PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Oral contraceptive use is associated with prostate cancer: An Ecologic Study
AUTHORS	David Margel and Neil E Fleshne

VERSION 1 - REVIEW

REVIEWER	Song Yao, PhD Research Assistant Professor of Oncology Department of Cancer Prevention and Control Roswell Park Cancer Institute Buffalo, NY 14263 USA I have no competing interests to disclose.
REVIEW RETURNED	07/09/2011

THE STUDY	The cited references 2-4 were three studies published a number of years ago, and there have been a lot more studies on the relationships between estrogen and prostate cancer risk in the literature, with a majority of them, including a pooled analysis of 18 prospective studies (JNCI 2008;100:170-183), and a most recent study from the PCPT (Cancer Causes and Control 2011;22:1121-1131, do not support such a relationship. The authors may consider a more balanced view based on the available literature on this topic.
RESULTS & CONCLUSIONS	In both Figures 1c and 2c, it is not clear how the results were presented by continent. It will help by providing some more detailed figure legends for better understanding this part of the results.
GENERAL COMMENTS	The authors presented an interesting ecologic study on the proportion of oral contraceptive (OC) use and prostate cancer incidence and mortality at a country level. Their hypothesis was that by-products of endocrine disruptive compounds from OCs may be dispersed into the enviroment, contaminate drinking water and finally get into men's bodies, causing increased everyday levels of estrogen and thus elevated prostate cancer risk. Their findings of positive correlations between proportion of OC use and prostate cancer incidence/mortality support this hypothesis. I have some concerns that the authors may find helpful to improve their manuscript. 1. The relationship between estrogens and prostate cancer risk is very inconsistent based on the published studies, and a large pooled-analysis concluded with null association (JNCI 2008;100:170-183). This may undermine the validity of their hypothesis, as well as the interpretation of the findings via mechanisms of estrogen. The authors may consider presenting a more balanced view in regards to the association of estrogen with prostate cancer risk, and discuss the possibility that their findings may be explained by mechanisms other than estrogens.

	2. Another point to consider in order to test if OC use is associated with increased risk of prostate cancer may be correlations of the time trends between OC use and prostate cancer incidence. If the hypothesized association existed, we would expect an increase in prostate cancer incidence a number of years after the wide use of OCs. If such historical data are available, this type of analysis may provide more definite answers. 3. It is unclear in Figures 1c and 2c how the analyses by continent were performed or presented. 4. It is unclear why the authors chose to randomly select 60 nations for the analysis. It may provide a more complete picture by analyzing data from all available nations. 5. On page 3, the Key Message, language revision "...female use of oral contraceptive use...".

REVIEWER	Dr Jane Melia Honorary research position Institute of Cancer Research UK I have no conflict of interest.
REVIEW RETURNED	01/09/2011

THE STUDY	This is a very interesting hypothesis. Although the authors recognise the limitations of their methods, it is possible that more data could have been included to test the hypothesis more rigorously against confounding factors. This should be discussed and assessed. This ecological study only studies use of contraceptives methods in relation to incidence and mortality from prostate cancer. The completeness and quality of these data are not fully described i.e representativeness of eac country populations with regard to the survey of contraceptive use, areas within countries where incidence data were provided by local registries . Other factors such as diet and diagnostic practices/ level of screening related to prostate cancer etiology are not included - this should be explored further by the authors. The countries included in the analyses should be listed in an appendix.The comparisons between oral contraceptives and other contraceptive methods is informative.
RESULTS & CONCLUSIONS	The authors make it clear that the paper is hypothesis generation. However, some attempt should have been made to include other factors related to variation in prostate cancer between countries eg diet, diagnostic practices, level of screening, use of the PSA test etc. In addition although the authors mention that chronic exposure to relatively low levels of environmental EDCs could be important, how low are these compared with those reported in the industrial exposure studies? Without knowning all the countries included in the study, it is not possible to make an informed assessment. Re. the comment on the last page of the Discussion it should be noted that even mortality rates may be affected by more diagnosis so they are not necessarily free of diagnistic bias. The results should have included the number of countries by continent which separately provided incidence, mortality and contraceptive data, and then the proportion of countries by continent which were included in the final analyses. The overall trends in incidence appear to be largely driven

	by trends in Europe (see Figures 1a and 1b). Mechanisms for the hypothesis need further discussion: Could oral contraceptives and their presence in the environment affect the unborn child as well as during lifetime? What are the environmental levels of EDCs in the food chain and drinking water?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 : Dr Jane Melia
Honorary research position
Institute of Cancer Research
UK

