Author’s response to reviews

Title: Use of skincare products and risk of cancer of the breast and endometrium: a prospective cohort study

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Author’s response to reviews:

Dear Reviewers,

Sincerest thanks for your comments and suggested improvements of our manuscript. We are grateful for the time you have spent on revising our manuscript and providing valuable comments.

Below, we have addressed the comments and revisions requested point-by-point. Changes in the manuscript are shown with track changes. The page and line numbers included below refer to the manuscript with track changes.

In addition to revisions according to your suggestions, we have added information about a newly published (September 2019) study about paraben concentrations in breast cancer cases and controls. (pp-5-6, ll. 97-99 and p.14, 311-317).

Reviewer #1:

In a previously published study, involving women from the Norwegian Women and Cancer cohort, the authors demonstrated that women who heavily used body cream products had elevated levels of several plasma parabens. In the current study, using the same population-based cohort, the researchers examined whether or not high use of the same body products was associated with an altered incidence of either breast or endometrial cancer. Average age of entry into the study was 54-55 years of age, and participants were followed, on average, for just over 10 years.
Covariates included in final regression models included numerous demographic and reproductive health factors associated with risk for developing breast and/or endometrial cancers. Analyses resulted in no positive associations between heavy use of skin lotions and development of either breast (premenopausal, postmenopausal, total, ER+, ER-) or endometrial cancers. A statistically non-significant inverse relationship between total skin lotion use and postmenopausal breast cancer was reported.

This is a clearly written, well-presented report. The authors have done a fine job introducing their study, and have presented an especially strong discussion of the limitations and contexts for understanding their epidemiological results in light of other, mainly laboratory studies which might have predicted different (positive association) results.

It is important to publish both positive and negative outcomes of major studies, and this report will be an important contribution to a complex literature on possible associations between exposures to potential endocrine disrupting compounds and risk for developing cancer.

Response: Thank you for your feedback. We fully agree that it is important to publish negative results as well.

Reviewer #2:

- On line 49 it says the questionnaire, "included questions on use of skincare products such as hand cream, facial cream and body lotion." Does this mean that there were other skincare products included in the questionnaire not included in this analysis? If so, why were these other products excluded?

Response: Thank you for pointing out this potential misunderstanding. In addition to questions on use of body lotion, facial cream and hand cream, our questionnaire included questions on frequency of perfume use and showering with and without soap. Furthermore we asked if the participants used sunscreen and during what time of the year it was used (never, up in the mountains during Easter, or during summer time). There were no questions about frequency of use of sunscreen.

In our previous publication of measured plasma concentrations of parabens in relation to self-reported use of skincare products (Sandanger et al. 2011), we observed no associations between perfume use or frequency of showering with soap and plasma concentrations of parabens. However, showering with soap was strongly correlated with use of body lotion, facial cream and hand cream. Because of the lack of associations, and the fact that soap is a rinse off product, we decided to focus the current manuscript on skincare lotions only, and not include perfume (beauty product).

Sunscreen is used highly irregular and only during short periods of the year in Norway due to our climate. Since our questionnaire contained no questions on frequency of sunscreen use or what parts of the body that was covered in sunscreen, it was impossible to identify heavy users of sunscreen. Due to these reasons, the present manuscript did not include use of sunscreen.

To clarify these points, we have rephrased the sentence referred to by the reviewer (p. 7, ll. 123-125)
and it now reads:

“Questionnaires that included questions about usage frequencies of hand cream, facial cream and body lotion were mailed to 192,648 women, of which 114,794 responded.”

• Authors say that questionnaires were distributed in waves. What were the differences in questions related to personal care product use by wave? What level of detail did this study include compared to the individual waves? Considering the very large number of participants, why not also look at some of the more detailed information if available?

Response: It is correct that the questionnaire in the whole NOWAC study was distributed in waves. The NOWAC study started including women in 1991 (p. 6, ll. 112-113). Since then, the cohort has been expanded several times by including new women (p. 6, ll. 117-118). In the present study, we have included all waves with questions regarding skincare product use and all available details on skincare product use (frequency). Thus, none of the waves contained any more details than described in this paper.

We have clarified this by adding one sentences on p. 7, l. 125:

“The questions regarding usage of skincare products were the same in all questionnaires.”

