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A Systematic Review and Meta-Analysis on Night Shift Work and Breast Cancer Risk for Female Employees

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By

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BA
University of North Carolina at Chapel Hill
2010

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An abstract of
A thesis submitted to the Faculty of the
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2013

Abstract

A Systematic Review and Meta-Analysis on Night Shift Work and Breast Cancer Risk for Female Employees

By Christina Wu

Background: In 2007, the International Agency for Research on Cancer (IARC) came to the conclusion based on "limited evidence for humans on the carcinogenicity of shift work that involves night shift work" that "shift work [that] involves circadian disruption is probably carcinogenic to humans." Breast cancer is one of the most common forms of cancer for women in Western societies where up to 15 to 20% of the total workforce is involved in night shift work. Consequently, female employees that have night shift work may have an increased risk of breast cancer.

Objectives: This study reviewed case-control and cohort studies on night shift work and breast cancer risk. It will assess if current research is conclusive on the association between night shift work and increased breast cancer risk amongst female night shift workers.

Methods: A systematic review and meta-analysis was performed on previous research studies measuring the association between night shift work and breast cancer risk. Study characteristics were extracted independently from each study. Weighted mean effect sizes, using both fixed- and random effects models, were calculated; greater effect sizes indicated an increased risk of breast cancer associated with night shift work. Heterogeneity between studies was also evaluated.

Results: Fixed- and random effects models found a significantly elevated breast cancer risk among female night shift workers in case-control studies and only a slightly elevated breast cancer risk in cohort studies. Case-control studies were found to be moderately homogeneous whereas cohort studies showed significant heterogeneity. The difference in aggregated breast cancer risk and heterogeneity between the two types of studies may be a consequence of the varying definitions for night shift work exposure and the differences in sample population.

Conclusions: Although this study suggests that night shift work may increase the risk of breast cancer, the association between night shift work and breast cancer risk is still inconclusive due to the limited evidence. The possible increase in the risk of breast cancer observed in the meta-analysis is not robust enough due to some heterogeneity observed in the case-control studies and the great variability and heterogeneity observed in the cohort studies.

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Tables, Figures, and Equations

1. INTRODUCTION:

1.1. Background

Breast cancer is one of the most common forms of cancer for women in Western societies, with the incidence rates consistently increasing in the past few decades. As early as 1987, Richard G. Stevens "hypothesized that exposure to light at night not only suppressed melatonin production but it also paralleled an increase in estrogen levels."[2] This, in turn may "induce a higher breast cancer risk among women who are frequently exposed to light at night"[2] in his article, "Electric Power Use and Breast Cancer: A Hypothesis," his hypothesis was based on experimental evidence that displayed an effect of light and extremely low frequency electric and/or magnetic (ELF) fields on pineal melatonin production, and on the relationship of melatonin to mammary carcinogenesis.[16]

According to Stevens, there is a discernible difference between breast cancer incidence and mortality across different populations. Incidence rates of breast cancer are lower in Africa and Asia, intermediate in southern Europe and South America, and highest in northern Europe and North America.[16] However, Stevens did note in his 1987 article that the rates for Japanese women were "one-fifth the rate of US women" but the "rates were rising fast." Additionally in the 50 years prior to his 1987 article, Iceland's rates were stated to have risen from the lower level to a level "approaching that of Connecticut."[16] For Stevens, he believes that race is not a factor in the geographic variation in breast cancer incidence rates. Instead he believes that "westernization may be the cause of this variation in rates."[16]

In an earlier early study conducted by M. Cohen, et al. in 1978, Cohen suggested that "reduced pineal melatonin production might increase human breast cancer risk," where "environmental lighting" was one of the factors that would accomplish this.[16] As

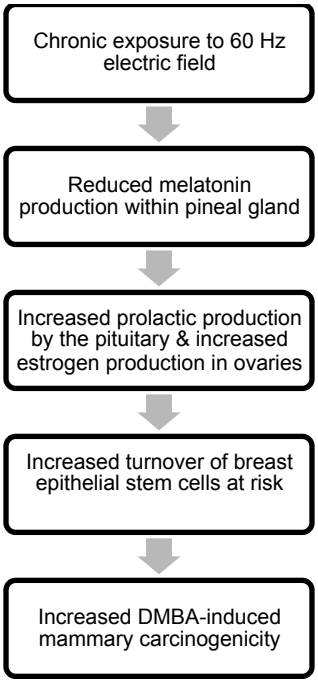
electricity is used to provide light at night, electric power increases with further westernization. This is because "high electric-use communities" like metropolitan centers do not have nights as dark as rural areas and incidentally breast cancer mortality rates are higher in urban areas than in rural areas.[16] In more recent years it is still hypothesized that the increasing risk of breast cancer observed in industrialized societies was partly due to the use of electrical lighting at night.[1]

Indirect evidence from previously observational studies on flight attendants have also suggested that there was an association between melatonin suppression and breast cancer risk. The original rationale for the studies on flight attendants was based on the assumption that their occupational exposure to cosmic radiation caused in excess in cancer risk. Subsequently, it was also assumed that the increase in breast cancer risk could also be due to melatonin deficiency resulting from work exposure to light at night.[15] It was assumed that light at night could possibly suppress melatonin output and increase estrogen levels, increasing the risk of breast cancer.[1]

In fact, the pineal gland secretion of melatonin displays a distinct circadian rhythm.[16] Melatonin is a "hormone of the dark" which is produced primarily at night. It is part of a circadian rhythm that is heavily determined by the day/night light exposure. Melatonin production tends to peak towards the middle of night and has relatively lower circulating levels during the day.[2] Based on the experimental evidence conducted on rodents there is a suggestion that constant light had an effect on mammary tumorigenesis, the production or formation of a tumor or tumors. In Stevens' article it was found that chronic exposure to a 60 Hz electric field suppressed the normal nocturnal rise of pineal melatonin in rats. This reduced melatonin lead to an increase in

dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis within the rats.[16] This is due to the pineal gland is transposing the light stimuli of the retina into a hormonal response of the sex glands. This melatonin formed by the pineal gland suppresses prolactin production by the pituitary and estrogen produced by the ovary.[16] Figure 1 displays a summary outline of the events that follow rats are exposed to chronic exposures of a 60 Hz electric field.

Figure 1 - Mechanisms by which chronic exposure to a 60 Hz electric field leads to an increased DMBA-induced mammary carcinogenesis in rats



In B.W. Wilson's study, 56-day old male rats were exposed to 60 Hz electric fields at constant strengths between 1.5 and 65 kV/m for 20 hours a day for 30 days. At the end of the 30 days the animals were killed in groups of 10 at four different times in the light-dark cycle of the day.[16] The rats' pineal glands were removed and assayed for melatonin and *N*-acetyl transferase (NAT), a rate limiting enzyme during the synthesis of melatonin from serotonin. Wilson's results declared that the rats that were exposed did

not exhibit the normal nocturnal rise in pineal melatonin levels and there was a significant reduction in melatonin in exposed rats compared to control rats.[16]

Further evidence was shown in L. Tamarkin et al.'s study that concluded melatonin affected DMBA mammary carcinogenicity. In his study, Tamarkin administered 15 mg of DMBA to 50-day old rats divided into four groups: a control group 1, DMBA plus daily vehicle injection of four percent ethanol in a phosphate-buffered saline; group 2, DMBA plus daily melatonin injections beginning at day 50; group 3, DMBA plus the removal of the pineal gland; and group 4, DMBA plus the removal of the pineal gland but daily melatonin injections. Group 2 had significantly less mammary tumors than the control group 1. This indicated that melatonin inhibited carcinogenesis. Group 3 had more tumors than group 1, indicating that the complete removal of the pineal gland further enhanced carcinogenesis. Group 4 had fewer tumors than groups 1 and 3. This showed that melatonin at least alleviated the adverse affects of the complete removal of the pineal gland altogether.[16]

P.N. Shah et al.'s study concluded that light suppresses melatonin production by the pineal gland. Shah et al. used "constant-light exposure" from birth to simulate the removal of the pineal gland in rats. The rats exposed to "constant-light" displayed significantly greater DMBA mammary tumors than rats on a normal light-dark cycle. Shah et al. also found that melatonin-treated rats with intact pineal glands showed lower plasma estradiol and prolactin levels than the vehicle-treated rats. Both Shah et al.'s study and another study by MC Mhatre et al. concluded that "constant-light" from birth effectively deprived female rats of melatonin, leading to a constant availability of estrogen and elevated circulatory prolactin. This constant availability of both hormones

would then increase the turnover of breast epithelial cells, making the breast tissue more susceptible to DMBA carcinogenicity.[16]

Various other epidemiologic results from highly-industrialized societies, the highest in risk for breast cancer, have also noted that light at night suppressed melatonin levels and thus contributed to an escalatd breast cancer risk due to an increase of estrogen levels.[1] This exposure to artificial lighting at night and the suppression of melatonin could also possibly inhibit tumor anti-proliferative mechanisms.[3] Cohen et al.'s study concluded that "reduced pineal function (in humans) may increase breast cancer incidence because lower melatonin output would lead to an increase in circulating estrogen levels, and stimulate proliferation of breast tissues."[16]

The hypothesis of light at night suppressing melatonin output has evolved to a more complex question of whether or not light at night disrupted the circadian rhythm and the interaction with clock genes. These genes drive the circadian rhythm and are the central players in gene regulation throughout an organism, particularly for life-cycle regulatory genes and the genes of cell death.[17] Circadian rhythms are controlled by a select group of eight genes that exert control over key cell-cycle checkpoint genes and cell death genes. However, circadian rhythms can be easily disrupted by ill-timed artificial lighting and by the lack of sunlight.[17]

