findings were supported by another publication, however it argued that human LHR are less sensitive than rat LHR (69).

The risk for testicular cancer among firefighters was examined within a study from the Nordic countries. Firefighters are a professional group that is considered to be exposed to multiple carcinogens. The study proved no increased risk to testis cancer in firefighters (70).

Table 9: Summary for PBDEs and PFAS *and the risk of TGCT*

Studied pollutant	***Article*/type of article**	**Positive findings**	**Negative findings**
	Cancer incidence among firefighters: 45 years of follow-up in five Nordic countries (70)/ **Cohort**		The study proved no increased risk to testis cancer in firefighters
BDE-47	***2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) decreases progesterone synthesis through cAMP-PKA pathway and P450scc downregulation in mouse Leydig tumor cells (67)/*** **Cohort**	BDE-47 decreased progesterone production in mLTC-1 cells. This may be associated with the decline of intracellular cAMP level. The site G protein in cAMP-PKA pathway may be involved in this process. BDE-47 reduced the activity of P450 side chain cleavage enzyme (P450scc), which was companied with the decline of P450scc mRNA and protein level in mLTC-1 cells.	
PFOA	***Biphasic effects of perfluorooctanoic acid on steroidogenesis in mouse Leydig tumour cells (68)/*****Cohort**	PFOA altered the fatty acid transportation into mitochondria and disturbed cholesterol synthesis in mLTC-1.	
	Mode of Action analysis of perfluorooctanoic acid (PFOA)		Human Leydig cells are not as sensitive as rat Leydig cells to LH stimulation. The relevance of the induction

	tumorigenicity and Human Relevance (69)/Review		of Leydig cell tumors in the rat by PFOA to humans is still not understood.

Dimethylformamide

Dimethylformamide (DMF) is a hydrophilic solvent with a high boiling point and used in the production of acrylic fibers and plastics and a couple of other applications (e.g. pesticides, pharmaceuticals, adhesives).

A review (42) mentioned a case-control study with 11 men diagnosed with TGCT, only three men had been exposed to DMF (71). Thus, no higher risk was evident.

Table 10: Summary for DMF and the risk of TGCT

***Article*/type of article**	**Positive findings**	**Negative findings**
***Environmental toxicology of testicular cancer (42)/* Review**		From a case-control study: Out of 11 patients with TGCT, only three were exposed to DMF. No higher risk of TGCT compared with controls (71).
***Occupational causes of testicular cancer in adults (36)/* Review**		No epidemiological study have confirmed a link between TC risk and DMF exposure (72, 73).

Phthalates

Phthalates are a group of chemicals used in a broad range of products as for example blood bags, toys, vinyl floor and wall covering, lubrication oils, food packaging, pharmaceuticals and personal care products. They have a short elimination half-life and are therefore not

regulated under the Stockholm convention. A couple of them are known to have endocrine disrupting effects.

Diethylhexyl phthalate (DEHP) and di-n-butyl-phthalate (DBP) are the most abundant phthalates, and when ingested by humans, they turn into the metabolites mono-(2-ethylhexyl) phthalate (MEHP) and mono-n-butyl phthalate (MBP) (63). MEHP was proven to have several effects that enhanced tumour progression and metastasis in a study on a human testicular embryonal carcinoma cell line NTERA-2 cl (74). Following MEHP exposure, NT2/D1 cells had an increased ability to digest and migrate into the basement membrane matrix (Matrigel in this case). This phenomenon was dependent on an increased activity of MMP2, caused by MEHP. MEHP also downregulated the expression of genes for tight-junction and adherens junction, which also enabled the increased migration. The Myc transcription factor was activated after MEHP exposure, which could be an underlying mechanism for MMP2 activation after MEHP exposure.

In rats, phthalates have been proven to have in utero effects, and induce germ cell loss in the early stage of the developing testis, and multinucleated germ cells were observed at a later stage in the developing of the testis (63). In humans, only germ cell loss and apoptosis were observed when exposed to MEHP at very low doses. When exposed to DBP, multinucleated germ cells were after short time observed in the fetal xenograft testis, but no decrease in density of germ cells was found. Though demonstrated that in utero phthalate exposure has a proven effect to impair the testosterone production in rats testis, both in vitro and in vivo, this has not been verified in humans (63). Interestingly, in other animals, like mice, phthalate exposure has *increased* the testosterone secretion.

A study from Sweden found that men working with polyvinylchloride (PVC) had a 6-fold higher rate of seminoma than controls (OR 6.6), and that men working with plastic containing PVC and phthalates had a OR of 2.9 of developing TGCT (42). However, in a larger case-control study, exposure to PVC did not show an increased risk for testicular cancer, and the same finding had a Danish study with 3,700 workers.

It has been proven that human testis are less sensitive to synthetic estrogen than testis of rodents (63).

Table 11: Summary for phthalates and the risk of TGCT

Studied pollutant	***Article*/type of article**	**Positive findings**	**Negative findings**
Phthalates	***Country-specific chemical signatures of persistent environmental compounds in breast milk (54)*/Ecological study**		No country-specific pattern regarding non-persistent phthalates in breastmilk.
MEHP	***Mono-(2-Ethylhexyl) Phthalate (MEHP) Promotes Invasion and Migration of Human Testicular Embryonal Carcinoma Cells (74)/ Cell line study***	Multiple cellular mechanisms triggered by MEHP indicate that exposure may act to enhance tumor progression/metastasis in testicular embryonal carcinoma cells. Findings related to this were: - Upregulation of MMP2 - Upregulation of MYC - Down regulation of GJA1 - Reduction of vinculin mRNA	
	***Effects of endocrine disruptors on the human fetal testis (63)/* Review**	In the developing human testis in organ culture MEHP induce germ cell apoptosis. Observed after a short exposure (three days) and at mild doses (10^{-5}M), compatible with the human exposure (75). Multinucleated germ cells in human fetal testis xenografts after a short exposure of DBP (76).	There has been no proven effect of testosterone impairment in human fetal testis after phthalate exposure, although it has been observed in rat testis (76-78). In mice, phthalates can induce a positive effect on testosterone secreted in cultured fetal cells (79).

	***Occupational causes of testicular cancer in adults (36)/* Review**	Phthalates have been shown to be associated with anomalies of the male reproductive tract after in utero exposure in rats (80) and in humans (81).	
PVC	***Environmental toxicology of testicular cancer (42)/* Review**	A significantly higher rate of TGCT was found in men who were plastic workers (OR 2.9, 95% CI 1.3–6.5). When the chemical compounds were evaluated in detail, men who worked with PVC had a 6-fold higher rate of seminoma compared with controls (OR 6.6, 95% CI 1.4–32) (82).	In one Swedish and one Danish case-control study no association was found between PVC exposure and development of TGCT (83, 84).

Discussion

All in all, the articles presented above were divided in whether there is an association between the research environmental pollutant and testicular cancer or not. This discussion part will first briefly go through the different study design of the articles yield from the literature search and evaluate the scientifically value of each design. Thereafter, the results of the cohort studies, cell line studies and the studies with serum measurements will be presented and discussed. In relation to the findings of the literature search the Hill's criteria for causation will be assessed. At the end of this chapter the findings will be discussed in relation to earlier studies and hypothesis on testicular cancer, as well as the weaknesses and strengths of this study.

Study designs

The results from the research where studies of different designs. Study designs are standardized ways of performing a research. There are strengths and weaknesses with all study designs and some are viewed of higher scientifically value than others.

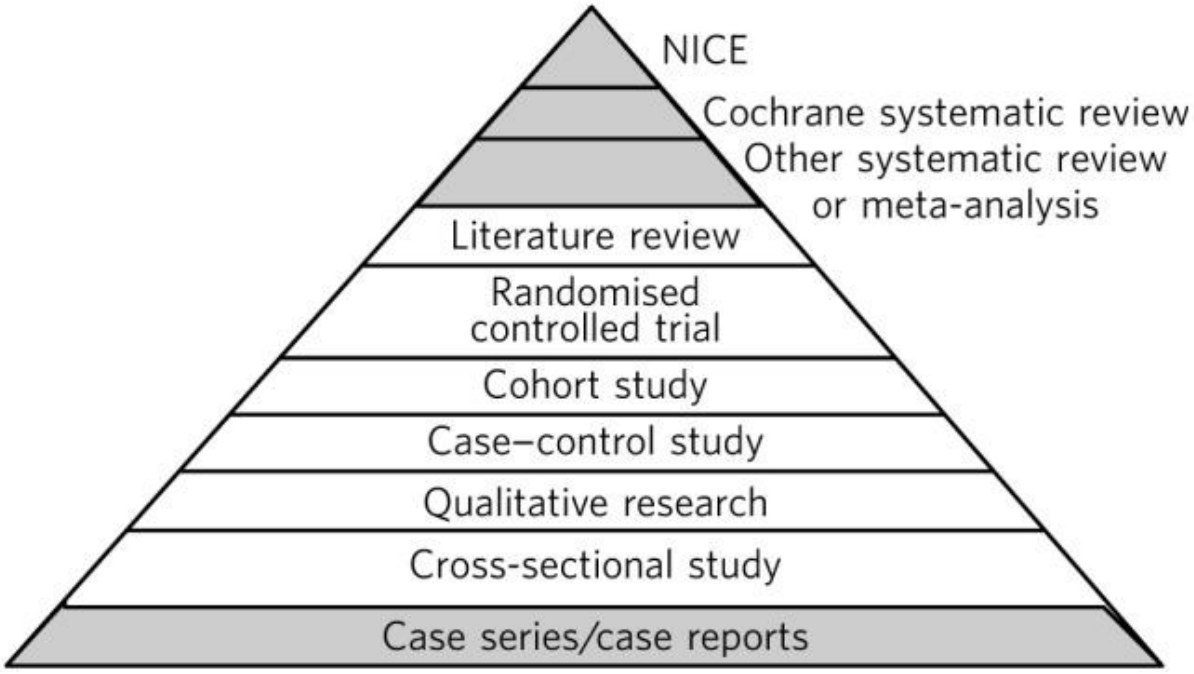


Figure 6: This pyramid illustrates the value of different study designs. The study designs at the top are the ones highest valued, while the ones at the bottom are least valued. Source: (85)

Ecological study

Only one study in this review, that assessed the difference in environmental pollutants in breast milk of Finnish and Danish mothers (54), was an ecological study. This study design is often used for generating hypothesis as they study risk-modifying factors on outcomes in populations. They may include many people and multiple risk modifying factors and is not very time consuming. This design is not shown in the pyramide above and is valued as having a low value as they may not show true association between the risk factor and outcome. When a group of people show a high incidence of a certain disease and a high labor of a suspected risk factor, it does not necessarily mean that it is the studied risk factor that causes the disease. Correlation does not necessarily mean causation. This could be summed up as cause-effect bias.