• The authors have not discussed measurement error in the exposure variable which may be important.

a. Did the questionnaire ask about which areas of the body women applied body lotion or did it just ask about general use?
b. Why is it assumed that using body lotion corresponds to complete coverage of the entire body (other than face and hands) when it may just be used on arms or legs and not also, for example, back and feet. Is it possible that women are mis-categorized as "heavy" users when they are actually "moderate users"? For example, if a woman reports using face, hand and body lotion twice per day, but only uses body lotion on her lower arms, exposure would be vastly overestimated. It looks like hand and face lotion makes up <10% of the body (Sandanger 2011), so there seems to be lots of room for measurement area by assuming use of body lotion equals 90% coverage.
c. What impact might potential measurement error have on the association/lack of association seen? This should be covered in the discussion section.

Response to a, b and c: Thank you for raising these important questions. We fully agree with the reviewer that potential misclassification deserves more attention in the manuscript.

As correctly pointed out, the participants did not report where they applied body lotion. They were only asked to report frequency of use. Therefore, we had to make some assumptions regarding the surface area covered with the different skin lotions, as the blood concentrations of EDs from skincare product use are influenced by the frequency of use and the percentage of body surface area that is covered in lotion. On this basis, we estimated “% skin covered in cream per day” by applying the The Lund and Browder Chart for skin burns that counts the body as 91% of total body surface. The same estimation was used in our previous publication by Sandanger et al. (2011), that showed strong positive associations between “% skin covered in cream” and plasma concentrations of parabens. Therefore, we
believe that this estimation will reflect blood concentrations of EDs from skincare product use better than only assessing the associations between frequency of use and cancer. However, we agree that this estimation may overestimate the actual percentage of skin covered in cream from body lotion, although we think that is less important as we already have confirmed that participants that reported the highest usage frequency of body lotion (which translated into the highest % skin covered in cream), had the highest plasma concentrations of parabens. Thus, we find it highly unlikely that the heavy and frequent users are not experiencing elevated plasma concentrations of parabens and likely other EDs from skincare product use.

To assess the associations between frequencies of skincare product use and cancer risk, we conducted several additional analyses that are included in the additional files. First, we estimated the associations between usage frequencies of body lotion and breast/endometrial cancer risk (Additional files 4). Then, we summarized the usage frequencies of hand cream, facial cream and body lotion and studied the associations with breast and endometrial cancer risk (Additional file 5). These analyses confirmed the results from the main analyses using % skin covered in cream, thus no associations between skincare products use and breast or endometrial cancer.

However, it is possible that some participants over- or under-reported their usage frequencies of skincare products. As all information was collected before cancer diagnosis, this measurement error is likely non-differential. In our age-adjusted models, assuming non-differential, nonsystematic errors, misclassification would attenuate the HR of the higher skincare category, but the test for trend would be valid. As we have several exposure categories and many confounding variables included in our multivariable models, non-differential misclassification can bias the estimates both towards and away from the null.

Based on the reviewer’s comments and the additional analyses we have made thorough revisions to the manuscript:

- Included information about the additional analyses in the methods section (p. 11, ll.225-231):

“Several additional analyses were performed. We assessed the associations between recorded usage frequencies of body lotion (categorized as never/seldom, 1-4 times/month, 2-6 times/week and 1≥2 times/day) and breast and endometrial cancer incidence. Furthermore, we summarized the usage frequencies of skincare products (scores from 0 [no use of any of skincare products] to 18 [use of hand cream, facial cream and body lotion ≥2 times/day]; categorized as low [0-5], moderate low [6-10], moderate high [11-14] and high [15-18]) and studied the associations with risk of breast and endometrial cancer.”