"Clock genes," circadian rhythm genes or circadian genes, have become a hot topic for individuals in the circadian and cancer research communities especially in areas pertaining to the "relation of the circadian genes in the master circadian pacemaker of the suprachiasmatic nuclei to the peripheral clock mechanism in cells and tissues." The suprachiasmatic nuclei are responsible for controlling the circadian rhythm. Many

researchers are also interested in the circadian genes in the "master circadian pacemaker" that controls the expression of a wide variety of genes, in particular those for cell-cycle regulation and cell death.[17] Thus far there has been eight core circadian genes identified: *Clock, casein kinase le (CKle), cryptochrome 1 (Cry1)* and *cryptochrome 2 (Cry2), Period1 (Per1), Period2 (Per2),* and *Period3 (Per3),* and *Bmall*.[17]

Phototransduction from the retina causes neuronal signaling to the suprachiasmatic nuclei (SCN). From the SCN there may be clock genes that can control the neuroendocrine transduction (of the pituitary, ovary, and pineal glands) that can affect hormones relevant to an increased breast cancer risk such as those associated with melatonin receptors. In addition, the SCN may also have clock genes that can cause a direct effect on the cell-cycle regulatory genes in the mammary tissue leading to an increased breast cancer risk. Through both mechanisms, the mammary tissue may have altered cell proliferation and tissue development.[17]

There also have been a limited number of epidemiological studies' directed towards examining polymorphisms in clock-related genes and phenotype expressions such as morning/evening preference. Although there have been reports that a polymorphism in the *Clock* gene is associated with the morning/evening preference as assessed by the Horne-Östberg scale, a widely used questionnaire to determine diurnal preference.[17] In addition, reports on *Per3* polymorphisms have been associated with delayed sleep-phase syndrome or diurnal preference that also used the Horne-Östberg scale.[17] This diurnal preference may predict tolerance to evening or overnight shift work and thus be related to melatonin levels; for example, individuals who are considered to be "morning types" may be less tolerant to such types of evening or overnight shift work,[17]

but without further study it is unsure if this may be a factor in an individuals risk of breast cancer in association with her night shift work.

1.2. Significance

More recently, the International Agency for Research on Cancer (IARC) assessed the possible association between night shift work and cancer. In 2007, the IARC concluded that "shift work [that] involves circadian disruption is probably carcinogenic to humans."[4] Such a hypothesis could lead to dire repercussions, especially for Western societies where 15 to 20% of the total workforce is estimated to be involved in night shift work and other types of shift work.[5] Even societies in developing countries where night shift work may be increasing for employees could potentially be at risk. In fact, approximately 429,000 European women were diagnosed with breast cancer in 2006. Roughly 20% of employees or the self-employed in the European Union have worked at least one night a month, between 10:00PM and 5:00AM, 10% work one to five nights a month, and even 10% have worked greater than five nights a month.[1] As much as 0.4% of those with occupations in the European Union have permanent night shift work. Night shift work appears to be the most prevalent in agriculture, hotels, restaurants, transport, communication, and health industries.[1] Breast cancer, incidentally, has also become the most common form of cancer in Europe, making up 13.5% of all cancer cases in 2006.[1] Fifty percent of these breast cancer cases can be attributed to known risk factors such as prolonged exposure to endogenous and exogenous female sex hormones, alcohol, obesity, and excessive weight gain.[1]

The IARC came to their conclusion "on the basis of limited evidence for humans on the carcinogenicity of shift work that involves night shift work and sufficient evidence

on experiments on animals for the carcinogenicity of light during the daily dark period (biological night).[4]" The IARC working group assessed human data based on the results from eight epidemiological studies: Hansen (2001), Schernhammer (2001), Schernhammer (2006), O'Leary (2006), Lie (2006), Tynes (1996), Davis (2006), and Schwartzbaum (2007). Amongst these studies, two of the most recently published studies, Davis (2006) and Schwartzbaum (2007), reported no increased breast cancer risk.[4] However, two large cohort studies, Schernhammer (2001) and Schernhammer (2006), based on the United States Nurses' Health Study I and II, observed that breast cancer risk increased with increasing number of night shifts.[4]

However, the shortcomings in study design, lack of control for potential confounders, and crude assessment in shift characteristics and definitions for night work in the IARC's evaluation of the eight epidemiological studies did pose as limitations for their conclusion.[4] In Megdal's 2005 systematic review and meta-analysis, night shift work was defined broadly as "any shift schedule that included overnight work" based on the studies reviewed.[16] In addition, in Megdal's systematic review and meta-analysis, Megdal found that the incomplete adjustment for confounding remained a large limitation in a majority of the studies. Some studies would only be adjusted for one confounder and disregard other possible confounders considered in other studies. The assessment of employment time or night shift work exposure may have been misclassified because it was based on crude estimates. This misclassification may have led to the decrease in the effect measurements between night shift work and breast cancer.[15]

According to the IARC statement, studies on the association between night shift work and breast cancer risk for humans have been "limited." But there is substantial and

"sufficient" evidence conducted on animal models based on the carcinogenicity of light exposure during the biological night period. Previous human studies assessing the association between night shift work and breast cancer have mostly consisted of cohort or case-control studies. There have also been several cross-sectional studies and other forms of observational studies based on this topic. Many of the studies that have been conducted dealing with the association of night shift work and breast cancer risk increase are comprehensive biomarker studies measuring the metabolite levels of melatonin in blood and urine samples.

The previous systematic and critical review conducted on this topic only accounted for one or a few confounders. This review will account for a larger number of potential confounders that are commonly found with breast cancer risk studies such as parity, family history of breast cancer incidence, hormone replacement therapy use, age, alcohol use, and BMI. This review will also strive to determine a more specific definition of night shift work based on the studies assessed as well as include more recent studies than those that have been reviewed before on this topic.

1.3. Research Objective

This study reviewed case-control and cohort studies on night shift work and breast cancer risk. It will assess if current research is conclusive on the association between night shift work and increased breast cancer risk amongst female night shift workers.

1.4. Aim and Hypothesis

The main aim of this project is to evaluate if there is an association between night shift work and breast cancer based on previously conducted research studies or if further research must be conducted in this area to better ascertain an association.

Female employees who have night shift work will have an increased risk oft breast cancer when compared to female employees who do not have night shift work.

2. METHODS:

2.1. PRISMA Statement

PRISMA stands for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. The aim of the PRISMA Statement is to improve the reporting of systematic reviews and meta-analyses. It focuses on ways in which authors can ensure transparent and complete reporting of systematic reviews and meta-analyses. The PRISMA Statement[23] consists of a 27-item checklist and a four-phase flow diagram. In addition, the PRISMA Explanation and Elaboration Paper[24] was developed that included examples that highlight how to best report each checklist item, and identifying a comprehensive evidence base to support the inclusion of each checklist item.

The reporting for this systematic review and meta-analysis will follow the guidelines established by the PRISMA Statement in order to allow for as much transparency and reciprocity as possible. As a result, the format in which the results are reported and discussed will also follow the PRISMA Statement. However, there are some items on their checklist and Explanation and Elaboration Paper that were not applicable and were thus not included.

2.2. Searching strategy

Studies were identified through searching electronic databases and scanning reference lists of articles for any epidemiologic literature applicable to night shift work and breast cancer risk from PUBMED and Thomson Reuter's Web of Science from the

June 2012 to April 2013. Limits were placed for language (English only), publication date (within the last 15 years), and for human studies only. Search terms used on each electronic database are listed in Table 1.

Table 1 - Search terms used on electronic databases (PUBMED and Web of Science)

Search Terms
1. *night shift AND breast cancer*
2. *rotating night shift AND breast cancer*
3. *night shift AND cancer*
4. *night shift AND carcinoma*
5. *circadian disruption AND breast cancer*
6. *melatonin AND breast cancer*
7. *breast cancer neoplasm AND night shift*
8. *breast cancer neoplasm AND work schedule tolerance*
9. *breast cancer epidemiology AND night shift*
10. *breast cancer epidemiology AND work schedule tolerance*
11. *breast cancer incidence AND night shift*
12. *breast cancer incidence AND work schedule tolerance.*

Eligibility assessment and screening on the risk of bias in the studies was performed solely and independently using the Newcastle-Ottawa Quality Assessment Scales for case-control and cohort studies. A copy of the Newcastle-Ottawa Quality Assessment Scales for case-control and cohort studies can be found in Appendix A.

Data extraction from each study was also conducted solely and independently. Information gathered from each study included: year of publication, type of study, location, number of participants (cases and controls/total population in cohort), measure of association, 95% confidence interval, and any potentially confounding covariates. This information can be found on Table 3 and 4 in the Results section.

2.3. Eligible studies

Observational studies that studied any type of night shift work and breast cancer risk were included. No restrictions were placed on eligible studies in regards to place of

origin, race of female population, or occupation. It was a likely a coincidence that the majority of the occupations for the female night shift workers tended to be nurses because many of the studies chose to extract data from populations from previous studies or registries specifically for nurses. Observational studies were included if they reported measures of associations for breast cancer risk in relation to night shift work. This included odds ratios, risk ratios, and hazard ratios.

2.4. Ineligible studies

Any animal studies were excluded as well as any reviews of previous studies conducted. However, reviews of previous studies were used as additional reference sources and as background information sources for the purposes of this document. Studies that were not published or translated into English or published in the last fifteen years were not included into the systematic review and meta-analysis. Additionally, studies that did not provide a direct measure of association for breast cancer were also excluded. Studies that did not directly address or answer in some part the problem statement were also excluded from further analysis.