Case-control study

Four of the studies included in this review are case-control studies. In these studies, the researchers find cases (i.e. testicular cancer patients) and investigate earlier exposures or experiences to later compare them with controls. In this manner the odds of having an experience with the outcome to the odds of having an experience without the outcome can be compared. Case-control studies are inexpensive and are not that time consuming.

A weakness with most of the case-control studies included in the present review seems to be that the studies are estimating the EDC concentrations the subjects have been exposed to through their life-spans. They rely on estimates and recollection of samples of the subject or parents. This cannot tell the exact concentration the subjects actually have been exposed to, neither the actual total body burden since a part of the pollutants are stored in lipid rich tissue.

For instance, in the review article investigating Agent Orange, the authors admit that there are no objective data to quantify TCDD exposure, such as for example serum measurements of TCDD (34). The review based their research mostly on case-control studies as there are few other data. Most of the studies included in the review relied on geographical models to extrapolate exposure. Due to a financial compensation for those who have been exposed and later developed malignancy, one should also take into account a potential bias for patients to admit to Agent Orange exposure. This is known as recall bias and weakens the value of this study design.

Cohort study

Two of the articles in this review are cohort studies. This design is more valued than of the two previous ones. Of the strengths of this type of study is that the exposure is measured before outcome (disease) which makes it good for measuring rare exposures. Although, cohort studies do have weaknesses. Contrary to case-control studies cohort studies can be very expensive and time consuming. They are also prone to bias due to loss of follow-up, and to confounding. Further, depending on for how long the study is lasting, the participants may move between exposure groups. The participants` awareness of being a part of a study may also alter their behavior. The knowledge of exposure may bias the classification of the outcome.

Literature review

As illustrated in the pyramid above literature studies are viewed as having a high quality and their findings of high value. These types of studies, like this paper, summarize previous research done at specific topic. Eight papers in this review were literature reviews. The most prominent weakness of this study design is that it is limited by the writer and could be heavily influenced by selection bias. This means that since the writer is the one selecting the articles to be used in the review, and his or her choices would be heavily affected by background,

interests and knowledge of the writer. Articles that could be of relevance for the study could therefore be left out of the review, or not so relevant articles included, due to selection bias.

The Cohort studies

The cohort studies will each be discussed separately in this subchapter. This is due to the strong scientific value cohort studies have compared to other studies, and that they evaluate one single exposure and its outcomes. However, the cohort studies presented here do not provide measured concentrations of environmental pollutants in blood serum, urine or other body fluids. Therefore, the quality of these studies is not as high as studies that are providing such measurements. The studies follow the participants over years, which is a strength.

Mortality and cancer incidence among British agricultural pesticide users (27)

In this cohort the group studied were individuals with Certificates of Competence issued by City & Guilds Land Based Services since 1987 and that had given their consent to give their personal details. It was a total of 62 960 participants. They were followed up during the time period between 1987 and 2004 for cancer incidence (2005 for mortality). The incidence and mortality for the types of cancers investigated were then compared to the incidence with the rest of the British population. For the incidence of testicular cancer for British agricultural pesticide workers the CI was 1.04–1.53. The real exposure to each individual was not measured.

The authors of the study highlight a weakness to the study; the database did not have information on potential confounding factors, such as smoking history, physical activity and hours spent outdoors, which would need to be collected through additional research. The study only included individuals that were certified to use agricultural pesticides and could therefore have excluded individuals that use agricultural pesticides on a commercial basis without certification.

Cancer incidence among firefighters: 45 years of follow-up in five Nordic countries(70)

Male fire fighters from the five Nordic countries were followed up during the period of 1961-2005 to check for cancer incidence through the cancer registries of their respective country. The incidence of testicular cancer was decreased for fire fighters (SIR=0.51, 95% CI 0.23 to 0.98). Neither in this study was real exposure measured.

Studies with serum measurements

A few studies measured serum levels when studying the risk of testicular cancer and the researched chemical. These are viewed as the studies of highest value in this present review. The researchers have more clear estimates when evaluating true exposure to the chemical when measuring chemicals. They have enough cases although it is hard when the diagnosis is this rare. They also control for confounders. Only the findings related to measured levels of chemicals will be discussed in this part. Additional findings relating to self-reported exposure will not be discuss, only mentioned. Nor will findings related to other conditions be discussed. All the studies found somewhat of an association between high concentrations of chemicals and testicular cancer. The studies are commented below.

Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls (37)

This Italian case-control study investigated the relation between serum concentration organochlorine pesticides and the risk of having testicular cancer. 50 testicular cancer cases and 48 controls, all males aged 18-45 years, were all recruited between October 2006 and September 2008 at the Laboratory of Seminology and Sperm Bank, Department of Medical Pathophysiology, by the local Institutional Review Board.

Cases were confirmed by histological examinations and evaluated ~1 month after orchidectomy. Blood samples were collected and the organochlorine pesticides included in this study were *p,p'*-DDE and HCB. Cases and controls were well balanced for age, BMI and place of recidence and did not have any other type of cancer. There were detectable values of at least one chemical in 32 % of the samples from TC patients and 11 % in the control group. The number of detectable samples for HCB was small but all were measured in cases. Only 3 cases measured ≥0.2 ng/ml, giving a p Fisher <0.01. The maximum detected value for HCB was 0.83 ng/ml.

The maximum measured serum concentration of *p,p'*-DDE, was 1.2 ng/ml, also measured in a case. The risk for testicular cancer when having detectable values of *p,p'*-DDE ≥0.2 ng/ml; OR =3.02, 95 % CI: 1.00-9.27. The association was less significant when adjustment for educational level and prenatal factors like mother's age at birth and parity ($OR_{adjusted}$ =3.21 95% CI: 0.77-13.30).

When evaluated together higher serum concentrations of *p,p'*-DDE +HCB were significantly associated with higher risk of testicular cancer (OR =3.15. 95 % CI: 1.00-9.91; $OR_{adjusted}$ =3.34, 95 % CI: 1.09-10.17)

The authours also discussed that the results may have biological confounding factors as there were more reproductive birth defects in cases than controls (P<0,01). This is supported by earlier litterature.

This study partially supports the hypothesis of Endocrine Disrupting Chemicals beeing the cause of testicular cancer. However, the sample is small, and the rarity of the tumour is a weakness for the study. Balancing the cases and controls with background and BMI and analyzing the samples in the same time periode are strengthens to the study.

Exposure to polychlorinated biphenyls and hexachlorobenzene, semen quality and testicular cancer risk (32)

A later case-control study was performed by many of the same scientists as from the study describes above. This study focused on serum concentrations of PCBs and HCB in testicular cancer cases versus controls, and also semen quality. It was performed in pretty much the same manner as the previous study. In this study the individuals included were 125 testicular cancer patients aged 29.6 ± 5.9 years (seminoma and non-seminoma) and 103 controls aged 31.3 ± 6.6 years. The cases were recruited from the Laboratory of Seminology-Sperm Bank at the Department of Experimental Medicine, University of Rome 'La Sapienza'. The testicular cancer cases were evaluated, and seminal fluid and blood serum were collected approximately 1 month after orchiectomy and before beginning chemo- or radiotherapy. The healthy controls were recruited in the same department undergoing an andrological examination and semen analysis in a national screening campaign. Cases and controls were balanced for BMI and residence.

Detected concentration of the sum of the 9 analyzed PCB congeners were found in 16 cases. None of the PCB congeners were detected in controls giving a statistically significant difference ($p < 0.001$). HCB was detected in five cases and one control; this difference was not statistically significant (OR 4.25; 95 % CI 0.49–36.97; $p = 0.22$). When looking at total detectable values of PCBs and HCB there was a statistically significant increase in testicular cancer risk (14.4 %) compared to controls (1.0 %) ($p < 0.001$).

Again, this study partially supports the theory that some chemicals may have an impact when it comes to the development of testicular cancer.

Country-specific chemical signatures of persistent environmental compounds in breast milk (54)

This study analyzed a total of 121 chemicals in the breast milk of Danish and Finnish mothers to newborn sons. The data set for this analysis was obtained between 1997 and 2001. Only breast milk of the 68 mothers (36 Danish and 32 Finnish) who gave birth to boys without malformations of dysfunctions were included in the study. Laboratories and technicians were blinded for country of origin when analyzing the samples.

54 chemicals had significantly higher concentrations in Danish than in Finnish breast milk samples before correction of multiple testing ($p < 0.05$). After correction the following chemicals were still differing significantly in concentration: Dieldrin, HCB, 1,2,3,4,7,8-HCDD, PCB-156, PCB-157 and PCB-209. After correction none of the chemicals measured were significantly higher in Finnish breast milk compared to Danish. Before correction MBP, methoxychlor and PCB-51 were higher in Finish breast milk than Danish. Other of the measured chemicals did not show a significant difference between the two countries.

Since the incidence of cancer is three to four folds higher in Denmark compared to Finland the findings of chemicals in human breast milk was viewed as highly interesting.

Prenatal DDT exposure and testicular cancer: a nested case-control study (49)

This study is based on existing data available from The Child Health and Development Studies (CHDS). A 40- year follow-up of over 20,000 pregnancies in Northern California between 1959 and 1967. Fifteen of 9,744 live-born sons were diagnosed with germ cell testicular cancer later in life. Each case was matched to three controls with regard to ethnicity and birth year. The maternal serum samples obtained postpartum and were compared between cases and controls investigating DDT and related residuals and associated with the son's risk of testicular cancer.

The measurements showed that mothers of cases had lower serum concentrations of *p,p′*-DDT, *o,p′*-DDT and *p,p′*-DDE than to their matched controls. A significantly higher ratio of *p,p′*-DDT to *p,p′*-DDE in 11 of 15 cases was observed. $p<0.03$ for the Wilcoxon matched

pairs signed ranks test and was significantly associated with testicular cancer risk in exact conditional logistic regression analyses ($p<0.01$). Testicular cancer cases had significantly shorter gestations. The study has low statistical power due to the low number of cases.

Cell culture studies

Four of the studies yield from the literature search were cell culture studies. It's not suggested to draw a definite conclusion concerning carcinogenic properties from these studies. However, these types of experiments show possible biological mechanisms the investigated chemical may have on cells. All of the cell studies in this review indicate that the studied chemical actually has an effect on the cellular mechanisms in the cells. Some of the mechanisms might even suggest effects leading to the development of testicular cancer. Especially mechanisms that lead to alteration of the steroidogenesis are of interest as this process is of importance for the health development of the testicles. The findings on cellular mechanisms are summed up below and in a table under the appendix.

2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) decreases progesterone synthesis through cAMP-PKA pathway and P450scc downregulation in mouse Leydig tumor cells(67)

BDE-47 is one of the congeners of PBDEs and is the most prevalent one in abiotic and biotic environments.

The study was performed on mLTC-1 cell line cultured in RPMI 1640 medium containing 100 IU/ml penicillin, 100 g/ml streptomycin sulfate, 2 g/l sodium bicarbonate and 10% heat-inactivated fetal bovine serum at 5% CO_2 and 37 °C.