1. Comment: This is a very interesting hypothesis. Although the authors recognise the limitations of their methods, it is possible that more data could have been included to test the hypothesis more rigorously against confounding factors. This should be discussed and assessed. This ecological study only studies use of contraceptives methods in relation to incidence and mortality from prostate cancer.
Response: We thank the reviewer for this comment we unfortunately don't have information regarding screening tendencies and PSA use world-wide and therefore cannot include this in our analysis. However we indeed agree with the reviewer that many confounders of our association exist and therefore added a multivariable analysis controlling for Gross domestic product (GDP) per capita. GDP refers to the market value of all final goods and services produced in a country in a given period. GDP per capita is often considered an indicator of a country's standard of living. In our multivariable analysis oral contraceptive use was associated with both incidence and mortality even when controlling for GDP. We believe this analysis has strengthened our hypothesis considerably, however additional confounding does exist.
Changes Made: Abstract Page 1 lines 12-13, 22-23, Methods Page 6 lines 9-16; Page 7 lines 6-10; Results Page 8 lines 11-1, Discussion Page 9 lines 7-8, Page 11 lines 10-17. Table 1

2. Comment: 2. The completeness and quality of these data are not fully described i.e representativeness of each country populations with regard to the survey of contraceptive use, areas within countries where incidence data were provided by local registries
Response: Indeed, we agree with the reviewer and now added required information on the data retrieval.
Changes Made: Methods page 5 lines 9-13; 21-23.

3. Comment: Other factors such as diet and diagnostic practices/ level of screening related to prostate cancer etiology are not included - this should be explored further by the authors.
Response: As discussed earlier we agree many confounders to our association exist and further research is needed to prove a true association with OC use and PCa. However this is the first study to suggest such an association and we now also control for countries wealth in a multivariable model. Regretfully information of world wide tendencies of screening and dietary hobbits which indeed may confound the association between OC and PCa are not available to us at this point, we hope that in the future we will be able to account for these tendencies.
Changes Made: Abstract Page 1 lines 12-13, 22-23 Methods Page 6 lines 9-16; Page 7 lines 6-10; Results Page 8 lines 11-1, Discussion Page 9 lines 7-8, Page 11 lines 10-17. Table 1

4. Comment: The countries included in the analyses should be listed in an appendix
Response: We agree and now added in Appendix 1 all countries explored
Changes Made: Appendix 1

5. Comment: The comparisons between oral contraceptives and other contraceptive methods is informative.
Response: We thank the reviewer for this kind comment

6. Comment: The authors make it clear that the paper is hypothesis generation. However, some attempt should have been made to include other factors related to variation in prostate cancer between countries eg diet, diagnostic practices, level of screening, use of the PSA test etc
Response: We have now included adjusting for GDP and regret that information on further confounders such as PSA screening and dietary contents are unavailable to us.
Changes Made: Abstract Page 1 lines 12-13, 22-23 Methods Page 6 lines 9-16; Page 7 lines 6-10; Results Page 8 lines 11-1, Discussion Page 9 lines 7-8, Page 11 lines 10-17. Table 1

7. Comment: In addition although the authors mention that chronic exposure to relatively low levels of environmental EDCs could be important, how low are these compared with those reported in the industrial exposure studies? Without knowing all the countries included in the study, it is not possible to make an informed assessment
Response: we agree and have added the countries explored in appendix 1

6. Comment: The results should have included the number of countries by continent which separately provided incidence, mortality and contraceptive data, and then the proportion of countries by continent which were included in the final analyses.
Response: Indeed this vital information was added.
Changes Made: Methods Page 6 lines 22-23; Page 7 lines 1-2

7. Comment: The overall trends in incidence appear to be largely driven by trends in Europe (see Figures 1a and 1b).
Response: We believe the trends in incidence are true world wide but the correlation is strongest in Europe
Changes Made: Discussion Page 9 lines 8-9.

8. Comment: Mechanisms for the hypothesis need further discussion: Could oral contraceptives and their presence in the environment affect the unborn child as well as during lifetime? What are the environmental levels of EDCs in the food chain and drinking water?
Response: We thank the reviewer for this comment and added to the discussion the pathophysiology and exposure data.
Changes Made: Discussion Page 11 lines 7-9; lines 20-21.