2.5. Definitions

The outcome of this systematic review and meta-analysis was histologically confirmed breast cancer. For the purposes of this review, “night shift work” was defined as any shift schedule that included working between the hours of 9:00PM to 7:00AM that could potentially disrupt the employee’s circadian rhythm and sleep patterns. This definition also would include any shifts that included overnight shift work or graveyard shift work.

2.6. Effect measures

Adjusted effect measures were used in the analysis where they were included in the source studies, under the assumption that adjustment was performed to remove bias in the estimate of the association between the exposure to night shift work and the risk of breast cancer. A majority of the case-control studies and cohort studies used odds ratios or risk ratios respectively. One cohort study reported hazard ratios. The Pronk[5] study reported hazard ratios and its confidence intervals but as a result of the proportional hazard assumption, it was assumed that the hazard ratios reported could be considered almost equal or synonymous to risk ratios. In the proportional hazard model, it is assumed that "changing a stress variable (or explanatory variable) has the effect of multiplying the hazard rate by a constant."[18] Based on consultation with Dr. Matthew Strickland of the Environmental Health Department of the Rollins School of Public Health at Emory University, he has stated that "fundamentally a hazard ratio and a risk ratio are different…the proportional hazards assumption lets you go from hazard ratios to rate (risk) ratios. But there is really no way to get from hazard ratios to risk ratios unless disease is rare." Breast cancer as a disease is rare enough (accounting for 22.9% of all cancers in women[19]) that both assumptions could apply in order for the hazard ratios presented in Pronk to be considered as risk ratios for the meta-analysis. Table 2 and Table 3 both include a column to show what risk measure was used in the analysis.

2.7. Statistical analysis

2.7.1. Calculating effect sizes

All effect measures obtained from original studies were for dichotomous data so only odds ratios or risk ratios were used to calculate effect sizes depending on what type

of study was conducted. This analysis only focused on case-control and cohort studies. A separate meta-analysis will be conducted for each type of study. Information provided in each study pertaining to the number of cases of breast cancer for night shift workers (disease+exposed) or the number of cases of breast cancer for non-night shift workers (disease+unexposed) versus the number of those who worked night shifts but did not develop breast cancer (no disease+exposed) or the number of those who never worked night shifts or developed breast cancer (no disease+unexposed) were used to create 2x2 tables for both case-control and cohort studies.

Odds ratios and risk ratios range from zero to infinity. When the null value equals 1, the odds ratio or risk ratios represent no effect based on exposure. This is not symmetric and represents a multiplicative scale. To correct this, both the odds ratios and risk ratios were log-transformed using the natural log function. This allows the effect measures to become more symmetric, ranging from negative infinity to infinity with a null value of zero. Log-transforming the effect measures also puts them into an additive scale, a necessity for the meta-analysis.

Variances of the log odds ratios (LnOR) and log risk ratios (LnRR) were calculated to determine the relative weights of the studies.

Equation 1 - Calculating the variance of LnOR

$$Var(LnOR) \approx \frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}$$

Equation 2 - Calculating the variance of LnRR

$$Var(LnRR) \approx \frac{b}{a(a+b)} + \frac{d}{c(c+d)}$$

The weight of a study in the meta-analysis is calculated by taking the inverse of the variance. The more subjects in a study the lower the variance and leading to an

increased weight in the meta-analysis. In order to find the relative weights (as a percentage) the sum of the weights for each type of study was calculated (cohort and case-control) separately, then each individual study's weight was divided by the total weight and multiplied by 100.

Equation 3 - Calculating the weight

$$w = 1/Var(LnOR) \text{ or } 1/Var(LnRR)$$

Equation 4 - Calculating the relative weight

$$RelativeWeight = \left[1/Var(LnOR)\right] \times 100 \text{ or } \left[1/Var(LnRR)\right] \times 100$$

The standard error for the LnOR and LnRR of each study was also calculated using Equation 5 and 6 respectively. The 95% confidence intervals for both the LnOR and LnRR were also calculated. The 95% confidence interval is the log-transformed effect measure plus or minus 1.96 multiplied by the standard error for each study.

Equation 5 - Calculating the standard error of LnOR

$$SE(LnOR) = \sqrt{Var(LnOR)}$$

Equation 6 - Calculating the standard error of LnRR

$$SE(LnRR) = \sqrt{\left[1/a + 1/c\right] - \left[1/(a+b) + 1/(c+d)\right]}$$

Equation 7 - Calculating the 95% confidence interval

$$95\%CI = LnOR \pm 1.96 \times SE(LnOR) \text{ or } LnRR \pm 1.96 \times SE(LnRR)$$

Effect sizes were calculated by multiplying the weight by the log-transformed effect measure. Since none of the studies selected for meta-analysis through the systematic review performed follow up, no baseline adjustments for the effect sizes were performed. All effect size measurements were calculated using Microsoft Excel.

Equation 8 - Calculating the effect size

$$E = LnOR \times (1/Var(LnOR)) \text{ or } LnRR \times (1/Var(LnRR))$$

2.7.2. Testing for heterogeneity

To test for heterogeneity, fixed effects and random effects were conducted. All calculations were conducted through Microsoft Excel. In a fixed effects analysis, the combined effect measurement (either a combined OR measurement or a combined RR measurement) is given by the weighted average of the observed effect from each individual study as shown in Equation 9. The combined effect variance is given by the inverse of the sums of all the weights. The combined effect standard error and 95% confidence intervals were also calculated using the same equations for each study but applied to the combined effect measurement calculations.

Equation 9 - Calculating the combined effect measurement

$$E_C = \frac{\sum wE}{\sum w}$$

Equation 10 - Calculating the combined effect variance

$$Var_C = \frac{1}{\sum w}$$

The homogeneity statistic, Q, was also computed. The Q statistic assesses whether there is true heterogeneity in the meta-analysis. It is the summing of the standard deviations of each study's effect estimate from the overall effect estimate, weighting the distribution of each study by its inverse variance (Equation 11).[20] Not rejecting the homogeneity hypothesis usually leads the meta-analyst to adopt a fixed-effects model because it is assumed that the estimated effect sizes only differ by sampling error. On the other hand, if the studies are found to be heterogeneous, random effects model that includes both within- and between-studies variability can be applied.

Equation 11 - Calculating the Q statistic

$$Q = \sum[w(LnOR)^2] - \frac{E^2}{\sum w} \text{ or } \sum[w(LnRR)^2] - \frac{E^2}{\sum w}$$

Another test for quantifying the heterogeneity in a meta-analysis consists of estimating the between-studies variance, T^2 (tau squared), assuming a random effects model; the between-studies variance reflects how much the population effect sizes estimated in the single studies of a meta-analysis differ. If the T^2 value is zero then, under the random effects model, all effect size measurements would be the same as those under the fixed effects model.

Equation 12 - Calculating the T^2 statistic

$$T^2 = \frac{Q - df}{C} \text{ where } C = \sum w - \frac{\sum w^2}{\sum w}$$

Two other statistical tests were performed to test for heterogeneity: the I^2 index and the H. The I^2 index measures the extent of true heterogeneity, dividing the difference between the result of the Q statistical test and its degrees of freedom (the number of studies minus one) by the Q statistic value itself and multiplying by 100 forming a percentage. Typically the cut off point between homogeneity and heterogeneity between studies is at 50% with 100% being entirely heterogeneous between studies. The H is the ratio of confidence interval widths for single summary estimates for random effects versus fixed effects analysis. It is taken as the square root of the Q divided by the degrees of freedom.

Equation 13 - Calculating the I^2 index

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100$$

Equation 14 - Calculating H

$$H = \sqrt{Q/df}$$

In order to perform a random effects analysis the T^2 is added to each study variance and the summary mean and summary variance is recalculated. Generally, a random effects analysis is preferred over a fixed effects analysis because the random effects model yields a wider confidence interval but if in the presence of publication bias, it tends to yield a more favorable point estimate of effect. Therefore, we cannot directly assume that the random effects model is "more conservative."

2.7.3. Determining outliers

Additional calculations for the standardized residuals of each study in the fixed effects and random effects analysis were also conducted. Standardized residuals demonstrate the differences between an individual study with the overall effect or result. It is used to calculate whether a study's effect measure is an outlier or not through the z-score value. Typically z-score values of less than negative 2.5 and greater than positive 2.5 are considered outliers.

Equation 15 - Calculating the standardized residual

$$Fixed\ effects\ standardized\ residual = \frac{(LnOR - E_C)}{SE(LnOR)} \text{ or } \frac{(LnRR - E_C)}{SE(LnRR)}$$

$$Random\ effects\ standardized\ residual = \frac{SE(LnOR) - E_{C^*}}{\sqrt{Var(LnOR)_*}} \text{ or } \frac{SE(LnRR) - E_{C^*}}{\sqrt{Var(LnRR)_*}}$$

$$\text{where } Var(LnOR)_* = Var(LnOR) + T^2 \text{ or } Var(LnRR)_* = Var(LnRR) + T^2$$

Equation 16 - Calculating the Z score

$$zscore = \frac{LnOR - E_{C^*}}{SE(LnOR)_*} \text{ or } \frac{LnRR - E_{C^*}}{SE(LnRR)_*}$$

2.8. Evaluation for heterogeneity and publication bias

The values for the LnOR (or LnRR) and the standard errors of each study were inputted into the Cochrane Collaboration's RevMan 5.2 computer software evaluate heterogeneity. In addition, RevMan 5.2 was used to produce forest plots in fixed effects and random effects models for both types of studies. Funnel plots were also created to evaluate potential publication bias using this computer software.