- Cell viabilities were not affected by BDE-47 exposure after 12, 24 and 48 hours.
- BDE-47 at 5 μmol/l and higher significantly decreased hCG-stimulated progesterone production in a dose-dependent matter. The effect was reversible after removing BDE-47.
- Did not affect CT-induced progesterone production or intracellular cAMP-formation
- Progesterone were reduced in a concentration-dependent matter when stimulated by forskolin, after BDE-47 exposure.
- exposure reduced progesterone production and decrease the intracellular cAMP level induced by hCG or forskolin. The results also indicated that BDE-47 could reduce the mRNA and/or protein expressions of P450scc and 3β-HSD.

P450scc and 3β-HSD are both enzymes that are important in the steroidogenesis. P450scc is a mitochondrial enzyme that catalyzes the side-chain cleavage reaction of cholesterol to produce pregnenolone. Pregnenolone is further converted by 3β-HSD to progesterone. 3β-HSD is also responsible for other reactions in the production of hormones.

The masculinization process in the development of the testicles is fully dependent on hormones (21). This study shows that in vitro exposure to BDE-47 is disturbing the production of progesterone in mouse Leydig tumor cells when stimulated by hCG or forskolin. However, if the same effect is reproducible in vivo in humans needs further research.

Biphasic effects of perfluorooctanoic acid on steroidogenesis in mouse Leydig tumour cells(68)

In this study a MLTC-1 cell line was cultured in RPMI-1640 medium, supplemented with 100 unit/mL penicillin, 100 unit/mL streptomycin and 10% (v/v) foetal bovine serum. Then, the cells were grown at 37°C with 5% CO_2 in a humidified incubator. The cells were incubated for 48 hours in 96-well plates at different concentrations of PFOA (0–300 μM), then 50 μL MTT (5 mg/mL) was added to each well and the cells were incubated for 4 h at 37°C. Cells that had not been treated with PFOA were used as controls.

The study investigated multiple cellular molecules. Some of the most important findings are summarized below:

- The following was not altered:
    - PPARα, controls the peroxisomal β-oxidation pathway of fatty acids
    - androgen receptor (AR) binds androgenic hormones and regulates male sexual phenotype gene expression.
    - carnitine-acylcarnitine translocase (CACT), a component of the mitochondrial inner membrane and transfers fatty acylcarnitines into the mitochondria.
    - P450scc (catalyses cholesterol side-chain cleavage to pregnenolone) and 17β-HSD (catalyses androstenedione to testosterone) mRNA expression.
- The following was induced:
    - Pregnane X receptor (PXR) regulates a broad range of genes involved in the transport, metabolism and elimination of foreign toxic substances.

    - Carnitine-palmitoyltransferase ii (CPTii) responsible for connecting carnitine to long-chain fatty acids, which facilitates them crossing the outer mitochondrial membrane. CPTii, coding for the inner mitochondrial membrane protein that converts acylcarnitine to acyl-CoA for further fatty acid metabolism, was significantly induced following 100 μM or 200 μM PFOA.
    - Mitochondrial matrix enzyme carnitine acetyltransferase (CRAT), that catalyses the inter-conversion of acetyl-CoA and acetylcarnitine, after 200 μM
- The following were significantly reduced:
    - Scavenger receptor B1 (SR-B1) mRNA (regulates cholesterol uptake).
    - Sterol regulatory element-binding protein 2 (SREBP2)
    - The StAR gene, responsible for cholesterol transport to the inner mitochondrial membrane.
    - 3β-HSD gene, responsible for converting pregnenolone to progesterone.
    - CYP17α, has a significant role in steroid hormone synthesis.
- The levels of 17-OH progesterone and of testosterone were increased at 100 μM, however at 200 μM the levels were significantly reduced.
- Luteinizing hormone receptor (LHR) triggers Leydig cells to produce androgens when activated. At low levels no significant alteration was observed. 100 μM PFOA significantly induced LHR expression. At 200 μM PFOA exposure it was reduced.

The results showed that exposure to PFOA is transported into and accumulate in cytoplasm and mitochondria. It alters fatty acids transport into mitochondria, the site of steroidogenesis, in the MLTC-1 cells and disturbs cholesterol synthesis transcriptional profile and secretion. PXR, LHR and SR-B1 genes were sensitive to PFOA. The cytoplasmic cholesterol transport was disturbed through inhibiting the SR-B1 uptake function. Steroidogenesis was inhibited through the LH binding. PFOA can accumulate in mitochondria and alter cholesterol precursor (cholesterol) production.

The site for steroidogenesis is in the mitochondria of Leydig cells. Inhibiting transport of cholesterol, of which the sex hormones is produced from, the whole process of synthesis is inhibited. Whether the same effect is on humans in vivo should be further researched.

Mechanism of bisphenol AF-induced progesterone inhibition inhuman chorionic gonadotrophin-stimulated mouse Leydig tumor cell line (mLTC-1) cells(60)

Mouse Leydig tumour cell line (mLTC-1) cells are cells derived from a Leydig cell tumour and is commonly used in cell experiments. The cells were exposed to 0, 0.1, 1, 10, 30, 50 and 70 µM BPFA for 24 hours. The study found that BPAF exposure inhibits progesterone secretion in hCG-stimulated mLTC-1 cells by reducing expression of scavenger receptor class B type I (SR-B1) and cytochrome P450 (P450scc) due to the adverse effects of cAMP. The study therefore concluded that BPAF is a potential endocrine disruptor in testicular Leydig cells. The study did the following findings:

- Progesterone levels decreased with increasing BPAF concentration.
- Progesterone secretion was unchanged at BPAF concentration at 0.1, 1, 10, 30 µM compared to controls. With increasing doses, progesterone levels also started to drop.
- Exposure to high doses BPAF decreased the cAMP production – reductions were 86% at 30 µM, 96.8% at 50 µM and 98.2% at 70 µM.
- The effects on steroidogenic genes: mRNA of luteinizing hormone receptor LHR, PBR or 3β-HSD was not altered (transport cholesterol to mitochondria and convert pregenenolone to progesterone).
- Levels of SR-B1 at 70 µM and P450scc protein expression were reduced in cells exposed to 50 and 70 µM BPAF. StAR was markedly decreased at70 µM.
- 22R-HC is a hydrosoluble form of cholesterol that can cross the mitochondrial membrane without the assistance of StAR. Adding 22R-HC led to an increased production of progesterone compared to controls. However, by introducing BPAF to the cell the progesterone levels were dramatically decreased again at 50 µM and 70 µM.

SR-B1 is a receptor on the mitochondria that is facilitating the uptake of lipoproteins – an important component in the production of steroid hormones. The P450scc is an enzyme in the cascade of steroidogenesis. The cellular effects proven in this study show that exposure to BPAF affects the steroidogenesis in multiple ways. Scientists hypothize that disruption of the steroidogenesis is crucial in the later development of testicular cancer (21).

Mono-(2-Ethylhexyl) Phthalate (MEHP) Promotes Invasion and Migration of Human Testicular Embryonal Carcinoma Cells(74)

Human testicular embryonal cell line NTERA-2 cl. D1 (NT2/D1) were treated with designated concentrations of MEHP diluted in dimetyl sulfoxide (DMSO) for various time periods. Cells treated with DMSO only were used as control groups.

MEHP exposure proved to have multiple effects on NT2/D1 cells that could indicate that exposure to this phthalate may enhance tumor progression and metastasis in these cells. The findings post MEHP exposure were the following:

- Matrix metalloproteinases (MMP) are enzymes capable of degrading extracellular matrix. They have been marked as one of the main factors for the progression of tumour cells. Low doses of MEHP had no significant effect, while 200 µM strongly induced MMP2. MMP9 had no significant change in expression post MEHP exposure.
- 200 µM MEHP exposure caused upregulation of MYC. MYC controls cell proliferation, cell differentiation and self-renewal, thus upregulation after exposure may indicate that MEHP affects normal cell differentiation and tumor cell development. MYC expression increased after 12hrs of incubation and in a dose-dependent matter.
- Post exposure the cells had a stronger ability to digest and pass through the Matrigel, the substance that were to resemble extracellular environment in which the cells were incubated in.
- MEHP also decreased GJA1 (a gene that codes for Cx43 protein, forming the main structure of the gap junction) mRNA expression in NT2/D1 cells. It also showed a reduction in vinculin mRNA level in testicular cancer cells. This is important for maintaining the structure of adherence junction through interaction with actin filament and integrin.

The scientists view the increased ability of the cells to migrate through the Matrigel as a result of the other effects presented: Upregulation of MMP and MYC, and the downregulation of GJA1 and vinulcin. This experiment shows that MEHP exposure has carcinogenic effects on these cells. However, some of these effects were only evident after high doses of MEHP, and there is a lower probability that humans are being exposed to that high amounts. Of course, the study must also be reproduced in vivo to conclude a similar effect in human cells.

Allethrin induces oxidative stress, apoptosis and calcium release in rat testicular carcinoma cells (LC540)(39)

The study investigates the toxicity of the chemical on cellular level with the main focus on the male reproductive system, meaning Leydig cells.

The cells were cultured in Eagle's Minimal Essential Medium containing 5% newborn calf serum, 1.2 g/L $NaHCO_3$ and antibiotic-antimycotic in a 5% humidified incubator at 37 °C. In the cohort cells were treated with varying concentrations of allethrin dissolved in DMSO for different time periods. Cells were also treated with DMSO only for 48 h to see if the solvent had any effect on the cells and did not prove to contribute to any cell killing. The cells were treated with allethrin for 24 h.

The cells activity was measured using MTT assay which is a colorimetric assay to check for cell toxicity.

- Allethrin did not prove any toxicity to 50 μM. At concentrations above 100 μM, cell killing was observed IC_{50} was found to be 125 μM.
- Allethrin was found to be cytotoxic to primary Leydig cells in a dose-dependent manner with an IC_{50} value of 59 μM.
- ROS was significantly increased after allethrin treatment compared to controls, although not in a time dependent way.
- Time dependent increase in the lipid peroxidation products was observed.
- Presence of apoptotic bodies in LC540 cells increased after allethrin treatment.
- Decrease of MMP in cells treated with 125 μM
- Increased p53 gene expression

LC540 cells were preferred over primary Leydig cells when studying the mechanism of allethrin due to the difficulty in obtaining a large number of Leydig cells. Allethrin is cytotoxic to isolated Leydig cells and testicular cancer cells.

Of the highlights presented in the study regarding the cytotoxic mechanisms were free radical generation and altered antioxidant status of the exposed cells. Apoptotic properties of allethrin could be due to the induction of BCL-2, caspase-3 activation and release of intracellular

calcium (possibly mediated by voltage gated channels). The researches also observed loss of MMP, which is important in cell proliferation, and increased p53 gene expression, which is of importance in cell apoptosis. This showed toxicity to these Leydig tumour cells.