Reviewer 2: Song Yao, PhD
Research Assistant Professor of Oncology
Department of Cancer Prevention and Control
Roswell Park Cancer Institute
Buffalo, NY 14263
USA

1. Comment: The relationship between estrogens and prostate cancer risk is very inconsistent based on the published studies, and a large pooled-analysis concluded with null association (JNCI 2008;100:170-183). This may undermine the validity of their hypothesis, as well as the interpretation of the findings via mechanisms of estrogen. The authors may consider presenting a more balanced view in regards to the association of estrogen with prostate cancer risk, and discuss the possibility that their findings may be explained by mechanisms other than estrogens.
Response: Indeed recently several studies have demonstrated that PCa may not be related to

endogenous androgens. The Endogenous Hormones and Prostate Cancer Collaborative Group (ref 5) analyzing 18 prospective studies of 3886 men with PCa and 6438 control subjects, found no associations between PCa risk and serum concentrations of testosterone, calculated free testosterone, dihydrotestosterone, dehydroepiandrosterone sulfate, androstenedione, androstanediol glucuronide, estradiol, or calculated free estradiol. However this study associated serum hormonal levels. EDCs may increase the risk of PCa by affecting tissue levels or causing genetic or epigenetic changes that may not be found using serum levels. Li Tang et al (ref 6) studied the association between repeat polymorphisms of three key estrogen-related genes (CYP11A1, CYP19A1, UGT1A1) and risk of prostate cancer in the Prostate Cancer Prevention Trial (PCPT), The results indicate that repeat polymorphisms in genes involved in estrogen biosynthesis and metabolism may influence risk of PCa. Further studies are needed to determine the role of EDCs in PCa.
Changes Made: Introduction: Page 4 lines 5-6 ; Discussion: Page 11 lines 10-23; references added 5,6

2. Comment: 2. Another point to consider in order to test if OC use is associated with increased risk of prostate cancer may be correlations of the time trends between OC use and prostate cancer incidence. If the hypothesized association existed, we would expect an increase in prostate cancer incidence a number of years after the wide use of OCs. If such historical data are available, this type of analysis may provide more definite answers.
Response: Indeed data of change over time in PCa incidence and mortality as related to use of OC would be of value regretfully such data is not available to us.

3. Comment: 3. It is unclear in Figures 1c and 2c how the analyses by continent were performed or presented.
Response: We have now added in Appendix1 the continents considered in Figure 1c and 2c. We hope this point is clearer now.
Changes Made: Appendix 1

4. Comment: It is unclear why the authors chose to randomly select 60 nations for the analysis. It may provide a more complete picture by analyzing data from all available nations.
Response: We thank the reviewer for this comment we have now added many nations to the analysis which now consists of 87 different nations for the survey ensuring to sample each continent (List of countries included in the analysis can be found in Appendix 1).
We used 50% of countries available from each continent (25 of 50 Africa; 25 of 50 Asia; 24 of 47 Europe; 11 of 23 America and Australia and Newzeland were also included). We did not use all available countries since we aimed at a equal representation of developed an under developed countries (using the entire sample would have caused over-representation of under-developed countries and may have biased our results)
Changes Made: Abstract Page 1 line 18 Methods Page 6 lines 21-23; Page 7 lines 1-5; Results Page 8 line 4 and line 8; Figure 1A and 2A.

5. On page 3, the Key Message, language revision "...female use of oral contraceptive use...".
Response: this typo was corrected

VERSION 2 - REVIEW

REVIEWER	Song Yao, PhD Research Assistant Professor Department of Cancer Prevention and Control Roswell Park Cancer Institute Buffalo, NY 14226 USA
REVIEW RETURNED	07/10/2011

GENERAL COMMENTS	The authors responded well the reviewers' comments. I have just one minor issue. In the introduction, the cited reference 6 was not the original report for associations of estrogen and prostate cancer risk in PCPT. The correct reference should be Yao S, Till C, Kristal AR, et al. Serum estrogen levels and prostate cancer risk in the Prostate Cancer Prevention Trial: a nested case-control study. Cancer Causes & Control. Aug 2011. 22:1121-1131.