3. RESULTS:

3.1. Studies identified

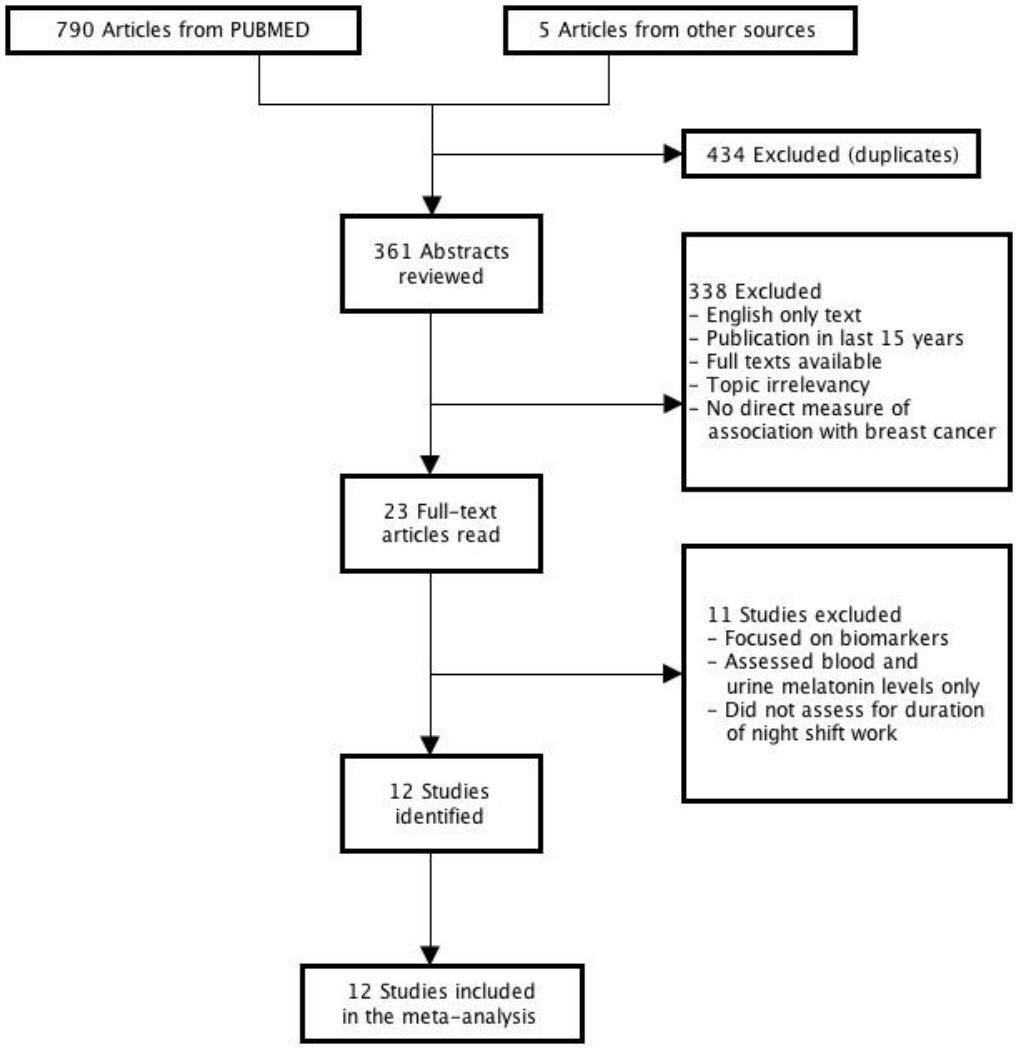
Figure 2 displays a flow diagram summarizing the systematic process of literature selection for assessment and meta-analysis. A total of 795 articles were identified initially. Five of these articles were found through hand-searches of the references in review articles conducted on the topic. After application of the search criteria as described earlier, 359 articles were retrieved and 434 were excluded. Titles and abstracts were reviewed and 23 papers were selected primarily for full-text review, excluding 338 articles if their titles, abstracts, or topic focus proved irrelevant to the research question.

These 23 articles underwent full-text and a total of eleven studies were deemed not fitting for this review's research focus and criteria. These studies focused primarily on biomarkers in assessing melatonin levels in blood or urine samples or were studies that did not assess the duration of night-shift work for female night-shift workers in association to breast cancer risk.

A total of twelve studies were selected for this systematic review and meta-analysis. Three cohort studies were selected and a total of nine case-control studies were

selected. The risk ratios, odds ratios and hazards ratios were identified for inclusion into the meta-analysis.

Figure 2 - Flow diagram: night shift work and breast cancer risk



3.2. Study characteristics

Table 2 and Table 3 summarize the main characteristics of the case-control and cohort studies considered for this analysis. There were nine case-control studies[3,4,6,7,8,9,10,21,22] from five different countries. The majority of the case-control studies were conducted in Europe in either Norway, Denmark, Germany, or France, and

two were conducted in the United States. The remaining articles were cohort studies[5,11,12] from the either the United States or China. Half of the studies had study populations taken directly from employment registries, such as for female military employees[9] or nurses[6,7,8,11,12]. The other half were from population-based cohort studies or registries[3,4,5,10,21,22]. Majority of the studies reported odds ratios or risk ratios as their measures of association. Pronk's[5] Chinese cohort study reported a hazard ratio. All studies reported a 95% confidence interval for any measures of associations listed. Based on all the articles reviewed for analysis, six specific possible confounders were selected. Studies were assessed to see if it had adjusted for any in their own analysis. These potential confounders are: parity, a family history of breast cancer, use of hormone replacement therapy, age, body weight index (BMI), and alcohol use. Most of the studies accounted for at least four out of six of these potential confounders with the exception of Hansen's 2001[21] and Lie's 2006[6] case-control studies that only adjusted for parity and age. Both studies' failure to account for the remaining four potential confounders may potentially weaken the studies' strength in validity. However, four studies did account for all the potential confounders: Schernhammer's 2001[11] and 2006[12] studies, Hansen's 2011[7] study, and Meneguax's[10] study. Most of the studies listed a maximum of number of years of night shift worked over 10 years except for Hansen[21], Davis[22], O'Leary[3] and Menegaux[10]. Having a lower maximum number of years night shift worked for these studies may have resulted in a less accurate measure of association for breast cancer risk.

Table 1 - Study characteristics of case-control studies

Study	*Year*	*Location and population*	*Number of participants*	*Years of night shift work (maximum)^*	*Covariates that were considered confounding variables**	*OR*	*95% Confidence Intervals*
Hansen et al.	2001	Denmark, women identified in the Danish Cancer Registry	7035 cases/ 7035 controls	>6	Parity, age	1.70	1.30 to 1.70
Davis et al.	2001	USA, women identified by the Cancer Surveillance System of the Fred Hutchinson Cancer Research Center, Seattle, WA	812 cases/793 controls	≥5.7	Parity, family history, hormone replacement therapy use, alcohol use	2.30	1.00 to 5.30
Lie et al.	2006	Norway, female nurses registered with the Norwegian Board of Health	537 cases/2148 controls	≥30	Parity, age	2.21	1.10 to 4.45
O'Leary et al.	2006	USA, female participants of the Electromagnetic Fields and Breast Cancer on Long Island Study	487 cases/509 controls	≥5	Parity, family history, hormone replacement therapy use, age	1.24	0.86 to 1.80
Pesch et al.	2010	Germany, female participants in the Gene Environment Interaction and Breast Cancer Study	857 cases/892 controls	≥20	Parity, family history, hormone replacement therapy use, BMI	2.49	0.87 to 7.18
Hansen et al.	2011	Denmark, women identified through the Danish Nurses Association	267 cases/1035 controls	≥20	Parity, family history, hormone replacement therapy use, age, BMI, alcohol use	2.10	1.30 to 3.20
Lie et al.	2011	Norway, female nurses registered with the Norwegian Board of Health	699 cases/895 controls	≥12	Family history, age, BMI, alcohol use	1.30	0.90 to 1.80
Hansen et al.	2012	Denmark, female military employees registered under the national pension fund and with the a military company	218 cases/899 controls	≥15	Parity, hormone replacement therapy use, age, BMI, alcohol use	2.10	1.00 to 4.50

Menegaux et al.	2012	France, newly identified breast cancer cases residing in French departements of "Cote d'Or" and "Ille-et-Vilaine"	1232 cases/1317 controls	≥3	Parity, family history, hormone replacement therapy use, age, BMI, alcohol use	1.13	0.76 to 1.68

***There were a total of six covariates considered as confounding variables: parity, family history, hormone replacement therapy use, age, BMI, and alcohol use.**
^Only the maximum years of night shift work was chosen as the exposure for all studies because majority of the studies stratified the years worked in different lengths.

Table 2 - Study characteristics of cohort studies

Study	*Year*	*Location and population*	*Number of participants*	*Years of night shift work (maximum)^*	*Covariates that were considered confounding variables**	*RR*	*95% Confidence Intervals*
Schernhammer et al.	2001	USA, female participants in the Nurses' Health Study I	2441 cases/ 78562 total	≥30	Parity, family history, hormone replacement therapy use, age, BMI, alcohol use	1.36	1.04 to 1.78
Schernhammer et al.	2006	USA, female participants in the Nurses' Health Study II	1352 cases/ 115022 total	≥20	Parity, family history, hormone replacement therapy use, age, BMI, alcohol use	1.79	1.06 to 3.01
Pronk et al.	2010	China, participants in the Shanghai Women's Health Study	717 cases/ 74792 controls	>17[$]	Parity, family history, age, BMI	0.80[#]	0.05 to 1.20

***There were a total of six covariates considered as confounding variables: parity, family history, hormone replacement therapy use, age, BMI, and alcohol use.**
^Only the maximum years of night shift work was chosen as the exposure for all studies because majority of the studies stratified the years worked in different lengths.
[$]Pronk et al. reported two types of exposure measurements, job matrix and a self-reported account of how many years worked in night shift. The self-reported account of years of night shift work was used for this analysis on the assumption that it was most closely matched with the other studies' interviews/questionnaire responses.
[#]Pronk et al. reported hazard ratios instead of risk ratios. But based on the rare disease assumption and the proportional hazards assumption, it was assumed that the HR was synonymous with the RR.