This study was performed on rat Leydig cells, and further research is necessary to assess whether the same effect is evident in human cells.

Hill's criteria for causation and the articles

As mentioned in the introduction part of this review Hill's criteria for causation is a useful tool when evaluating a true causality between an exposure and disease. Below each of the criteria have been gone through and evaluated on basis of the articles in this review:

1. Strength of association: The supposed cause must be significantly present in the exposed compared to the non-exposed.

This first criteria is difficult to confirm or discharge. As environmental pollutants can be measured almost anywhere in the environment there is no clear separation between exposed to non-exposed. Only in cell studies this can be clearly manifested. In population studies the scientist may separate exposed to non-exposed by assessing workplace, living area, hobbies and so on. But true exposure will be hard to trace and confirm without measuring the concentration of a chemical in serum or other body secretions. These studies were particularly discussed in the section above. Hence, association observed in some studies that rely on interviews or estimates may be due to systematic error, random error, chance or there may be a true causality.

Four studies in this present review that did measure concentrations of chemicals in blood serum or breast milk. They all produced results that partly supports the hypothesis that there is an association between organic environmental pollutants and testicular cancer:

Higher blood serum concentrations of *p,p'*-DDE +HCB were significantly associated with higher risk of testicular cancer. HCB was detected in three cases and no controls, and elevated concentrations of *p,p'*-DDE was associated with an increased risk of testicular cancer (37).

When measuring blood serum concentration 9 analyzed PCB congeners were found in 16 testicular cases, and none were detected in controls. This gave a statistically significant difference ($p < 0.001$). HCB was detected in five cases and one control, which was not a statistically significant difference. Detectable values of PCBs+HCB in serum gave a statistically significant increase in testicular cancer risk (14.4 %) compared to controls (1.0 %) ($p < 0.001$) (32).

Breast milk from Danish mothers measured higher concentrations (after correction) of the following chemicals than breast milk from Finnish mothers: Dieldrin, HCB, 1,2,3,4,7,8-HCDD, PCB-156, PCB-157 and PCB-209 (54). The fact that Denmark has a higher incidence of testicular cancer than Finland might be related to this.

Mothers of boys that later in life developed testicular cancer had lower serum concentrations of *p,p′*-DDT, *o,p′*-DDT and *p,p′*-DDE, and a higher ratio of *p,p′*-DDT to *p,p′*-DDE than did mothers of controls (49).

2. Consistency: The result must reproducible in other studies.

The question of consistency is challenging to clearly give an answer to as there has not been done too much research on testicular cancer and environmental pollutants. Testicular cancer is still a rare disease, which makes it difficult to study.

This review has failed to prove consistency. Although the studies cited mostly can refer to other studies that show similar findings, the number of confirming studies is still too low to conclude on consistent findings. The tables summing up all the findings indicate varying conclusions on whether there is association or not. While some prove increased risks of testicular cancer with environmental exposure, others has shown no or even preventive effects (24). The consistency criteria needs further research to be conducted to be confirmed or discharged.

A suggestion to a new study on this topic would be to have a prospective (over at least 30 years), transnational study with blood serum measurements of EDC in the objectives. The studied group of individuals should be big and diverse enough to represent each nations population and get enough cases. This is however hard as testicular cancer is an uncommon diagnose. The group should also be controlled for confounders like cryptorchidism,

hypospadias, ethnicity and others. The measurements should be done from early on in life (maybe even prenatally) and throughout the study. This study would be very time consuming and costly of course, but it would possibly provide an interesting result.

3. Specificity: Association is casual when the exposure only causes one disease.

The specificity criteria will not be extensively discussed here. One exposure could cause different conditions, and this is an accepted fact in today's research. All pathological effects of environmental pollutants are not yet fully understood, but it is believed that it could be the cause of a variety of diseases. Different populations are exposed to the variety of the pollutants and unfortunately there is still no studies that addressed the effect of pollutant mixtures on the incidence of testicular cancer.

4. Temporality: The phenomenon has to occur *after* the exposure for there to be a causality.

Cell experiments showed a significant change in cell mechanisms after the cells were exposed to the different chemicals. Unexposed cells did not show the same effect as the exposed cells and the cells were strictly controlled during the experiments. The effects observed could therefore most certain be due to the chemical exposure only. In these cell studies the strength of association must be valued as strong.

Temporality is much harder to confirm when studying populations as the environmental pollutants can be measured almost anywhere in the environment. It is also not easy to know which chemical to control and evaluate. Self-reported exposure is also not to be given a high value as this is prone to recall bias.

The incidence of testicular cancer is on the rise and some scientists view this as related to the increased concentration of environmental pollutants in the environment. It is postulated that well developed countries also have introduced more environmental pollutants into their environment. Epidemiological studies show that countries that score high on HDI also have a higher incidence of testicular cancer than countries that score low on HDI. As mentioned earlier in this review correlation does not necessarily mean correlation. This view does not take into account the incidence difference in-between countries with high HDI. This could be due to under-reporting and a lack of health services in the countries with a low HDI score.

These countries also score higher on mortality due to testicular cancer than do countries at the other end of the scale. There are very few prospective cohort studies with serum measurements of POPs that assessed association between environmental pollutants and TC.

5. Biological gradient: An increase in the exposure of the supposed cause would also cause an increased incidence or severity of the phenomenon.

The literature research yielded studies that investigate cells response to different environmental pollutants. Allethrin was found to be cytotoxic to primary Leydig cells in a dose-dependent manner (39). The same was for the other studies – the higher the exposure the more cytotoxic effect the chemical was to the cell.

The biological gradient is harder to prove for studies on humans. Many of the studies were based on self-reported exposure. The true exposure would have to be measured to be further evaluated. In the environment, but also in each individual to assess how much is accumulated and metabolized in the body and over a life span. None of the studies did evaluate the degree of disease in humans. The individuals were classified in cases or non-cases.

POPs have been restricted the latest years through the Stockholm Convention on Persistent Organic Pollutants. With this in mind, one might expect that if there is a causality between testicular cancer and environmental pollutants that the incidence of testicular cancer would decrease over the years. If this is the case, the outcome would probably take a few years. POPs remains in the environment for many years and would still be around for a while, also after regulations. Also, if the case is that the prenatal exposure is the most crucial for developing testicular cancer, the incidence would depend on the exposure of mothers, rather than males. This would cause a further delay in any manifestations of the incidence curve. Whether the incidence of testicular cancer decreases after some years of regulation of environmental pollutants remains to see.

6. Plausibility: Based on the up-to-date knowledge accessible about the disease, a causality would be probable, and may be explained through biological studies.

A variety of environmental pollutants have proven to persist in the environment and have endocrine effects on human cells. As the formation and development of testis are hormonal dependent there is a likelihood that these chemicals also could affect this process in some way

and further contribute to the development of testicular cancer. The cell studies presented in this present review support the hypothesis of association between organic environmental pollutants and testicular cancer. So do the studies that include serum measurements.

7. Coherence: coherent finding between epidemiological studies and the results in laboratory findings.

Some of the epidemiological studies show increased risk when exposure but not all. Although laboratory findings prove carcinogenic effects by different chemicals on cells these are not necessarily reproduced in the epidemiological studies. However, as mentioned earlier, there are few studies of high quality with a prospective cohort design that measured POPs concentrations in blood of the participants. Studies with estimated exposure or self-reported exposure are prone to many biases.

8. Experiment: Experiments performed on cells that indicate a carcinogenic effect when exposed to organic environmental pollutants.

The studies on cells yield in this literature search prove that different chemicals affect cells and alter their properties. Hence, the experiment criteria is by far confirmed in this review, although more extensive research would strengthen the association.

The studies proving that exposure to the studied chemical disturbs the steroidogenesis is of particular interest. Steroid hormones are important in the masculinization process in the development of the testicles and alterations is the hormone production could therefore be of significance when this process goes wrong. The chemicals, BDE-47, PFOA, BPAF and MEHP, had different effects that indicate induction of increased cell proliferation and metastasis – common traits of carcinogenic cells. Allethrin showed to have toxic properties and apoptotic effects on testicular Leydig cells. Allethrin exposure might disturb the steroidogenesis and possibly have carcinogenic effects.

9. Analogy: A phenomenon or exposure that resemble or behave in the similar way as the studied objects

The simultaneous increased incidence of genital abnormalities in boys that also increase the risk of developing testicular cancer could also be a part of the explanation for the trends.

Whether genital abnormalities are also due to exposure to environmental pollutants remains to be explored.

Further discussion of the results

The results of the literature search proved that there is not done much research on the cause of testicular cancer and whether it could be related to environmental pollutants. Most of the findings were review articles, which could imply that most of the research done on this field is old (older than 10 years as the search was limited to).

One of the difficulties studying the incidence and mechanisms of testicular cancer is that it is still a rare disease, although the most common type of cancer in young men. In the review article investigating Agent Orange and the incidence of cancer incidence (34) the authors highlight the fact that testicular cancer most often occurs in young men and that the Vietnam veterans are well outside this age range.

Additionally, one must keep in mind that real-life exposure is never as simple as assessing the toxicity of a single compound in the laboratory. The effect a chemical has on a cell in vitro is not necessarily as reproducible in vivo and in real life.

Another real-life implication is the fact that we are exposed to a variety of chemicals on an every-day basis. As one chemical may not pose a harmful effect, the mix of all these could. This is known as the mixture effect, also called “the cocktail effect”. *The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis* (50) highlights the fact that epidemiological studies often only address individual chemicals and receive extensive criticism for this. Though, the review argues that some POPs are highly correlated, and when studying one chemical it is not necessarily possible to distinguish it from another, and some chemicals, like, PCB-153, are in fact reflecting exposure to a mixture of numerous other chemicals (50). The cocktail of pollutants effect would be more complex to study than the effect of each individual chemical, but it is much more relevant due to the simple fact that different populations may be exposed to different mixtures of chemicals.

Denmark and Norway are countries with high incidence of testis cancer as well as areas with poor semen quality. Vice versa countries like Estonia and Finland have a low incidence of

testis cancer and good semen quality in men (86-88). This would suggest that the whole picture of the testicular cancer development is more complex and a following effect of genital abnormalities. In the Italian research on serum concentrations of PCBs and HCB in testicular cancer cases versus controls (32), they found that the sperm quality of testicular cancer cases was worse compared to controls. The samples of semen presented by cases had lower mean values for sperm concentration, lower total sperm number and total motility and a higher percentage of abnormal forms than those with undetectable levels compared to controls. This difference was not statistically significant though.