- Included the results from the additional analyses in the result section (p.13, ll.270-280):

“In the additional analyses, we found no significant association between usage frequencies of body lotion and the risk of pre- or postmenopausal breast cancer, endometrial cancer, ER+ or ER- breast cancer (body lotion: 0.30≤p-trend≤0.55) (Additional files 4). However, there was a significant inverse trend for usage frequencies of skincare products combined and postmenopausal breast cancer and ER+ breast cancer (0.02≤p-trend≤0.046), although we did not find significantly reduced HRs for any of the categories (postmenopausal breast cancer: moderate low versus low use: HR=1.01, 95% CI: 0.88,1.17; moderate high versus low use: HR= 0.94, 95% CI: 0.81,1.08; high versus low use: HR= 0.89, 95% CI: 0.77,1.03; ER+ breast cancer: moderate low versus low use: HR=1.07, 95% CI: 0.91,1.24; moderate
Revised the discussion (p. 18, ll. 396-412):

“The NOWAC questionnaires did not include questions about which part of the body that was covered in body lotion; therefore we assumed that one application of body lotion covered nearly the whole body (91%). This may be seen upon as a limitation as many women only use body lotion on arms and legs, suggesting that our measure “% skin covered in cream per day” may overestimate the exposure. However, we have previously shown that “% skin covered in cream per day” was strongly associated with plasma concentrations of parabens within the NOWAC cohort (24), and we believe that this measure better reflect blood concentrations of EDs from skincare product use than the frequency of use. Nevertheless, we conducted several additional analyses of reported usage frequencies of skincare products (Additional file 4-5). These analyses confirmed our main results, i.e. no increased risk of breast or endometrial cancer by frequent use of skincare products. Still, it is possible that some NOWAC participants over- or under-reported their usage frequencies of skincare products. As all information was collected before cancer diagnosis, this measurement error is likely non-differential. In our age-adjusted models, assuming non-differential, nonsystematic errors, misclassification would attenuate the HR of the higher skincare category, but the test for trend would be valid (50). As we have several exposure categories and many confounding variables included in our multivariable models, non-differential misclassification can bias the estimates both towards and away from the null (51).”

In the discussion, authors say "our exposure measure "% body surface creamed per day" does not differentiate between frequent low exposure (e.g. daily application of facial cream) and less frequent higher exposure (e.g. use of body lotion once a week) which may be important if dermal absorption of EDs on specific areas is more relevant for breast/endometrial cancer than continuously elevated systemic concentrations."

This is an important point. Because the data is available, why not conduct a secondary analysis looking at the frequency of skincare product use (total and stratified by location and breast/endometrial cancer use)?

Response: Unfortunately, we have no information about location where the body lotion/creams were applied. To avoid misunderstandings, we have now emphasized this in the discussion on p. 18, ll. 396-398:

“The NOWAC questionnaires did not include questions about which part of the body that was covered in body lotion; therefore we assumed that one application of body lotion covered nearly the whole body (91%).”

As described above, we have now conducted several additional analyses on usage frequencies of skincare products (Additional files 4-5).

In the discussion, the authors make comparisons with Taylor et al. Taylor et al 2017 and 2018 included nine different skincare products (cleansing cream, anti-aging cream, body lotion, hand lotion,
face cream, foot cream, petroleum jelly, talcum powder applied under arms, and talcum powder applied elsewhere) compared to this study's three. Author's should mention these differences in their discussion and address whether this may have contributed to the differences in results.

Response: Thank you for pointing this out. We have revised the discussion accordingly, pp. 14, ll. 302-310:

“Notably, Taylor and colleagues included nine different skincare products (face cream, cleansing cream, anti-aging cream, foot cream, body lotion, hand lotion, petroleum jelly, and two different talcum powders) in comparison to three in the present study, which could explain differences in results. In the analyses stratified by breast cancer type, Taylor et al. observed a borderline significant increased risk of in situ breast cancer among frequent users of the nine skincare products compared to infrequent users, which may suggest that the association with skincare product use may be different in in situ and invasive breast cancer. Additionally, skincare product constituents in the United States and in Europe may vary due to differences in regulations, which could also affect the results. “

• Other than PCPs being a source of both parabens and phthalates, other potential endocrine disrupting chemicals found in PCPs, and mixtures in general, are not mentioned by the authors. This is a particularly important topic, as these products are inherently chemical mixtures.

Response: We agree with the reviewers and have revised the introduction and discussion accordingly.