3.3. Assessing the quality of studies

3.3.1. Comparison groups

Seventy-five percent of the studies chosen for review were case-control studies, as a result a possible confounding factor may exist due to the comparison groups chosen. Some of these case-control studies chose to select cases of breast cancer and controls from a cohort of registered occupations in an area or country. This is usually done because such a population is usually standardized in terms of education, socioeconomic status, easily accessible, and other factors. Such populations are also registered so contacting and obtaining information on them can be relatively efficient. Both of Lie's[6,8] studies and Hansen's 2011[7] case-control studies chose to gather cases and controls from the a population of nurses. The 2012 Hansen study[9] focused its cases and controls from a population of female military employees. This can be seen as a potential confounder because unlike the general population such occupations have a higher degree of risk of developing breast cancer, or any types of cancer, because of their increased exposure to harmful chemicals and situations such as radiation due to their occupation. This also implies that the study cannot act as a representative of the general female population who work night shifts because they may or may not have the same varying types of exposures, other than night-shift work and light-at-night exposure that could lead to cancer. The remaining other studies[3,4,10,21,22] all selected cases and controls from a more generalized or widespread population. Of the cohort studies two was from a cohort of nurses, whilst the remaining were cohorts taken from the general population of where the study took place.[5,11,12]

3.3.2. Quality assessment of studies

Most of the case-control studies scored relatively high on the Newcastle-Ottawa Quality Assessment scale. The average score was 7.56 out of 9 points, with nine being the highest score and signifying a study of best quality. The highest-ranking case-control study was O'Leary's[3] with a total score of nine. The second highest-ranking case-control studies were tied between Pesch[4], Hansen (2011)[7], and Menegaux[10] with a score of eight each. The remaining case-control studies all scored a seven. Majority of the points were docked in the following categories: adequacy of case definition, representativeness of the cases, the ascertainment of exposure, and non-response rate.

Cohort studies were also ranked according to the Newcastle-Ottawa Quality Assessment scale. Cohort studies ranked even higher with an average of 8.3 out of 9 points, with nine being the highest score and signifying a study of the best quality. The highest ranking study was the Pronk[5] study with a score of nine but the two Schernhammer studies[11,12] both received high ranking scores of eight as well. Most points were taken off for representativeness of the exposed cohort in both Schernhammer studies. However, the higher average score for quality assessment may not be entirely indicative of better quality in study when compared to the case-control studies. The fewer number of cohort studies may have resulted in the higher average when compared to case-control studies. Results from both case-control and cohort studies in the Newcastle-Ottawa Quality Assessment scale can be seen in Table 4 and Table 5.

Table 1 - Quality assessment of case-control studies

Study	*Hansen (2001)*	*Davis (2001)*	*Lie (2006)*	*O'Leary (2006)*	*Pesch (2010)*	*Hansen (2011)*	*Lie (2011)*	*Hansen (2012)*	*Menegaux (2012)*	**Mean Score**
						Selection				
Case definition adequate	0	0	1	1	1	1	1	1	1	0.78
Representativeness of the cases	1	1	0	1	1	0	0	0	1	0.56
Selection of controls	1	1	1	1	1	1	1	1	1	1
Definition of controls	1	1	1	1	1	1	1	1	1	1
						Comparability				
Comparability of cases and controls on the basis of the design or analysis	2	2	2	2	2	2	2	2	2	1.78
						Exposure				
Ascertainment of exposure	0	0	1	1	1	1	1	1	1	0.78
Same method of ascertainment for cases and controls	1	1	1	1	1	1	1	1	1	1
Non-response rate	1	0	0	1	0	1	0	0	0	0.33
Total	7	7	7	9	8	8	7	7	7	7.56

Table 2 - Quality assessment of cohort studies

Study	*Schernhammer (2001)*	*Schernhammer (2006)*	*Pronk (2010)*	**Mean Score**
		Selection		
Representative of the exposed cohort	0	0	1	0.33
Selection of the non-exposed cohort	1	1	1	1
Selection of controls	1	1	1	1
Demonstration that outcome of interest was not present at start of study	1	1	1	1
		Comparability		
Comparability of cohorts on the basis of the design or analysis	2	2	2	2
		Outcome		
Ascertainment of outcome	1	1	1	1
Was follow-up long enough for outcomes to occur	1	1	1	1
Adequacy of follow-up for cohorts	1	1	1	1
Total	8	8	9	8.33

3.3.3. Night shift work definitions

According to the United States Department of Labor's Occupational Safety and Health Administration a normal work shift is defined as "a work period of no more than eight consecutive hours during the day, five days a week with at least an eight-hour rest." Any shift that extends beyond this eight-hour maximum, requires more consecutive days of work, or involves work in the evening would be considered as "extended" or "unusual." Night shift work would fall under the "unusual" or "extended" work definition. Unfortunately the United States OSHA does not provide any standard protocol or definition in regards to such types of "unusual" or "extended" work.[13] However, the International Agency for Cancer Research has gathered various definitions of night-shift work and night-time work from major European countries such as Austria, Belgium, Finland, France, Germany, Greece, Ireland, Italy, the Netherlands, Portugal, Spain, Sweden, and the United Kingdom. Most of these countries defined night shift or nighttime work to start at the earliest 8:00PM in the evening to the latest of 7:00AM in the morning. The IARC also went on to gather the definition of "night shift workers" from these countries. The general definition of a "night shift worker" from the IARC is any worker who has to work at least three hours daily solely in night shift work or nighttime work.[14]

Some of the studies selected for this review also held their own definitions for defining night shift work. Menegaux[10] defined night shift work as working at least one hour between the hours of 11:00PM and 5:00AM. For Hansen's 2012[9] study, she defined night shift work as working at least one year during hours beginning after 5:00PM and ending before 9:00AM. Pronk[5] defined night shift work according to a job matrix and

also defined it as starting work after 10:00PM at least three times a month for over a year. In Pesch's[4] GENICA study, night shift work was defined according to the International Labor Organization, which defined night shift work as working the fulltime period between midnight and 5:00AM. O'Leary[3] defined night shift work vaguely with no real time frames. Both of Schernhammer's[11,12] studies and Lie's[6] earlier study also had no mention of any definition for night-shift work. Davis' study[22] specified night shift work as "graveyard shift" but gave no specification as to what hours would qualify under this term. The failure to have a standardized definition for night-shift work in all the studies can be seen as a limiting factor to the reliability of the association between night-shift work and breast cancer but this also explains why there are were so many digressions in terms of results amongst the studies reviewed. The results in the studies range from declaring there is a distinct association between night shift work and breast cancer, there is a slight association, to none at all where night shift work accumulation may even appear to be a protective factor for employees against breast cancer. Several of the studies that failed to give a clear definition or any definition at all of night shift work was studies also based in the United States. As stated earlier, OSHA has yet to give an OSHA standard definition of night shift work, generalizing it loosely under the terms "unusual" or "extended" work. This could explain why such studies failed to give a discrete definition. The remaining studies that did not state a discrete definition for night shift work could be a result of their method of gathering information. These studies gathered information from standardized questionnaires given to participants; night shift work may have been generalized for the purpose of succinctness in the questionnaires or interviews given.

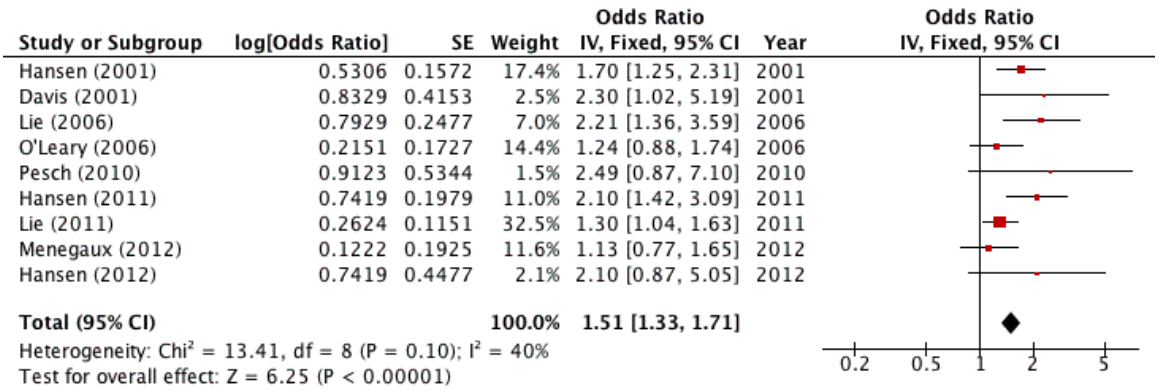
3.4. Meta-analysis

The fixed effects model found a significantly elevated breast cancer risk among female night shift workers (OR, 1.51; 95% CI, 1.33–1.71) in case-control studies. In the cohort studies, under the fixed effects models, a weak elevated breast cancer risk among female night shift workers (RR, 1.06; 95% CI, 0.90-1.24) was calculated. The manually calculated results for the fixed effects models for case-control studies and cohort studies can be found in Table 6 and Table 7. Forest plots and computer-generated results using the computer software RevMan 5.2 can be seen for comparison with the manually calculated results in Figure 3 and Figure 4.