In Caucasian men, men with cryptorchidism, a family history of testicular cancer and possibly men with tall statures testicular cancer is more common (89). This suggests that there may be genetic factors that affect the risk of developing testicular cancer. The review *Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): environmental connection* (90) discusses some aspects of genetical factors that may play a role in the development of testicular cancer. Mastermind Like Domain Containing 1 (MAMLD1) (or chromosome X open reading frame 6, *CXORF6*) is a gene in which mutations cause hypospadias and testicular dysgenesis (91). Fukami et al. writes about how this gene is affecting the synthesis of hormones and that mutations in MAMLD1 disrupt androgen production (92).

Although not scientifically confirmed, genetic polymorphisms can modify the susceptibility to environmental factors and development of Testicular Dysgenesis Syndrome (93-96). If this can be transferred to the development of testicular cancer is still not confirmed. Further research is required to validate these findings, preferably in larger populations, as other studies have reached contradicting results (97).

The significantly higher ratio of *p,p′*-DDT to *p,p′*-DDE in mothers of boys who developed testicular cancer later in life compared to controls (49) could imply a genetic variation that causes individual differences in the metabolism of DDT. Meaning the pathology could be a result of genetic variation and environmental factors combined. The difference in incidence seen in-between continents and countries could therefore also be due to genetic differences, or, even more likely, the combination of exposure and genetics.

Initially, this study describes how the incidence of testicular cancer differs between countries. Few studies answer this question. *Country-specific chemical signatures of persistent environmental compounds in breast milk* (54) investigated the national difference between Denmark and Finland in terms of the incidence of testicular cancer. Other than the study this present review failed to address the question why there are large international differences.

As mentioned earlier it is postulated that countries that score high on the HDI also have introduced extensively more chemicals and pollutants in the environment and also their micro-environments than have countries that score lower. Therefore, environmental pollutants could be the cause of a higher incidence of testicular cancer in these countries than in those that score lower. However, the countries that are at the poorer end of the HDI scale are often the sites of production of plastic and other materials. Waste is usually disposed in the environment and the inhabitants of the area and workers are usually unprotected from these. This observed trend could possibly be explained due to underreporting of cases in less developed countries.

Strengths and weaknesses of this study

As mentioned earlier, reviews like this are most likely influenced by selection bias. This review has been influenced by my (and my supervisors) background in terms of creating a search in PubMed and also in selecting relevant articles and discarding irrelevant articles.

The present review has thoroughly gone through cell line studies to analyze the exact mechanism of the studied organic pollutant. It also looked at studies with serum measurements to assess whether exposure to organic pollutants increase the risk of testicular cancer *in vivo*. Cohort studies were also gone through due to the high quality of this type of study design. In addition to this, results of studies summarized in other reviews were also mentioned. Rounding off, the study discusses the results in relation to earlier studies. In this way the possible carcinogenic properties of organic pollutants were studied from different perspectives and this present study gives a nuanced image of how they possibly affect testicular health.

Overall, the results were partly summarized and put in the context of the nine principles of the Hill's criteria for causation. This is commonly used in public health research to establish a

relationship between a presumed cause and a plausible health effect. Which is exactly what this study is investigating – organic pollutants and testicular cancer.

The search included multiple organic environmental pollutants. It would be extremely demanding to include all varieties of words and searches for different pollutants, as there are thousands of them. Therefore, inevitably some pollutants may have been excluded from the search. This might have affected the results to include fewer articles than are available with relevant research on this topic.

The search was not updated since the search was executed 23rd of October 2019 and possibly new relevant studies for this present review has emerged that is not included.

The research only included *Testicular cancer* as outcome as this was the main object of the research question. In this way only studies which investigated testicular cancer were included and made the search more specific for this outcome only. At the same time, studies that evolved around other genital dysfunctions that could possibly be caused by exposure to environmental pollutants were excluded. Conditions like hypospadias are linked to the later development of testicular cancer. Although, it is not absolute that boys born with genital dysfunctions will also develop testicular cancer, this research possibly excluded research articles that investigated whether there is an association between environmental pollutants and genital dysfunctions that could lead to testicular cancer.

Conclusion

This systematic review aimed at investigating whether there is an association between environmental pollutants and testicular cancer. The literature search yield very few articles of interest, and the results of these were discordant. Very few studies measured concentrations of environmental pollutants in blood and relate the results to testicular cancer. Therefore, the studies of highest values are underrepresented. Due to this lack of research data of high quality it is impossible to conclude whether there is any causal association or not. Nevertheless, all the studies with measured concentrations of chemicals presented, in some way supported the hypothesis of that organic environmental pollutants are associated with testicular cancer. The same goes for the cell studies. When studying the cellular mechanisms all the studied chemical had some sort of effect on the cell components.

Not all of the Hills criteria for the causal association are fulfilled according to the up-to date knowledge. However, there are several individual studies that showed a possible association between individual POPs and testicular cancer and studies in cell cultures showed plausible biological mechanisms for this association. Nevertheless, prospective studies measuring pollutant mixtures and their possible effect on testicular cancer development are absent.

Many factors have been investigated in the existing studies, but the reason for the increased incidence of TGCT remains unclear. Occupational exposures during adulthood are unlikely to be involved in TGCT etiology because of the young age of patients. Whether parental exposure to a mixture of pollutants could affect the testicular health of their offspring should be further investigated. Multiple risk factors such as hypospadias, being Caucasian and hydrocele suggest that prenatal events may affect testicular health, but the whole picture is still unclear. Genetic variations may also play a role in whether an individual develops testicular cancer or not. Further research should explore effects of different pollutants mixtures in a prospective design and have reliable multiple measurements of exposure such as blood or urine concentrations.

Works cited

1. Znaor A, Lortet-Tieulent J, Jemal A, Bray F. International variations and trends in testicular cancer incidence and mortality. European urology. 2014;65(6):1095-106.
2. Shanmugalingam T, Soultati A, Chowdhury S, Rudman S, Van Hemelrijck M. Global incidence and outcome of testicular cancer. Clinical epidemiology. 2013;5:417-27.
3. Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. The Journal of urology. 2003;170(1):5-11.
4. Observatory GC. Incidence – ASR (World) vs Human Development Index, testis, in 2018, males, all ages 2019 [Available from: http://gco.iarc.fr/today/online-analysis-scatter-plot?v=2018&mode=cancer&mode_population=continents&population=900&populations=900&key=asr&sex=1&cancer=28&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=5&group_cancer=1&include_nmsc=1&include_nmsc_other=1&fit_to_screen=1&same_scale=0&axis_indicators=%257B%2522x%2522%253A%2522hdi_value%2522%252C%2522y%2522%253A%2522inc%2522%257D&axis_keys=%257B%2522x%2522%253A%2522asr%2522%252C%2522y%2522%253A%2522asr%2522%252C%2522log_scale_x%2522%253Afalse%252C%2522log_scale_y%2522%253Afalse%257D.
5. Cheng L, Albers P, Berney DM, Feldman DR, Daugaard G, Gilligan T, et al. Testicular cancer. Nature reviews Disease primers. 2018;4(1):29.
6. Sciences NIoEH. Endocrine Disruptors May, 2019 [Available from: https://www.niehs.nih.gov/health/topics/agents/endocrine/index.cfm.
7. Maqbool F, Mostafalou S, Bahadar H, Abdollahi M. Review of endocrine disorders associated with environmental toxicants and possible involved mechanisms. Life sciences. 2016;145:265-73.
8. De Coster S, van Larebeke N. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. Journal of environmental and public health. 2012;2012:713696.

9. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrine reviews. 2012;33(3):378-455.
10. Oncolex. Testikkelkreft: Oncolex.no; 2019 [Available from: http://oncolex.no/Testikkel?lg=print.
11. Kreftlex. Symptomer ved testikkelkreft: Kreftlex.no; 2019 [Available from: https://kreftlex.no/Testikkelkreft/BAKGRUNN/Symptomer?CancerType=Testikkel.
12. Kreftlex. Utredning ved testikkelkreft: Kreftlex.no; 2019 [Available from: https://kreftlex.no/Testikkelkreft/ProsedyreFolder/UTREDNING/New-ksProcedureChapter.
13. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972-8.
14. Program UNE. Why do persistent organic pollutants matter? : UNEP; 2019 [Available from: https://www.unenvironment.org/explore-topics/chemicals-waste/what-we-do/persistent-organic-pollutants/why-do-persistent-organic.
15. Convention S. All POPs listed in the Stockholm Convention 2019 [Available from: http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx.
16. Convention S. History of the negotiations of the Stockholm Convention 2008 [Available from: http://www.pops.int/TheConvention/Overview/History/Overview/tabid/3549/Default.aspx.
17. Larsson K, Ljung Bjorklund K, Palm B, Wennberg M, Kaj L, Lindh CH, et al. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. Environ Int. 2014;73:323-33.
18. Silveira RS, Rocha BA, Rodrigues JL, Barbosa F, Jr. Rapid, sensitive and simultaneous determination of 16 endocrine-disrupting chemicals (parabens, benzophenones, bisphenols, and triclocarban) in human urine based on microextraction by packed sorbent combined with liquid chromatography tandem mass spectrometry (MEPS-LC-MS/MS). Chemosphere. 2019;240:124951.
19. Sumner RN, Tomlinson M, Craigon J, England GCW, Lea RG. Independent and combined effects of diethylhexyl phthalate and polychlorinated biphenyl 153 on sperm quality in the human and dog. Scientific Reports. 2019;9(1):3409.
20. Negri E, Metruccio F, Guercio V, Tosti L, Benfenati E, Bonzi R, et al. Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data. Crit Rev Toxicol. 2017;47(6):482-508.
21. Sharpe RM. Pathways of endocrine disruption during male sexual differentiation and masculinization. Best Pract Res Clin Endocrinol Metab. 2006;20(1):91-110.
22. Hill AB. THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION? Proc R Soc Med. 1965;58:295-300.
23. Beranger R, Le Cornet C, Schuz J, Fervers B. Occupational and environmental exposures associated with testicular germ cell tumours: systematic review of prenatal and life-long exposures. PLoS One. 2013;8(10):e77130.
24. McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Polychlorinated biphenyls and risk of testicular germ cell tumors. Cancer Res. 2009;69(5):1901-9.
25. Kelleher C, Newell J, MacDonagh-White C, MacHale E, Egan E, Connolly E, et al. Incidence and occupational pattern of leukaemias, lymphomas, and testicular tumours in western Ireland over an 11 year period. J Epidemiol Community Health. 1998;52(10):651-6.