Correction

After review of the data it appears that the authors accidentally miscoded several points in the data set, which have resulted in an error in the published article (*BMJ Open* 2011;**1**: e000311. doi:10.1136/bmjopen-2011-000311). In the abstract the year used to retrieve age standardised incidence and mortality rates was the 2008 and not 2007 dataset (the correct year is mentioned in the methods section and in the references), and the number of countries was 88 (as appears in the appendix) and not 87. The Pearson correlation between prostate cancer incidence in nations' world-wide and oral contraceptive use was 0.58 and not 0.61. The Pearson correlation between prostate cancer incidence in Europe and oral contraceptive use was 0.59 and not 0.55. Prostate cancer incidence correlated with condom use in nations worldwide (r=0.48) but not in Europe or by continent. Figure 1A,B have been corrected. In the multivariable mode the adjusted estimates for the association of oral contraceptive use with prostate cancer incidence is 0.65 (95% CI 0.3 to 1.01), p=0.001 (not 1.06 (95% CI 0.58 to 1.6)). Table 1 has been corrected. The correlation of prostate cancer mortality rates with oral contraceptive use was not statistically significant (r=0.16, p=0.1 not 0.53, p<0.05). Figure 2 has been changed. With hindsight, after correcting the data and the analysis, the title of the manuscript would have been less easily misinterpreted if it had been: 'Oral contraceptive use is associated with prostate cancer incidence: an ecologic study'.

Data deposited in the Dryad repository: doi:10.5061/dryad.ff6bd0pq (http://datadryad.org/).

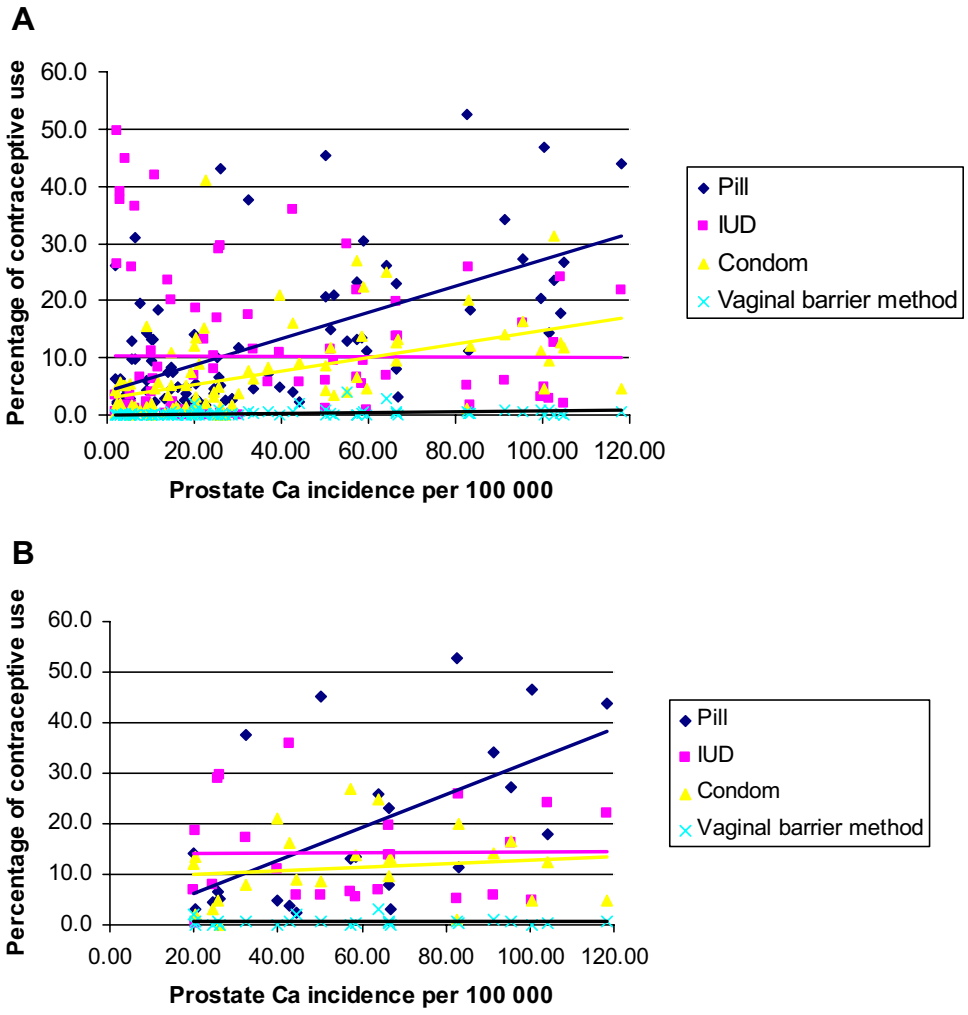

Figure 1 (A) Correlation between contraceptive mode and prostate cancer incidence. (B) Correlation between contraceptive mode and prostate cancer incidence in Europe.