Table 6 - Fixed effects model for case-control studies

	Mean	*Variance*	*Standard Error*	*95% Confidence Interval*
Log Scale	0.41	0.0043	0.07	0.28-0.54
Natural Scale	1.51			1.33-1.71

Figure 3 - Forest plot: fixed effects model for case-control studies

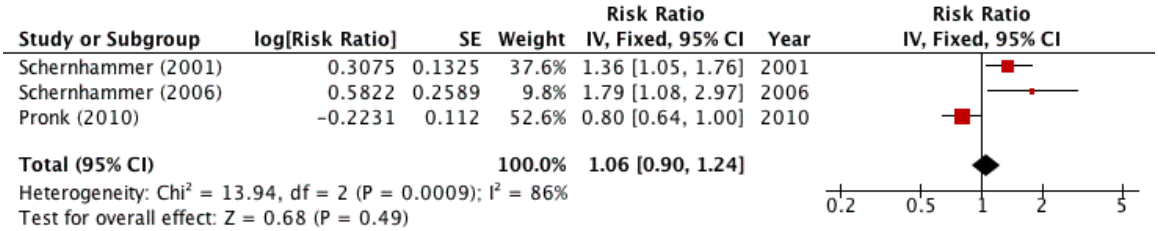


Values shown on the figure were calculated through the software RevMan 5.2 and vary slightly due to rounding within the software.

Table 7 - Fixed effects model for cohort studies

	Mean	*Variance*	*Standard Error*	*95% Confidence Interval*
Log Scale	0.06	0.0066	0.08	-0.10-0.21
Natural Scale	1.06			0.90-1.24

Figure 4 - Forest plot: fixed effects model for cohort studies



Values shown on the figure were calculated through the software RevMan 5.2 and vary slightly due to rounding within the software.

Tests of heterogeneity were performed on both types of studies. Q statistics were found for both types of studies. The case-control studies held a Q statistic value of 13.42 and the cohort studies held a Q statistic value of 13.94.

As a result, additional tests for heterogeneity were conducted. The case-control studies showed moderate homogeneity with an I^2 index of 40.37%. In contrast, cohort studies showed significant heterogeneity with an I^2 index of 85.65%. Values for each statistic and test used to test for heterogeneity in each study type can be found in Table 8 and Table 9.

Table 8 - Heterogeneity for case-control studies

Q	13.42
T^2	0.03
H	1.29
I^2	40.37%

Table 9 - Heterogeneity for cohort studies

Q	13.94
T^2	0.14
H	2.64
I^2	85.65%

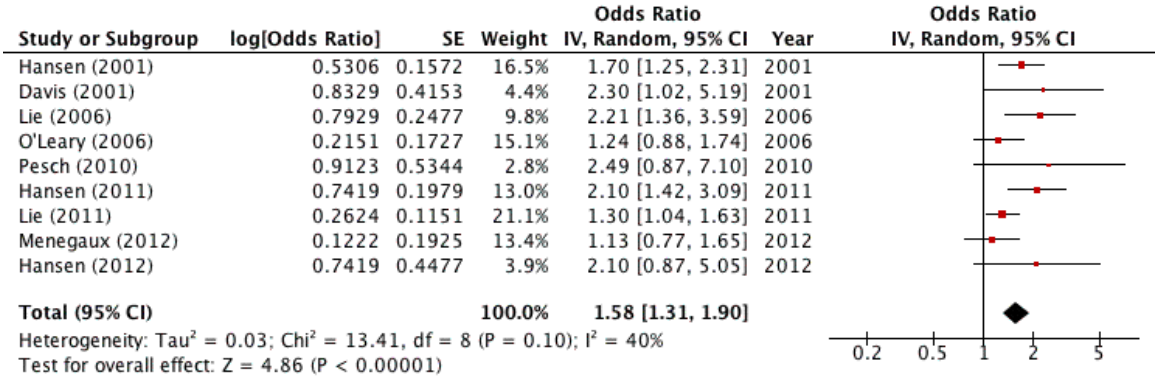
Random effects models were also conducted on both types of studies. The T^2 for case-control studies and cohort studies was found to be 0.03 and 0.14 respectively. The T^2 reflects how much the population effect sizes estimated in the single studies of a meta-analysis differ. In random effects models, there was slightly more significant elevated breast cancer risk (OR, 1.58; 95% CI, 1.31-1.90) for the case-control studies and a

slightly more significant elevated breast cancer risk (RR, 1.20; 95% CI, 0.76-1.91) for cohort studies. The manually calculated results for the fixed effects models for case-control studies and cohort studies can be found in Table 10 and Table 11 Forest plots and computer-generated results using the computer software RevMan 5.2 can be seen for comparison with the manually calculated results in Figure 5 and Figure 6.

Table 10 - Random effects model for case-control studies

	Mean	*Standard Error*	*95% Confidence Interval*	*Weight*
Log Scale	0.46	0.09	0.27-0.64	113.24
Natural Scale	1.58		1.33-1.71	

Figure 5 - Forest plot: random effects model for case-control studies

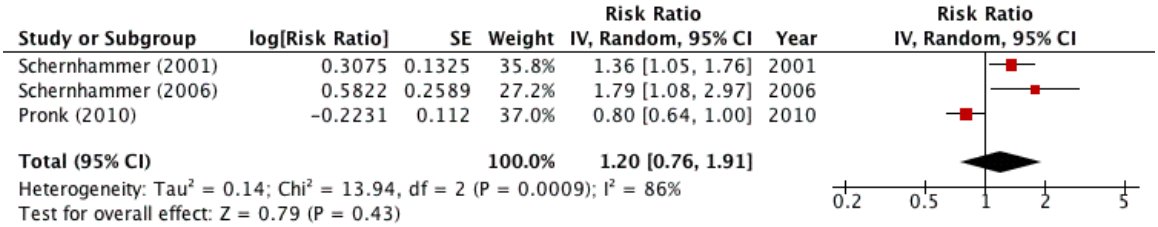


Values shown on the figure were calculated through the software RevMan 5.2 and vary slightly due to rounding within the software.

Table 11 - Random effects model for cohort studies

	Mean	*Standard Error*	*95% Confidence Interval*	*Weight*
Log Scale	0.19	0.24	-0.28-0.65	17.99
Natural Scale	1.20		0.76-1.91	

Figure 6 - Forest plot: random effects model for cohort studies



Values shown on the figure were calculated through the software RevMan 5.2 and vary slightly due to the rounding within the software.

Additional calculations were conducted to find the standardized residuals for each study in the meta-analyses. The Z score was then derived from each single studies' standardized residuals to determine outliers. Standardized residuals and Z scores can be found in Table 12. Ultimately, there were no distinctive outliers observed in any of the studies for either study type.

Table 12 - Standardized residuals and Z scores for case-control and cohort studies

Study	*Fixed effects standardized residuals*	*Randomized effects standardized residuals*	*Z score*	*Outlier*
Hansen (2001)	0.77	-1.30	0.32	No
Davis (2001)	1.02	-0.09	0.86	No
Lie (2006)	1.55	-0.70	1.18	No
O'Leary (2006)	-1.13	-1.18	-1.09	No
Pesch (2010)	3.89	0.14	0.82	No
Hansen (2011)	8.54	-0.99	1.17	No
Lie (2011)	7.73	-1.67	-1.07	No
Hansen (2012)	3.77	-0.02	0.61	No
Menegaux (2012)	7.74	-1.03	-1.40	No
Schernhammer (2001)	1.90	-0.13	0.39	No
Schernhammer (2006)	2.03	0.16	1.03	No
Pronk (2010	1.45	-0.19	-1.33	No

3.5. Evaluation for publication bias

Figure 7 and Figure 8 are funnel plots created through the RevMan 5.2 software for both case-control and cohort studies were evaluated for asymmetry that could signify publication bias. The sample effect measurements of breast cancer risk associated with

night shift is represented by the horizontal axis, against the standard error on the vertical axis. The standard error provides a measure of the precision of the effect measurements as an estimate of the population parameter. As the vertical axis of the funnel plot is inverted with zero at the top, the studies with less precise estimated effects scatter more widely at the bottom of the plot. The vertical dashed-line represents the total overall estimate of effect measurements for each meta-analysis, in case-control studies the odds ratio and in cohort studies the risk ratio. It is estimated that the estimated effects will scatter uniformly around the total overall estimate of the meta-analysis because of sampling error in the selection of samples from the population. But, as sample size increases, the precision of the estimated effects increases and the spread of points narrow. As seen on the two funnel plots for each type of study, there is some evidence of publication bias in either funnel plots created for the case-control and cohort studies due asymmetry found in both plots.