26. Fleming LE, Bean JA, Rudolph M, Hamilton K. Cancer incidence in a cohort of licensed pesticide applicators in Florida. J Occup Environ Med. 1999;41(4):279-88.
27. Frost G, Brown T, Harding AH. Mortality and cancer incidence among British agricultural pesticide users. Occup Med (Lond). 2011;61(5):303-10.
28. Dich J, Wiklund K, Holm LE. Testicular cancer in pesticide applicators in Swedish agriculture. Scand J Work Environ Health. 1996;22(1):66.
29. Alavanja MC, Sandler DP, Lynch CF, Knott C, Lubin JH, Tarone R, et al. Cancer incidence in the agricultural health study. Scand J Work Environ Health. 2005;31 Suppl 1:39-45; discussion 5-7.
30. Zandjani F, Hogsaet B, Andersen A, Langard S. Incidence of cancer among nitrate fertilizer workers. Int Arch Occup Environ Health. 1994;66(3):189-93.
31. Kristensen P, Andersen A, Irgens LM, Bye AS, Vagstad N. Testicular cancer and parental use of fertilizers in agriculture. Cancer Epidemiol Biomarkers Prev. 1996;5(1):3-9.
32. Paoli D, Giannandrea F, Gallo M, Turci R, Cattaruzza MS, Lombardo F, et al. Exposure to polychlorinated biphenyls and hexachlorobenzene, semen quality and testicular cancer risk. J Endocrinol Invest. 2015;38(7):745-52.
33. Le Cornet C, Fervers B, Dalton SO, Feychting M, Pukkala E, Tynes T, et al. Testicular germ cell tumours and parental occupational exposure to pesticides: a register-based case-control study in the Nordic countries (NORD-TEST study). Occup Environ Med. 2015;72(11):805-11.
34. Chang C, Benson M, Fam MM. A review of Agent Orange and its associated oncologic risk of genitourinary cancers. Urol Oncol. 2017;35(11):633-9.
35. Yi SW. Cancer incidence in Korean Vietnam veterans during 1992-2003: the Korean veterans health study. J Prev Med Public Health. 2013;46(6):309-18.
36. Mester B, Behrens T, Dreger S, Hense S, Fritschi L. Occupational causes of testicular cancer in adults. Int J Occup Environ Med. 2010;1(4):160-70.
37. Giannandrea F, Gandini L, Paoli D, Turci R, Figa-Talamanca I. Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls. J Environ Sci Health B. 2011;46(8):780-7.
38. Allethrin, CID=11442 National Center for Biotechnology Information: PubChem Database; [Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Allethrin.
39. Madhubabu G, Yenugu S. Allethrin induces oxidative stress, apoptosis and calcium release in rat testicular carcinoma cells (LC540). Toxicol In Vitro. 2014;28(8):1386-95.
40. W.R. Kelce CRS, S.C. Laws, et al. Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature. 1995(375):581-5.
41. Horsak RD, Bedient P.B., Hamilton, M.R., Thomas, F.B. Environmental Forensics - Contaminant Specific Guide: Elsevier Inc.; 2005. 576 p.
42. Meeks JJ, Sheinfeld J, Eggener SE. Environmental toxicology of testicular cancer. Urol Oncol. 2012;30(2):212-5.
43. J. Guo EP, P. Kyyršnen, et al. Testicular cancer, occupation, and exposure to chemical agents among Finnish men in 1971–1995. Cancer Causes Control. 2005;16:97-103.
44. Purdue MP, Engel LS, Langseth H, Needham LL, Andersen A, Barr DB, et al. Prediagnostic serum concentrations of organochlorine compounds and risk of testicular germ cell tumors. Environ Health Perspect. 2009;117(10):1514-9.
45. McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. J Natl Cancer Inst. 2008;100(9):663-71.

46. Biggs ML, Davis MD, Eaton DL, Weiss NS, Barr DB, Doody DR, et al. Serum organochlorine pesticide residues and risk of testicular germ cell carcinoma: a population-based case-control study. Cancer Epidemiol Biomarkers Prev. 2008;17(8):2012-8.
47. Hardell L, Bavel B, Lindstrom G, Eriksson M, Carlberg M. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. Int J Androl. 2006;29(1):228-34.
48. Hardell L, van Bavel B, Lindstrom G, Carlberg M, Dreifaldt AC, Wijkstrom H, et al. Increased concentrations of polychlorinated biphenyls, hexachlorobenzene, and chlordanes in mothers of men with testicular cancer. Environ Health Perspect. 2003;111(7):930-4.
49. Cohn BA, Cirillo PM, Christianson RE. Prenatal DDT exposure and testicular cancer: a nested case-control study. Arch Environ Occup Health. 2010;65(3):127-34.
50. Bonde JP, Flachs EM, Rimborg S, Glazer CH, Giwercman A, Ramlau-Hansen CH, et al. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. Hum Reprod Update. 2016;23(1):104-25.
51. McGlynn KA, Trabert B. Adolescent and adult risk factors for testicular cancer. Nat Rev Urol. 2012;9(6):339-49.
52. K.A. McGlynn SMQ, B.I. Graubard, et al. Polychlorinated biphenyls and risk of testicular germ cell tumors. Cancer Research. 2009;69(5):1901-9.
53. Gocmen A, Peters HA, Cripps DJ, Morris CR, Dogramaci I. Porphyria turcica: hexachlorobenzene-induced porphyria. IARC Sci Publ. 1986(77):567-73.
54. Krysiak-Baltyn K, Toppari J, Skakkebaek NE, Jensen TS, Virtanen HE, Schramm KW, et al. Country-specific chemical signatures of persistent environmental compounds in breast milk. Int J Androl. 2010;33(2):270-8.
55. Jensen OM, Olsen, J. H. & Osterlind, A. Testis cancer risk among farmers in Denmark. Lancet. 1984;1:794
56. Masuda Y. Health status of Japanese and Taiwanese after exposure to contaminated rice oil. Environ Health Perspect. 1985;60:321-5.
57. Reijnders PJ. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature. 1986;324(6096):456-7.
58. Helmfrid I BM, Löfman O, Wingren G. Health effects and exposure to polychlorinated biphenyls (PCBs) and metals in a contaminated community. Environment International. 2012;44:53-8.
59. Hardell Lea. Concentrations of polychlorinated biphenyls in blood and the risk for testicular cancer International Journal of Andrology. 2004;27:282-90.
60. Feng Y, Shi J, Jiao Z, Duan H, Shao B. Mechanism of bisphenol AF-induced progesterone inhibition in human chorionic gonadotrophin-stimulated mouse Leydig tumor cell line (mLTC-1) cells. Environ Toxicol. 2018;33(6):670-8.
61. Raloff J. Another plastics ingredient raises safety concerns. Science News. 2010;177(12):14.
62. T. N'Tumba-Byn DM, M. Lacroix, C. Lecureuil, L. Lesage, S.M. Prud'homme, et al. Differential effects of bisphenol A and diethylstilbestrol on human, rat and mouse fetal Leydig cell functionPLoS One. 2012;7(12):e51579.

63. Rouiller-Fabre V, Habert R, Livera G. Effects of endocrine disruptors on the human fetal testis. Ann Endocrinol (Paris). 2014;75(2):54-7.

64. Bouskine A NM, Brucker-Davis F, et al. . Low doses of bisphenol A promote human seminoma cell proliferation by activating PKA and PKG via a membrane G-protein-coupled estrogen receptor. Environ Health Perspect. 2009;117:1053-8.
65. Ohlson CG HL. Testicular cancer and occupational exposures with a focus on xenoestrogens in polyvinyl chloride plastics. . Chemosphere. 2000;40:1277-82.
66. Hardell L MN, Ohlson CG, et al. . Testicular cancer and occupational exposure to polyvinyl chloride plastics: a case-control study. . Int J Cancer. 2004;109:425-9.

67. Han X, Tang R, Chen X, Xu B, Qin Y, Wu W, et al. 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) decreases progesterone synthesis through cAMP-PKA pathway and P450scc downregulation in mouse Leydig tumor cells. Toxicology. 2012;302(1):44-50.
68. Tian M, Huang Q, Wang H, Martin FL, Liu L, Zhang J, et al. Biphasic effects of perfluorooctanoic acid on steroidogenesis in mouse Leydig tumour cells. Reprod Toxicol. 2019;83:54-62.
69. Klaunig JE, Hocevar BA, Kamendulis LM. Mode of Action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and Human Relevance. Reprod Toxicol. 2012;33(4):410-8.
70. Pukkala E, Martinsen JI, Weiderpass E, Kjaerheim K, Lynge E, Tryggvadottir L, et al. Cancer incidence among firefighters: 45 years of follow-up in five Nordic countries. Occup Environ Med. 2014;71(6):398-404.
71. J. Walrath WEF, P.G. Gilby, et al. A case-control study of cancer among Dupont employees with potential for exposure to dimethylformamide. J Occup Med. 1989;31:432-8.
72. Chen JL FW, Pell S.J Cancer incidence of workers exposed to dimethylformamide and/or acrylonitrile. . Occup Med (Lond). 1988;30:813-8.
73. Walrath J FW, Gilby PG, Pell S. A case-control study of cancer among du pont employees with potential for exposure to dimethylformamide. J Occup Med. 1989;31:432-8.
74. Yao PL, Lin YC, Richburg JH. Mono-(2-ethylhexyl) phthalate (MEHP) promotes invasion and migration of human testicular embryonal carcinoma cells. Biol Reprod. 2012;86(5):160, 1-10.
75. V. Muczynski JPC, A. Lehraiki, C. Levacher, D. Moison, C. Lecureuil, et al. Effect of mono-(2-ethylhexyl) phthalate on human and mouse fetal testis: in vitro and in vivo approaches. Toxicol Appl Pharmacol. 2012;261(1):97-104.
76. N.E. Heger SJH, M.A. Sandrof, E.V. McDonnell, J.B. Hensley, E.N. McDowell, et al. Human fetal testis xenografts are resistant to phthalate-induced endocrine disruption. Environ Health Perspect. 2012;120(8):1137-43.
77. R.T. Mitchell AJC, R.A. Anderson, S. van den Driesche, P.T. Saunders, C. McKinnell, et al. Do phthalates affect steroidogenesis by the human fetal testis? Exposure of human fetal testis xenografts to di-n-butyl phthalate. J Clin Endocrinol Metab. 2012;97(3):E341-E8.
78. R. Lambrot VM, C. Lécureuil, G. Angenard, H. Coffigny, C. Pairault, et al. Phthalates impair germ cell development in the human fetal testis in vitro without change in testosterone production. Environ Health Perspect. 2009;117:32-7.
79. A. Lehraiki CR, A. Krust, R. Habert, C. Levacher. Phthalates impair germ cell number in the mouse fetal testis by an androgen- and estrogen-independent mechanism. Toxicol Sci. 2009;111(2):372-82.
80. PM. F. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl. 2006;29:140-7.
81. Meeker JD SS, Swan SH. . Phthalates and other additives in plastics: human exposure and associated health outcomes. . Philos Trans R Soc Lond B Biol Sci 2009;364:2097-113.

