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)	Cancer risk with folic acid supplements: a systematic review and meta-analysis
AUTHORS	Tale Norbye Wien, Eva Pike, Torbjørn Wisløff, Annetine Staff, Sigbjørn Smeland and Marianne Klemp

This paper was submitted to the BMJ but declined for publication following peer review. The authors addressed the reviewer's comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted to BMJ Open. Only one of the BMJ reviewers gave permission to have their comments published. These are below accompanied by the authors' responses.

VERSION 1 - REVIEW

REVIEWER	Arduino A Mangoni, Professor of Medicine of Old Age, University of Aberdeen
REVIEW RETURNED	29/06/2011

GENERAL COMMENTS	GENERAL COMMENTS This work provides a useful update on the important issue of long-term safety of oral folic acid supplementation in relation to cancer risk in different cohorts (not just pts with high cardiovascular risk). It would be of interest to the journal readership. That said, I find the data interpretation and reporting (results and discussion) quite biased towards the very limited, and of doubtful clinical significance, evidence of increased cancer incidence and mortality. Negative findings are downplayed throughout the manuscript. Even more disappointingly perhaps is the underreporting of results from observational studies. This is of concern because such studies provide valuable information, as they are not confined to strict RCTs criteria, and complement randomized studies. Answer: We agree. We have rephrased our interpretation of the data throughout the manuscript, changing moderate to borderline and including the results from the observational studies in a much more detailed manner, as well as showing negative results more clearly. It should be further stressed that an important limitation of the meta-analysis is the lack of information on dietary patterns in the studied populations. Significant differences in folate content among different food types (and food processing) might have influenced the reported findings.

	Answer: Yes we agree to this possibility, but nevertheless this lacking information in the clinical studies might be randomly distributed across the study populations. A better justification of why studies on food fortification were not considered is needed in both introduction and discussion. The results of RCTs/observational studies in Canada and USA are likely to be influenced by the introduction of mandatory food fortification, particularly when this occurred during the conduct of such studies. Cancer incidence and mortality should be compared between those studies in countries where mandatory folate fortification was implemented from those studies where folate fortification was not introduced. Answer: Two of the RCTs[12;13] were done in USA after introduction of fortification. Sensitivity analysis of those two compared to the studies performed in the countries without fortification, showed no significant difference between the groups (Figure 2c); i.e populations with fortification did not show increased cancer risk with folate supplements compared to studies performed in countries before fortification/ countries without fortification. SPECIFIC COMMENTS Abstract_results_a: 95% CI cancer incidence data for 1-2 relevant cancer types other than prostate (e.g. colon and breast) are required. Negative data, in addition to positive associations, should be reported. Answer: This has been performed in the revised manuscript. Abstract_results_b: for the same reason 95% CI total cancer mortality data should be reported. Answer: This has been performed in the revised manuscript. Abstract_results_c: once again, 95% CI data for cancer risk in observational studies must be reported. Answer: This has been reported in the results chapter. Abstract_conclusions_a: 'FA might moderately increase total cancer incidence' is clearly an overstatement. With an RR of 1.07 and CI of 1.00-1.14 the risk is very mild at best. Also, the conclusions should reflect the findings (negative) from observational studies. Answer: we have rephrased and added findings from observational studies (please see our answer under General Comments above). Abstract_conclusions_b: the conclusions on prostate cancer should also reflect the negative findings of observational studies. Answer: There was no observational studies reporting on prostate cancer in our included studies. However one of the excluded studies (Shannon 2009) (excluded due to daily folic acid dose <0.4 mg) has now been analyzed and commented on in the discussion part. This study showed no significant difference between the groups. (Please

	see our first answer to Reviewer 1 for more details). Abstract_conclusions_c: the rest of the para (starting with 'A major limitation....") can be removed as it's not relevant. Answer: Done. What this study adds: once again, the first bullet point ('moderately increase') is an overstatement. Answer: We agree and this has been updated in the revised version. Introduction_a: data on prevalence of folate deficiency would be useful. Answer: We have rephrased this paragraph. Results_a: a full list of observational studies considered should also be provided in Table format. Answer: Done, please see table 2a and 2b. Results_b: were there any differences in cancer incidence and mortality in studies with folic acid alone vs. combination with other B-vitamins? Answer: No difference, please see new text in results part. Results_c: ASA is thought to be protective. Were the results of studies on folate + acetylsalicylic acid any different? Answer: Two of the studies administered folic acid together with aspirin[14;15]. A sensitivity analysis of those studies showed a borderline significant increased risk in the folic acid groups compared to the control groups without aspirin (RR 1.43, 95% CI 1.00-2.03). Results_d: full results from observational studies should be presented in the text and Table/Figure format. This should include data on cancer incidence as well as cancer mortality. Answer: Done. However, none of the observational studies reported on mortality. Discussion_a: the first para is very biased towards the very limited positive findings of an association between folic acid and cancer. You cannot state that folate may increase mortality, when no such a link is reported in the results (!). As previously discussed data interpretation should be much more balanced and include the lack of association with the vast majority of cancer types + negative findings from observational studies. Answer: We have corrected this in the revised manuscript. Discussion_b: similar (major) revision is needed for the para "Our finding of a possible adverse effect from folic acid.... Answer: This paragraph has been deleted. Discussion_c: the first 2 sentences (page 14, "folate may play a dual role...") don't make any sense and should be revised.