Table 1 Multivariable linear regression of the association of mode of contraception and GDP (a measure of country's wealth) with PCa incidence

	Estimate	95% CI	p Value
Oral contraceptive use	0.65	0.3 to 1.01	0.001
Intrauterine device	−0.12	−0.4 to 1.7	0.46
Vaginal barrier	2.2	−3.6 to 8.2	0.45
Condom use	0.59	0.02 to 1.2	0.04
GDP	0.01	0.009 to 0.011	<0.001

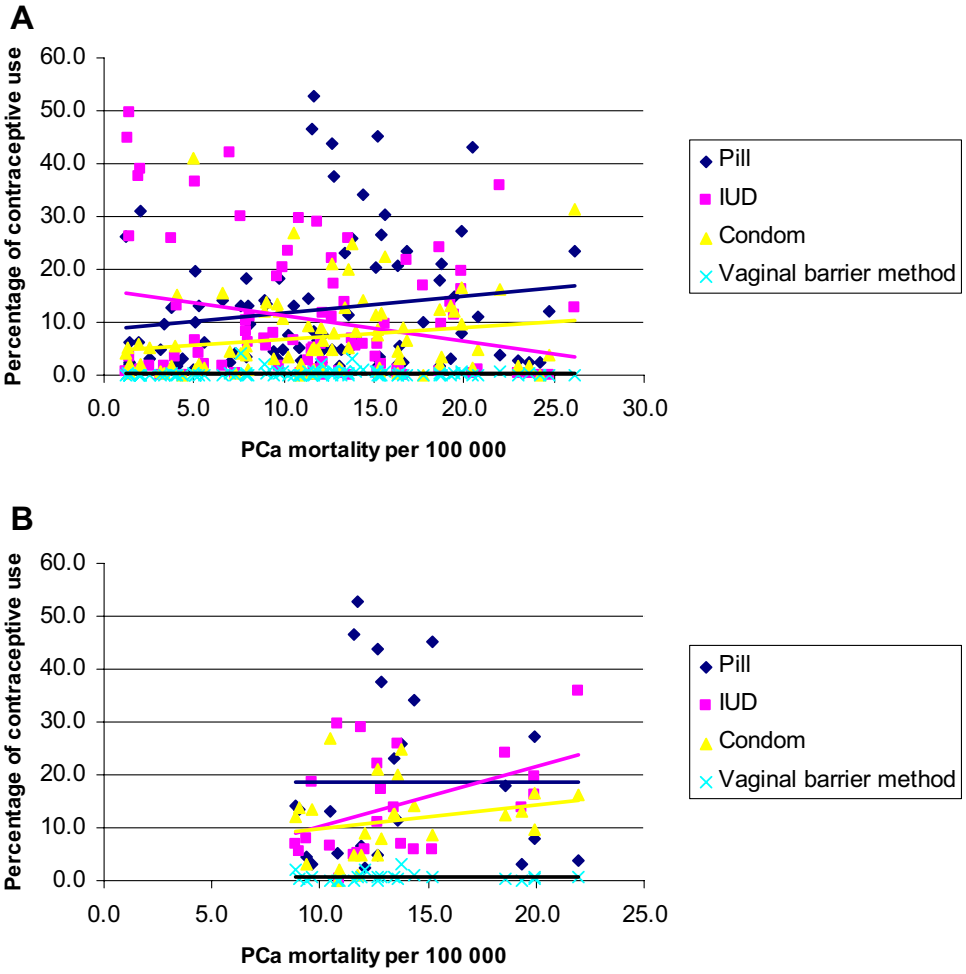

Figure 2 (A) Correlation between contraceptive mode and PCa mortality. (B) Correlation between contraceptive mode and PCa mortality in Europe.

BMJ Open 2012;**2**:e000311corr1. doi:10.1136/bmjopen-2011-000311corr1