Figure 7 - Funnel plot: case-control studies

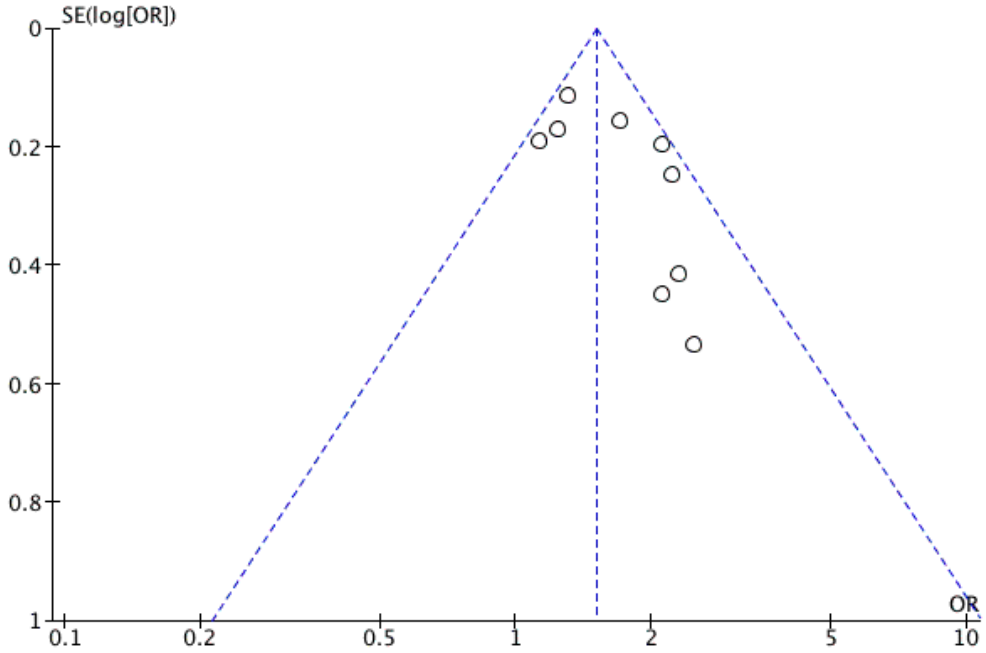
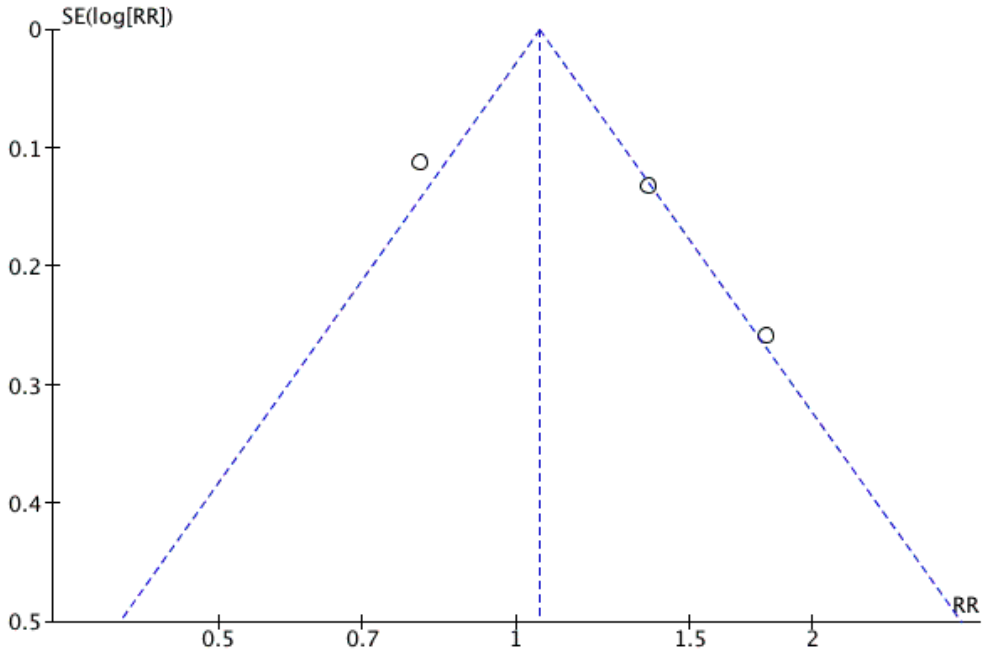


Figure 8 - Funnel plot: cohort studies



4. DISCUSSION:

4.1. Summary of key findings

The IARC came to the conclusion "on the basis of limited evidence for humans on the carcinogenicity of shift work that involves night shift work and sufficient evidence on experiments on animals for the carcinogenicity of light during the daily dark period." After the 2007 International Agency for Research on Cancer (IARC)'s conclusion that "shift work [that] involves circadian disruption is probably carcinogenic to humans,"[4] a greater number of research studies have been conducted to assess the possible association between night shift work and breast cancer although no conclusive association has been found. In this systematic review and meta-analysis, the association between night shift work and breast cancer risk was found to be stronger in case-control studies than in cohort studies.

This systematic review and meta-analysis included 12 observation studies (nine case-control studies and three cohort studies) that examined breast cancer risk among female shift workers. Pooled results found a 51% increase in the risk of breast cancer among night shift workers in the fixed effects model for case-control studies. Pooled results for cohort studies found a 6% increase in the risk of breast cancer among night shift workers in the fixed effects model. Under the random effects models a 58% and a 20% increase in the risk of breast cancer among night shift workers was found for case-control studies and cohort studies respectively.

Total overall estimates of effect measurements from both the fixed effects model and the random effects model were calculated and were presented to allow for comparison between both models. If only the fixed effects model analysis was used, it would be assumed that the studies were homogeneous and that the estimated effect sizes only differed by sampling error. In the random effects model, variability within- and between-studies are included.

In addition, the Q statistic for both types of studies was calculated, 13.42 for case-control studies and 13.94 cohort studies, but it was decided that the values were too similar to account for heterogeneity. The Q statistic also has poor power to assess heterogeneity with smaller numbers of studies and excessive power to detect negligible variability with a high number of studies.[20] Both types of studies had relatively smaller numbers of studies (nine case-control studies and three cohort studies), so it can be assumed that additional tests for heterogeneity should be conducted.

The I^2 index value was found for both meta-analyses. The I^2 index measures the extent of true heterogeneity. The case-control studies showed moderate homogeneity

with an I^2 index of 40.37%. With 50% being the general cut-off point of homogeneity and heterogeneity, the I^2 index for case-control studies can be seen as relatively homogeneous between studies. I^2 index can be interpreted as the percentage of the total variability in a set of effect sizes due to true heterogeneity, that is, due to between-studies variability.[20] However, cohort studies showed significant heterogeneity with an I^2 index of 85.65%, well past the 50% cut-off point of homogeneity.

The T^2 was also calculated for both types of studies. The T^2 for case-control studies was 0.03 and for cohort studies, 0.14. The T^2 (tau square) estimates the between-studies variance. Under the assumption that a random effects model will be conducted, this between-studies variance reflects how much the population effect sizes estimated in a single studies of meta-analysis differ. If the T^2 was equal to zero, then under the random effects model, all effect sizes would be the same as those under the fixed effects model. When comparing the effects sizes for the random effects and fixed effects model for case-control studies, it was discovered that there was less of a change between random and fixed effects models for case-control studies than for cohort studies. The case-control studies had a T^2 value closer to zero, so it was assumed that under random effects models the combined effect measurement and 95% confidence intervals would remain relatively similar when compared to those in the fixed effects model. The cohort studies also showed values greatly varying from those under the fixed effects model.

The significant difference in associated risk of breast cancer for female night shift workers case-control studies and cohort studies can be attributed to the sample population. The sample population that a majority of the case-control studies located their cases and controls from were from the general public of females that may or may not

have been recruited into a larger study dealing with cancer or breast cancer. Four of these case-control studies were employment specific, three having sample populations consisting of nurses' registries and one military registry. It was likely that the case-control studies displayed a lower I^2 index indicating greater homogeneity because a majority of the case-control studies were likely to be taken from similar sample populations for studies designed exclusively for breast cancer risk research. However, at 40.37%, the case-control studies' homogeneity was not significantly robust, being still relatively close to the 50% cut-off point between homogeneity and heterogeneity. The remaining case-control studies were employment specific to occupations in healthcare and military. This could have resulted in a greater number of confounders that were not accounted for such as other forms of exposures that are associated with breast cancer.

In contrast, a majority of the cohort studies had sample populations derived specifically from studies conducted on nurses and one on the general female public. The differences in sample population may be a large contributing factor to the high I^2 index value of 85.65% associated with greater heterogeneity. The heterogeneity between the cohort studies can be a consequence of lack of original design geared specifically for the purpose of assessing breast cancer risk associated with night shift work. Instead the cohort studies gathered cases from cohorts already established within a general health study for Shanghai woman and cohorts already established within a general heath study conducted specifically on nurses.

Also, differences between the two study types may have also been a contributing factor in the contrasting risks. A case-control study begins with people with the disease (cases) and compares them to people without the disease (controls). In contrast, a cohort

study begins with a group of exposed people and compares them to a non-exposed group. The exposure factor is hypothesized to influence the occurrence of a given disease (breast cancer). The main feature of a cohort study is the observation of large numbers over a long period with comparison of incidence rates of disease in groups that differ in exposure levels. Although cohort studies may be lauded as stronger study in assessing association, in the case of the association between breast cancer risk and night shift work it may be dependent on study design. If the cohort studies assessed in this review were taken from sample populations or previous studies designed specifically for breast cancer research, like the majority of those found in the case-control meta-analysis, the results would probably have been more homogeneous.

4.2. Previous research

Exposure to artificial light at night, with melatonin production at its peak, has been hypothesized to sharply reduce levels of melatonin and thus elevate cancer risk. Previous research have shown that the decreased melatonin production due to exposure to light at night leads to a rise in the levels of reproductive hormones. like estrogen, causing hormone sensitive tumors in the breast. *In vitro* experimental studies indicate that both pharmacological and physiologic doses of melatonin have been shown to reduce the growth of malignant cells of the breast.[15] Rat models have also showed that pinealectomy, removal of the pineal gland, boosts tumor growth whereas exogenous melatonin administration exerts anti-initiating and oncostatic activity in chemically induced cancers.[15]

4.3. Comparison with previous reviews

A previously conducted systematic review and meta-analysis was conducted by Megdal et al.[15] in 2005. Megdal found a significant elevation of breast cancer risk with an RR of 1.51 and a 95% confidence interval of 1.36-1.68 for female night shift workers and breast cancer risk.[15] Megdal also found evidence that suggests confounding due to the incomplete adjustment for breast cancer risk factors that remained a limitation in a majority of the studies done in her review.[15] Megdal's study also chose to assess the increased breast cancer risk of female flight attendant crew members in her meta-analysis with the original rationale for studies of flight attendants assuming that their occupational exposure to cosmic radiation caused an excess cancer risk. It was later reasoned that the observed increase in breast cancer risk could as well be due to a melatonin deficiency resulting from work-associated exposure to light at night.