82. C.G. Ohlson LH. Testicular cancer and occupational exposures with a focus on xenoestrogens in polyvinyl chloride plastics. Chemosphere. 2000;40:1277-82.
83. Hansen J. Risk for testicular cancer after occupational exposure to plastics. Int J Cancer. 1999;82:911-2.
84. L. Hardell NM, C.-G. Ohlson, et al. Testicular cancer and occupational exposure to polyvinyl chloride plastics: A case-control study. Int J Cancer. 2004;109:425-9.
85. Steele R, Tiffin PA. 'Personalised evidence' for personalised healthcare: integration of a clinical librarian into mental health services - a feasibility study. Psychiatr Bull (2014). 2014;38(1):29-35.
86. Jorgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen AG, et al. East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum Reprod. 2002;17(8):2199-208.
87. Jorgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, et al. Regional differences in semen quality in Europe. Hum Reprod. 2001;16(5):1012-9.
88. Bergstrom R, Adami HO, Mohner M, Zatonski W, Storm H, Ekbom A, et al. Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. J Natl Cancer Inst. 1996;88(11):727-33.
89. McGlynn KA. Environmental and host factors in testicular germ cell tumors. Cancer Invest. 2001;19(8):842-53.
90. Toppari J, Virtanen HE, Main KM, Skakkebaek NE. Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): environmental connection. Birth Defects Res A Clin Mol Teratol. 2010;88(10):910-9.
91. Fukami M, Wada Y, Miyabayashi K, Nishino I, Hasegawa T, Nordenskjold A, et al. CXorf6 is a causative gene for hypospadias. Nat Genet. 2006;38(12):1369-71.
92. Fukami M, Wada Y, Okada M, Kato F, Katsumata N, Baba T, et al. Mastermind-like domain-containing 1 (MAMLD1 or CXorf6) transactivates the Hes3 promoter, augments testosterone production, and contains the SF1 target sequence. J Biol Chem. 2008;283(9):5525-32.
93. Watanabe M, Yoshida R, Ueoka K, Aoki K, Sasagawa I, Hasegawa T, et al. Haplotype analysis of the estrogen receptor 1 gene in male genital and reproductive abnormalities. Hum Reprod. 2007;22(5):1279-84.
94. Yoshida R, Fukami M, Sasagawa I, Hasegawa T, Kamatani N, Ogata T. Association of cryptorchidism with a specific haplotype of the estrogen receptor alpha gene: implication for the susceptibility to estrogenic environmental endocrine disruptors. J Clin Endocrinol Metab. 2005;90(8):4716-21.
95. Aschim EL, Haugen TB, Tretli S, Daltveit AK, Grotmol T. Risk factors for hypospadias in Norwegian boys - association with testicular dysgenesis syndrome? Int J Androl. 2004;27(4):213-21.
96. Beleza-Meireles A, Omrani D, Kockum I, Frisen L, Lagerstedt K, Nordenskjold A. Polymorphisms of estrogen receptor beta gene are associated with hypospadias. J Endocrinol Invest. 2006;29(1):5-10.
97. van der Zanden LF, van Rooij IA, Feitz WF, Vermeulen SH, Kiemeney LA, Knoers NV, et al. Genetics of hypospadias: are single-nucleotide polymorphisms in SRD5A2, ESR1, ESR2, and ATF3 really associated with the malformation? J Clin Endocrinol Metab. 2010;95(5):2384-90.

Appendix

Table 12: Summary of cell line studies and molecules affected

Chemical / Molecule	*BDE-47*	PFOA	BPAF	MEHP	Allethrin

3β-HSD	↓ mRNA and/or protein expression	↓ gene expression	mRNA levels not altered at any concentration		
AR		↓ at 50 or 100 µM, but not in higher doses			
cAMP	↓ intracellular cAMP induced by hCG or forskolin		↓ at high doses		
CYP17α		↓ at 200 µM			
GJA1				↓mRNA levels	
LHR		No significant alteration at lower concentrations. ↑ at 100 µM ↓ at 200 µM	mRNA levels not altered at any concentration		
MMP				↑ MMP2 at 200 µM	↓ at concentrations over 125 µM
MYC				↑at 200 µM	
P450scc	↓ mRNA and/or protein expression	Not altered	↓ at 50 and 70 µM		
p53					↑ gene expression
PBR			mRNA levels not altered		
PPARα		mRNA expression not altered			
PXR		↑ in a dose-dependent matter			

SR-B1		↓ mRNA expression	↓at 70 µM		
SREBP2		↓ expression			
StAR		↓ gene expression	↓ markedly at 70 µM		
Vinculin				↓ mRNA levels	

exposure and serum organochlorine residuals among testicular cancer patients and healthy controls, Journal of Environmental Science and Health, Part B, 46:8, 780-787. https://doi.org/10.1080/03601234.2012.597704

Grade - quality | **Low**

Aim	Materials and method	Results	Discussion/Comments/Check list
Investigate whether patients with testicular cancer have been more exopsed to organochloride pesticides (including DDE and HCB) than healthy controls **Conclusion** There was a significant association between testicular cancer and reported use of household insecticide. There was also an association with higher total serum concentration of organochloride pesticides **Country** Italy **Year of collecting data** 2006-2008	**Population:** All individuals in this study were males aged 18-45 years and did not have any other type of cancer. Cases and controls were well balanced for age, BMI and place of recidence. All were recruited at the Laboratory of Seminology and Sperm Bank, Department of Medical Pathophysiology, by the local Institutional Review Board of University Hospital «Umberto I». **-cases:** 50 testicular cancer patients were evaluated ~1 month after orchidectomy. 28 cases were seminomas and 22 were non-seminomas. **-controls:** recruited from the same Department among healthy men undergoing an andrological examination in order to ascertain fertility status. **Main exposure:** Organochloride pesticides, including dichlorodiphenyltrichloroethane (DDE) and hexachlorobenzene (HCB) **Important cofunding factors:** - Occupation - Life style - Diet (meat, fish, dairy, fruits and vegetables) - Perinatal characteristics **Statistical methods:** - Chi-squared test - Fisher's exact test	**Key findings** Testicular cancer and household insecticide use: OR adjusted = 3,23 95% CI: 1,15-9,11 Testicular cancer and total measured Organochlorine pesticides: OR adjusted = 3,34 95% CI: 1,09-10,17 **Additional findings** Dairy (OR=2,15, 95% CI: 0,67-6,83) and fish (OR= 2,03, 95% CI: 0,86-4,77) consumption was identified as possible risk factors No association was found for meat consumption (OR=0,98, 95% CI: 0,43-2,21), and a protective effect was observed with high fruit and vegetable consumption (OR=0,50, 95% CI: 0,22-1,13)	**Check list:** • **The aim is clearly formulated** • **The case-control study design is suited for the study** • **Cases were recruited in a time period over 2 years from the same department. The authors write that cases and controls were balanced for age, BMI and recidence, but does not further elaborate how this was done without any selection bias. Although, they have take into account these confunding factors.** • **The diagnosis were confirmed through histological examination.** • **The controls were recruited from the same department as the cases. The controls were healthy men who had not been diagnosed with testicular cancer or any other cancer at the time of the study. However, the controls could develop testicular cancer after the study. The study did no such follow-up.** • **The study does not mention any non-responders** • **Cases-controls were balanced for age, BMI and recidence.** • **The main exposure, organochloride pesticides, were measured in blood serum and to assess exposure in daily life the cases and controls were interveiwed. This is not ideal to investigate whether the individuals have been exposed prior to the cancer diagnosis. The individual might answer incorrectly to how much they actually have been exposed to the chemicals and other.** • **The two groups have been treated the same in the study and exposures have been graded the same.** • **The study says nothing about blinding in the study and the interview was performed face-to-face.** • **It is a too little sample and too many uncertainties about the study that has not been accounted for that makes it challanging to conclude from the study and use the findings. More research is needed** • **Other studies support the results of this study** **What the autours discuss as:** • **Strengths:** balancing of cases and controls, cases were histologically confirmed, analyses were performed in the same laboratory and collection of blood was made during the same period for cases and controls • **Weaknesses:** serum consentrations of DDE and HCB were lower than those found in earlier studies from some Nordic countries, low power of the study due to rarity of the tumour The results may have biological confounding factors as there were more reproductive birth defects in cases than controls (P<0,01). This is supported by litterature

Reference: Barbara A. Cohn MPH, MCP, PhD, Piera M. Cirillo MPH & Roberta E. Christianson MA (2010) **Prenatal DDT Exposure and Testicular Cancer: A Nested Case-Control Study**, Archives of Environmental & Occupational Health, 65:3, 127-134, DOI: 10.1080/19338241003730887			**Study design: Case-control** **Grade - quality** **Low**
Aim	**Materials and method**	**Results**	**Discussion/Comments/Check list**
To investigated association between prenatal exposure to DDT and testicular cancer **Conclusion** mothers of sons with testicular cancer had a higher DDT/DDE ratio, but lower o,p'-DDT, but the number of cases in this study is too small to conclude from this. This gives the study low statistical power. **Country** USA **Year of collecting data** 1959-1967	**Population:** Participants were sons of women who participated in the Child Health and Development Studies (CHDS). The women included were members of the Kaiser Foundation Health Plan, residing in the Oakland, California area, and pregnant between 1959 and 1967. **-cases:** participants in the CHDS who develpoed testicular cancer later in life.. **-controls:** participants in the CHDS who did *not* develop testicular cancer **Main exposure:** DDT **Important cofunding factors:** - Race - Recidence - Years of birth **Statistical methods:** P-value was obtained by comparing the signed rank statistiv to its permutation distribution. The association between risk factors and case/control status were analyzed using exact conditional logistic regression.	**Key findings** mothers of sons with testicular cancer had a higher DDT/DDE ratio, but lower o,p'-DDT.	**Check list:** • **The aim is clearly formulated** • **The case-control study design is suited for the study** • **The laboratory was blinded as to case status of the samples** • **The participants were recruited over the same timeperiod and from the same area and development study.** • **False positives are highly unlikey, particular for testicular cancer where the diagnosis is made by examination of a biological specimen** • **Cases lacking maternal pregnancy or postpartum serum were excluded (N=5)** • **Cases-controls were balanced for year of birth and race.** • **The main exposure, DDT, were measured in maternal blood serum, which was collected during each semester of pregnancy and the early postpartum, 1 to 3 days after delivery.** • **The two groups have been treated the same in the study.** • **Controls were selected at random from the pool of matches (on race and year of birth) if more than three were available** • **It is highly unlikely that the controls would develop TC later** • **The study was not able to control for multiple confunding factors** • **It is a too little sample and too many uncertainties about the study that has not been accounted for that makes it challanging to conclude from the study and use the findings. More research is needed** • **Other studies could explain the metabolism of DDT, but there are to this day very few studies on how this affects the human body. Although, some studies show correlation to DDT and malignancies** **What the autours discuss as:** • **Strengths:** a sizeable source population • **Weaknesses:** Conclusions drawn from this study are limited to detection of sizable effects due to the rarity of testicular cancer and the small number of cases observed over four decades. Unable to control for multiple confounding factors due to sample size limitations.