Answer: This paragraph has been deleted.

Discussion_d: need also to explain why no associations were found for cancers other than prostate (and the potential biological mechanisms for the lack of such associations)
Answer: We have tried to explain this paragraph better in the updated version of the discussion.

Conclusions and policy implications: as previously discussed the first sentence does not reflect the findings of the study. Emphasis on negative findings is also needed.
Answer: This has been corrected in the revised manuscript.

Reference List

(1) Freudenheim JL, Marshall JR, Vena JE, Laughlin R, Brasure JR, Swanson MK et al. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. *J Natl Cancer Inst* 1996; 88(6):340-348.

(2) Maruti SS, Ulrich CM, White E. Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. *Am J Clin Nutr* 2009; 89(2):624-633.

(3) Oaks BM, Dodd KW, Meinhold CL, Jiao L, Church TR, Stolzenberg-Solomon RZ. Folate intake, post-folic acid grain fortification, and pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *The American journal of clinical nutrition* 2010; 91(2):449-455.

(4) Stolzenberg-Solomon RZ, Chang S-C, Leitzmann MF, Johnson KA, Johnson C, Buys SS et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr* 2006; 83(4):895-904.

(5) Slatore CG, Littman AJ, Au DH, Satia JA, White E. Long-term use of supplemental multivitamins, vitamin C, vitamin E, and folate does not reduce the risk of lung cancer. *Am J Respir Crit Care Med* 2008; 177(5):524-530.

(6) Skinner HG, Michaud DS, Giovannucci EL, Rimm EB, Stampfer MJ, Willett WC et al. A prospective study of folate intake and the risk of pancreatic cancer in men and women. *Am J Epidemiol* 2004; 160(3):248-258.

(7) Tjønneland A, Christensen J, Olsen A, Stripp C, Nissen SB, Overvad K et al. Folate intake, alcohol and risk of breast cancer among postmenopausal women in Denmark. *Eur J*

Clin Nutr 2006; 60(2):280-286.

(8) Shannon J, Phoutrides E, Palma A, Farris P, Peters L, Forester A et al. Folate intake and prostate cancer risk: a case-control study. *Nutr Cancer* 2009; 61(5):617-628.

(9) Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Micronutrient Intake and Risk of Urothelial Carcinoma in a Prospective Danish Cohort. *Eur Urol* 2009; 56(5):764-770.

(10) Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Source-specific effects of micronutrients in lung cancer prevention. *Lung Cancer* 2010; 67(3):275-281.

(11) Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Micronutrient intake and risk of colon and rectal cancer in a Danish cohort. *Cancer Epidemiology* 2010; 34(1):40-46.

(12) Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007; 298(10):1163-1170.

(13) Zhang SM, Cook NR, Albert CM, Gaziano JM, Buring JE, Manson JE. Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial. *JAMA : the journal of the American Medical Association* 2008; 300(17):2012-2021.

(14) Logan RFA, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. Aspirin and Folic Acid for the Prevention of Recurrent Colorectal Adenomas. *Gastroenterology* 2008; 134(1):29-38.

(15) Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA : the journal of the American Medical Association* 2007; 297(21):2351-2359.

VERSION 2 – REVIEW

REVIEWER	Arduino A Mangoni, Professor of Medicine of Old Age, University of Aberdeen
REVIEW RETURNED	25/11/2011

GENERAL COMMENTS	I think the authors have successfully addressed the points raised.

	The revised paper presents a more balanced interpretation of the available data.

REVIEWER	Pagona Lagiou Associate Professor of Hygiene and Epidemiology, University of Athens Medical School; Adjunct Professor of Epidemiology, Harvard School of Public Health
REVIEW RETURNED	29/11/2011

THE STUDY	Supplemental documents do not contain information that should be better reported in the manuscript.