Although this review did not include any studies on flight attendants, Megdal this current review did assess a few of the same studies. This included both Schernhammer studies[11,12], Davis[23], and Lie[6]. Megdal included articles from January 1960 to January 2005 found on MEDLINE, experts, bibliographies, and abstracts into her review. This systematic review and meta-analysis included literature from January 2001 to January 2013 searched through two different electronic databases. This review's goal was to include the most up-to-date studies conducted after the IARC statement on night shift work and cancer risk in 2005. Megdal conducted a combined meta-analysis on the flight attendant studies and the female night shift workers studies as well as separately. She also did not separate the meta-analyses based on study type. However for this review, the studies were divided into two types, case-control and cohort studies, for the ease of

calculation and to prevent having to transform between an RR to an OR and vice versa. It was assumed that the differences between the study types would result in differences in whether or not there was an increased risk for breast cancer or not.

Megdal's definition of night shift work was generalized as "any shift schedule that included overnight work."[15] This review chose to define night shift work more specifically as "any shift schedule that included working between the hours of 9:00PM to 7:00AM that could potentially disrupt the employee's circadian rhythm and sleep patterns." This definition also would include any shifts that included overnight shift work or graveyard shift work. Also, Megdal's review did not perform quality assessment on the studies included in her meta-analysis nor did she account for any potential confounders that could affect the measures of association. Megdal did however list what confounders each study adjusted for but did not state if she included the crude measures of associations or the adjusted measures of association. This review lead a quality assessment on each study based on the Newcastle-Ottawa Quality Assessment Scale for case-control and cohort studies. Only the adjusted measures of association were included in the systematic review and meta-analysis. A list of six of the most influential confounders for breast cancer risk was also taken into account when conducting the quality assessment.

In summary, the strengths of this review included the focus on quality assessment with the inclusion of particular potential confounders during the systematic review. In addition, unlike the previously conducted systematic review and meta-analysis by Megdal[15], this review performed literature searches on two different electronic databases: PUBMED and Thomson Reuter's Web of Science. Most of the publications included in

this review were published after Megdal et al's 2005 study, making this review as up-to-date as possible. In contrast to Megdal's more generalized definition of night shift work, a more distinct and descriptive definition of night shift work was utilized in this review. This review also conducted separate meta-analyses based on study type between cohort studies and case-control studies in order to prevent any miscalculations during transformation of effect measurements.

4.4. Potential significance of findings

This review suggests that industry or organizations such as the Occupational Safety and Health Administration that deal with occupational safety establish a more succinct and decisive definition of night shift work. This definition should include how long a shift must be to be considered "night shift work," when the shift occurs that counts it as night shift work, and establishes if there is a difference between night shift work and overnight shifts. In past studies, assessment of employment time or night shift work exposure may have been misclassified because it was based on crude estimates instead of a set definition. This misclassification may have led to the decrease of the relative risk ratios.[15] The establishment of a decisive definition of night shift work by an authority may prove beneficial especially for future research leading to greater homogeneity with the night shift work exposures.

In addition, establishing a more decisive definition of night shift work may affect industry and even policy in the future. Policies may be created that include public education of employees and a right-to-know policy where future employees are informed outright about the possible associated risks of working night shift work and breast cancer risk. These new policies may also force industry to have policies where future employees

sign a waiver informing them of the risks associated with night shift work and encourages or forces the employee to get regular mammography performed to ensure no detrimental health effects have occurred.

4.5. Limitations

With a limited number of observational studies, a majority of the studies included in this review were studies that obtained data on cases, controls, or cohorts from already established studies that may or may not have been designed originally with the intent to study breast cancer risk. This may have led to increased heterogeneity between studies. If already available data have to be used it would best to use data from registries of the general female public or shift workers or even previous studies that have been conducted on breast cancer risk.

Another limitation encountered in this review would be that conducting the quality assessment portion of a systematic review individually might have resulted in bias. Typically this bias is rectified by several other reviewers meeting in discussion and coming to a conclusion in assessing the quality of a study. Conducting this individually in a limited amount of time may have lead to a conflict of interest that resulted in some studies either not being included in the review or mistakenly included.

5. CONCLUSIONS:

In summary, although this systematic review and meta-analysis suggest that night shift work may increase the risk of breast cancer, the association between night shift work and breast cancer risk is still inconclusive due to the limited evidence from the small number of studies done on this topic. The possible increase in the risk of breast

cancer and its association with night shift work observed in the meta-analysis is not robust enough due to some heterogeneity observed in the case-control studies and the great variability and heterogeneity observed in the cohort studies. It is recommended that a more decisive definition of night shift work exposure be determined for future studies in order to better ascertain an association. In previous studies, the assessment of employment time or night shift work exposure may have been misclassified because it was based on crude estimates instead of a set definition. This misclassification may have led to the decrease of the relative risk ratios.[15] Closer attention to study design and sources of data should be put into greater consideration for future studies. Future studies should strive to use original data or obtain data from previous studies that are applicable to the research question or purpose of study. Future studies should also be conducted on more generalizable populations or cohorts instead of occupation specific populations or cohorts to increase the external validity of their results.

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APPENDICES:

Appendix A – Newcastle-Ottawa Quality Assessment Scales for case-control and cohort studies

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE - CASE CONTROL STUDIES

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

Selection

1) Is the case definition adequate?

a) yes, with independent validation *

b) yes, eg record linkage or based on self reports

c) no description

2) Representativeness of the cases

a) consecutive or obviously representative series of cases *

b) potential for selection biases or not stated

3) Selection of Controls

a) community controls *

b) hospital controls

c) no description

4) Definition of Controls

a) no history of disease (endpoint) *

b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

a) study controls for _____ (Select the most important factor.) *

b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure

a) secure record (eg surgical records) *

b) structured interview where blind to case/control status *

c) interview not blinded to case/control status

d) written self report or medical record only

e) no description

2) Same method of ascertainment for cases and controls

a) yes *

b) no

3) Non-Response rate

a) same rate for both groups *

b) non respondents described

c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE - COHORT STUDIES

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

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average _____ (describe) in the community *****

b) somewhat representative of the average _____ in the community *****

c) selected group of users eg nurses, volunteers

d) no description of the derivation of the cohort

2) Selection of the non exposed cohort

a) drawn from the same community as the exposed cohort *****

b) drawn from a different source

c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure

a) secure record (eg surgical records) *****

b) structured interview *****

c) written self report

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes *****

b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for _____ (select the most important factor) *

b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome

a) independent blind assessment *

b) record linkage *

c) self report

d) no description

2) Was follow-up long enough for outcomes to occur

a) yes (select an adequate follow up period for outcome of interest) *

b) no

3) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for *

b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) *

c) follow up rate < ____% (select an adequate %) and no description of those lost

d) no statement

Appendix B – Equations

Variance	$Var(LnOR) \approx \frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}$ $Var(LnRR) \approx \frac{b}{a(a+b)}+\frac{d}{c(c+d)}$
Weight	$w = 1/Var(LnOR)$ or $1/Var(LnRR)$
Relative weight	$RelativeWeight = \left[1/Var(LnOR)\right]\times 100$ or $\left[1/Var(LnRR)\right]\times 100$
Standard error	$SE(LnOR) = \sqrt{Var(LnOR)}$ $SE(LnRR) = \sqrt{\left[1/a + 1/c\right] - \left[1/(a+b) + 1/(c+d)\right]}$
95% confidence interval	$95\%CI = LnOR \pm 1.96 \times SE(LnOR)$ or $LnRR \pm 1.96 \times SE(LnRR)$
Effect size	$E = LnOR \times (1/Var(LnOR))$ or $LnRR \times (1/Var(LnRR))$
Combined effect measurement	$E_C = \frac{\sum wE}{\sum w}$
Combined effect variance	$Var_C = \frac{1}{\sum w}$
Q statistic	$Q = \sum[w(LnOR)^2] - \frac{E^2}{\sum w}$ or $\sum[w(LnRR)^2] - \frac{E^2}{\sum w}$
T^2 statistic	$T^2 = \frac{Q-df}{C}$ where $C = \sum w - \frac{\sum w^2}{\sum w}$
I^2 index	$I^2 = \left(\frac{Q-df}{Q}\right)\times 100$
H	$H = \sqrt{Q/df}$
Standardized residuals	*Fixed effects standardized residual* $= \frac{(LnOR - E_C)}{SE(LnOR)}$ or $\frac{(LnRR - E_C)}{SE(LnRR)}$ *Random effects standardized residual* $= \frac{SE(LnOR) - E_{C^*}}{\sqrt{Var(LnOR)_*}}$ or $\frac{SE(LnRR) - E_{C^*}}{\sqrt{Var(LnRR)_*}}$

	where $Var(LnOR)_* = Var(LnOR) + T^2$ or $Var(LnRR)_* = Var(LnRR) + T^2$
Z score	$zscore = \frac{LnOR - E_{C^*}}{SE(LnOR)_*}$ or $\frac{LnRR - E_{C^*}}{SE(LnRR)_*}$