Reference: Feng Y, Shi J, Jiao Z, Duan H, Shao B.**Mechanism of bisphenol AF-induced progesterone inhibition inhuman chorionic gonadotrophin-stimulated mouse Leydigtumor cell line (mLTC-1) cells**.Environmental Toxicology.2018;33:670–678. https://doi.org/10.1002/tox.22554			**Study design: Cohort study** Grade - quality: Moderate
Aim	**Material and methode**	**Results**	**Discussion/comments/checklist**
To investigate the mechanism of bisphenol AF (BPAF) on hCG-stimulated mLTC-1 cells **Conclusion** BPAF exposure inhibits progesterone secretion in hCG-stimulated mLTC-1 cells by reducing expression of scavenger receptor class B type I (SR-B1) and cytochrome P450 (P450scc) due to the adverse effects of cAMP. BPAF is a potantial endocrine disruptor in testicular Leydig cells. **Country** China **Years of collection data** Not specified in the article (around 2017)	**Population:** mLTC-1 cell line **Cohorts:** cells were exposed to 0, 0.1, 1, 10, 30, 50 and 70μM BPFA for 24 hours. **Main outcome:** progesterone secretion was reduced in cells exposed to BPAF **Main confunding factors:** The cells are from the same cell line **Statistical methods:** The results are presented as the mean ±S.E.M. (n53). Statistical analyzes of hormone production and gene transcription profiles between the control and exposed cells were evaluated by one-way analysis of variance, followed by post-hoc least significant difference tests. P-value<0,05 was considered statistically significant. The IC_{50} value of BPAF with regard to progesterone production was determined using Hill regression of the dichotomous model with Benchmark Dose Software.	**Key findings:** **BPAF reduce for progesterone production in mLTC-1 cells** **Additional findings:** • The expression levels of proteins involved in progesterone biosynthesis were measured. The results demonstrated that the levels of SR-B1 and P450scc protein expression were strongly decreased in cells exposed to 50 μM and 70 μM BPAF compared with that in control cells ($P < .01$). However, the levels of StAR were not altered with BPAF exposure at any concentration • In the 22R-HC treatment group, the progesterone levels were still dramatically decreased with exposure to higher doses of BPAF, by 47.9% and 57.3% at 50 μM and 70 μM of BPAF, respectively • BPAF, at any concentration, did not affect the mRNA levels of PBR and 3β-HSD • cAMP production was significantly decreased with treatment at high doses.	**Check list:** • The purpose is clearly stated? Yes • Are the groups recruited from the same population? (selection bias) Yes • Were the groups comparable to important background factors? (selection bias) * Yes • Were the exposed individuals representative for a defined population? Yes • Were exposure and outcome measured equally and reliably (validated) in the two groups? (Classification bias) Yes • Is the person who evaluated the results (endpoints) blind to group affiliation? Not specified in the study • Was the study prospectiv? Yes, over 24 hours • Were many enough individuals in the cohort followed up? (Attrition bias / follow-up bias) The cells were grown in 6-well plates at a density of 1.25 x 10^6 cell/well. • Has dropout analyzes been performed? (Eval. attrition bias) Not relevant • Was the follow-up time long enough to detect positive and / or negative outcomes? Yes • Have important confounding factors been taken into account in design / execution / analysis? Yes • Do you believe in the results? Yes • Is there other litterature that strengthen or weaken the results of the study? There are studies that support the finding that BPFA is an endocrine disruptor, but the exact mechanism has not been as thoroughly described before. • What does the results mean for change of practice? BPFA is shown to be a possible endocrine disruptor in testicular Leydig cells. These findings should be further investigated whether exposure has an effect on humans. **The autors discussed:** • **The autours do not discuss any weaknesses og strengths about the study. In the discussion-part they only discuss the findings of the study.**

Reference: Pei-Li Yao, Yi-Chen Lin, John H. Richburg **Mono-(2-Ethylhexyl) Phthalate (MEHP) Promotes Invasion and Migration of Human Testicular Embryonal Carcinoma Cells.** Biology of Reproduction, Volume 86, Issue 5, 1 May 2012, 160, 1-10, https://doi.org/10.1095/biolreprod.111.097295			**Study design: Cohort study** Grade - quality: Moderate
Aim	**Material and methode**	**Results**	**Discussion/comments/checklist**
Investigate the effect of MEHP on stimulation of cell invasion and migration in testicular embryonal carcinoma cells. **Conclusion** The findings of the current study indicate that cellular mechanisms triggered by MEHP exposure act to enchance tumor progression/metastasis in testicular embryonal carcinoma cells (NT2/D1). **Country** USA **Years of collection data** 2011	**Population:** Human testicular embryonal cell line NTERA-2 cl. D1 (NT2/D1) **Cohorts:** NT2/D1 cells were treated with designated concentrations of MEHP diluted in dimetyl sulfoxide (DMSO) for various time periods. Cells treated with DMSO were used as control groups. **Main outcome:** MEHP exposure proved to have multiple effects on NT2/D1 cells, indicating that exposure may enhance tumor progression/metastasis in these cells. **Main confunding factors:** Cells from the same cell line and both were treated with DMSO **Statistical methods:** All experiments were performed in triplicate and repeated using three independent sets of cell preparations. - Data were subjected to Student *t*-test or a parametric one-way ANOVA followed by the Tukey test for post hoc comparisons. - Statistical significance when P<0.05	**Key findings:** Multiple cellular mechanisms triggered by MEHP indicate that exposure may act to enhance tumor progression/metastasis in testicular embryonal carcinoma cells. **Additional findings:** • MEHP exposure gave the cells stronger ability to digest and pass through the Matrigel, likely to increased activation of MMP. • MEHP exposure leads to decrease in GJA1 (a gene that codes for Cx43 protein, forming the main structure of the gap junction) mRNA expression in NT2/D1 cells. • MEHP exposure reduces vinculin mRNA level in testicular cancer cells (important for maintainingthe structure of adherens junctin through interaction with actin filament and integrin)	**Check list:** • The purpose is clearly stated? Yes • Are the groups recruited from the same population? (selection bias) Yes • Were the groups comparable to important background factors? (selection bias) * Yes • Were the exposed individuals representative for a defined population? Yes, it's a human cell line. • Were exposure and outcome measured equally and reliably (validated) in the two groups? (Classification bias) Yes • Is the person who evaluated the results (endpoints) blind to group affiliation? Not specified in the study • Was the study prospectiv? Yes, cell RNA were isolated after 0, 3 and 24 hours of MEHP exposure. • Were many enough individuals in the cohort followed up? (Attrition bias / follow-up bias) Has dropout analyzes been performed? (Eval. attrition bias) Not relevant • Was the follow-up time long enough to detect positive and / or negative outcomes? Not relevant • Have important confounding factors been taken into account in design / execution / analysis? Yes • Do you believe in the results? Yes • Is there other litterature that strengthen or weaken the results of the study? Studies show that theese mechanisms could be tumor promoting. • What does the results mean for change of practice? The findings of this study show that cellular mechanisms triggered by MEHP exposure act to enhance tumor progression/metastasis in testicular embryonal carcinoma cells (NT2/D1). This should be further investigated in humans to see whether these effects are reproducable in vivo. **The autors discussed:** • **The autours do not discuss any weaknesses og strengths about the study. In the discussion-part they only discuss the findings of the study.**

Reference: Frost, G., Brown, T., Harding, A.H., **Mortality and cancer incidence among British agricultural pesticide users.** Occup Med (Lond). 2011 Aug;61(5):303-10. doi: 10.1093/occmed/kqr067.			**Study design: Cohort study** Grade - quality: Moderate
Aim	**Material and methode**	**Results**	**Discussion/comments/checklist**
To compare mortality and cancer incidence experienced by a cohort of British pesticide users to that of the Great Britain population. **Conclusion** This study suggests that pesticide users in the Pesticide User Health Study are generally healthier than the national population but may have excesses of non-melanoma skin cancer, testicular cancer and multiple myeloma. **Country** United Kingdom **Years of collection data** Between 1987-2004 for incidence and 2005 for mortality.	**Population:** All individuals with Certificates of Competence issued by City & Guilds Land Based Services since 1987 are eligible for inclusion in the PUHS. Those who consent to give their details to HSE are included in the study. **Cohorts:** Individuals on the PUHS are flagged for cancer and death registrations at the National Health Service Central Register (NHSCR) for England and Wales and the General Register Office for Scotland (GROS). Notifications were received quarterly from the National Health Service Information Centre. The HSE Research Ethics Committee approved the study **Main outcome:** cancer incidence and mortality **Main confunding factors:** Pesticide use and the cancer incidens and mortality **Statistical methods:** Standardized mortality ratios (SMRs) and Standardized incidence ratios (SIRs) were used to compare mortality and cancer incidence (respectively) among the pesticide users to that observed in the GB population	**Key findings:** All-cause mortality was lower for both men [SMR 0.58, 95% confidence interval (CI) 0.55-0.60] and women (SMR 0.71, 95% CI 0.52-0.98) compared to the GB population. Mortality and incidence were below those expected for all cancers combined among men (SMR 0.71, 95% CI 0.66-0.77; SIR 0.85, 95% CI 0.81-0.90), particularly for cancers of the lip, oral cavity and pharynx, digestive organs and respiratory system. The incidence of testicular cancer, non-melanoma skin cancer and multiple myeloma were above expected. For the incidence of testicular cancer the CI was 1.04–1.53. **Additional findings:** Mortality from injury by machinery was significantly above expected for men (SMR 4.21, 95% CI 2.11-8.42).	**Check list:** • The purpose is clearly stated? Yes • Are the groups recruited from the same population? (selection bias) Yes • Were the groups comparable to important background factors? (selection bias) * Uncertain • Were the exposed individuals representative for a defined population? Yes • Were exposure and outcome measured equally and reliably (validated) in the two groups? (Classification bias) Yes • Is the person who evaluated the results (endpoints) blind to group affiliation? Not specified in the study • Was the study prospectiv? Yes • Were many enough individuals in the cohort followed up? (Attrition bias / follow-up bias) • Has dropout analyzes been performed? (Eval. attrition bias) • Was the follow-up time long enough to detect positive and / or negative outcomes? • Have important confounding factors been taken into account in design / execution / analysis? • Do you believe in the results? Yes • Is there other litterature that strengthen or weaken the results of the study? Yes • What does the results mean for change of practice? No **The authors discussed:** • **Strengths:** Members of the PUHS were recruited from all those who have passed Certificates of Competence in applying agricultural pesticides since 1987, so the cohort should be representative of those using agricultural pesticides in GB. • **Weaknesses:** The database lacks information on potential confounding factors, such as smoking history, physical activity and hours spent outdoors, which would need to be collected through additional research. Information held by the issuer's database is restricted to the information provided at the time of application for the certificate for applying agricultural pesticides. Individuals may use agricultural pesticides on a commercial basis without certification