Introduction, pp.4-5, ll. 59-75:

“Among frequently used EDs in PCPs are phthalates, ultraviolet (UV) filters, triclosan and parabens (5). Phthalates are also commonly used as softeners in consumer products such as food packaging material, children’s toys and building material (6) and have been suggested to interfere with the testosterone production or action (2). Recently, phthalates have shown anti-estrogenic effect in breast cancer cell lines (7) and induced cell proliferation in normal breast cells (8). However, recent epidemiological studies reported no significant association between phthalate exposure and breast cancer (9, 10). UV-filters are a large group of compounds used as constituents in sunscreen as they are able to absorb UV-radiation. They are also included in other PCPs to increase shelf-life (2), and have been detected in human urine (11, 12), breastmilk (13) and breast tissue (14). Many UV-filters exert estrogenic activity in in vivo/in vitro experiments (15), however epidemiological studies of endocrine disrupting effects of UV-filters are rare. Triclosan is primarily used as an antimicrobial agent in soap, toothpaste, cosmetics and pharmaceuticals and has shown endocrine disrupting properties in experimental settings (2). Prenatal triclosan concentrations was recently reported associated with earlier menarche in American girls, whereas there was no effect of prenatal or peripubertal concentrations of the UV filter Benzophenone-3 (16).”

Introduction, p. 5, ll. 90-93:

“Other studies have found similar results (25), and there are reason to believe that women who use skincare products frequently experience higher body burdens of other EDs as well, for instance phthalates, UV filters and triclosan (12, 26).”

Discussion, pp. 16-17, ll. 369-380:
“Our previous study of plasma concentrations of parabens in relation to reported use of skincare products confirmed that individuals classified as frequent or heavy users of skincare products experience elevated circulatory concentrations of methyl-, ethyl- and propyl parabens compared to the non-users (24). Philippat et al. also confirmed a positive association between the number of PCPs used and increasing urinary paraben concentrations. They further suggested that questionnaires can be used to measure exposure to parabens (12). However, skincare products are not only sources of parabens; they are sources of other EDs with different properties and mechanisms of action, individually and as mixtures. As we have not measured other EDs than parabens in plasma from skincare products users in NOWAC, we cannot be certain that those classified as frequent or heavy users also experienced the highest concentrations of other EDs such as phthalates, UV filters and triclosan that individually or combined may exert estrogenic activity.”

• Authors say, "In fact, an inverse relationship between skincare product use modelled in continuous scale and postmenopausal breast cancer risk was observed, which may indicate that skincare product use is a proxy marker for other beneficial behavior that our questionnaire did not cover."

Would authors explain what other beneficial behavior skincare product use may be a proxy for?

Response: Thank you for highlighting an unclear sentence. Our analyses adjust for well-known risk factors for breast and endometrial cancer. Even though we find it highly unlikely, we cannot fully rule out residual confounding as frequent/heavy users of skincare products could have a certain beneficial behavior that our questionnaire did not cover, which could explain the inverse association between skincare products use (modelled in continuous scale with restricted cubic splines) and postmenopausal breast cancer. From previous research, we know for instance that women in NOWAC that uses skincare products frequently also shower often. It could be possible that these women are more likely to detect breast tumors as they touch their breasts more often during showering/application of body lotion than those not showering or using skincare products as frequent. To avoid misunderstandings, we have rephrased this sentence.

The sentence (pp. 12-13, ll. 291-294) now reads:

“ In fact, an inverse relationship between skincare product use modelled in continuous scale with restricted cubic splines and postmenopausal breast cancer risk was observed, which could be a chance finding or a result of residual confounding."

• Authors say, "In fact, given the advanced age of the study participants, it is likely that their use of skincare products during childhood and adolescence were lower than what is common among children and adolescents today."

Why would these study participants be expected to use less skincare products during childhood than children and adolescents today?

Response: The study participants were born in the 1940’s and 50’s, meaning they were children and adolescents in the 1950’s and 60’s. By that time, the Norwegian household economy was substantially weaker than nowadays, and there were not the same availability to a large number of different skincare products as it is today. We can therefore safely assume that our participants did not use skincare
products as frequent as children and adolescents that grow up today.

To clarify this, we have revised this sentence (p. 17, ll. 389-394), and it now reads:

“In fact, given the advanced age of the study participants, it is likely that their use of skincare products during childhood and adolescence were lower than what is common among children and adolescents today as the general Norwegian household economy was much weaker in the 1950’s and 60’s than nowadays and the availability to skincare products were much more limited.”

We look forward to your response to the revised manuscript. We would be glad to respond to any further questions and comments that you may have.

On behalf of the authors,
Charlotta Rylander