PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Isotretinoin exposure during pregnancy: a population-based study in the Netherlands
AUTHORS	Zomerdijk, Ingeborg; Ruiter, Rikje; Houweling, Leanne; Herings, Ron; Sturkenboom, Miriam; Straus, Sabine; Stricker, Bruno

VERSION 1 - REVIEW

REVIEWER	Christof Schaefer Pharmakovigilanzzentrum Embryonaltoxikologie Charité-Universitätsmedizin Berlin
REVIEW RETURNED	11-Jul-2014

GENERAL COMMENTS	The authors deal with the important issue of failure of pregnancy prevention programs (PPP) in context with the established teratogen isotretinoin. To conclude, the submitted paper is worth to be published in a shorter, succinct format to illustrate the failure of the isotretinoin PPP. Data on pregnancy outcome should be presented as secondary study goals in a much more comprehensive way addressing the critical aspects discussed below including separate evaluation of birth defects and spontaneous fetal deaths. The study approach using nationwide prescription/dispensing data may allow an estimate of the extent of failure of the PPP. For assessing embryotoxic or teratogenic risks these data are not really suitable. Essential information is lacking, such as precise time of exposure, infant's diagnosis, early pregnancy loss until week 16, maternal characteristics potentially relevant for pregnancy outcome. In addition, the no. of 28/45 women exposed during the first trimester is too low to reach adequate power. The results are based on only 45 exposed pregnancies documented between 1999 and 2007. Why has the study period been limited to 2007? It should be critically discussed that congenital anomaly rates were only adjusted for maternal age and not for additional contributing factors such as smoking, alcohol and concomitant medication. There is no rationale to combine fetal deaths and birth defects in one outcome variable. Even if spontaneous abortions and birth defects are both considered as "embryotoxic effects", evaluation should be performed separately. Furthermore, evaluation of fetal loss rates should consider spontaneous abortions/fetal deaths and ETOPs as competing risks. Assessing the PPP failure rate, exclusion of pregnancies until week 16 may substantially under-estimate the real failure rate. Other studies have shown that up to or even more than 70% of pregnancies with isotretinoin exposure were lost either spontaneously or by elective termination. Most of the ETOPs were performed until week 16. Since some of the ETOPs followed diagnostics of birth defects, the exclusion of pregnancies <17 weeks

	may also result in an underestimation of the risk of birth defects. With the exception of roughly estimating the occurrence of PPP failure, the author's statement is not correct that their data are superior to data from teratology information services (TIS). In contrary, TIS data on maternal characteristics and pregnancy outcome are much more detailed since TIS have the possibility to check the quality and plausibility of the clinical information due to their position being involved in the treatment. In addition, exposure data including concomitant medication are collected by TIS close to the real exposure time making recall and documentation bias less likely. Their concept of comparison cohorts including disease comparison cohorts ascertained with similar procedures may even allow controlling for (confounding) disease effects on pregnancy outcome. Specifics: P 4, L 10: a potential is no effect, replace by "…is an important characteristic of isotretinoin." L 22-23: It is debatable whether only exposure beyond week 10 leads to CNS defects and whether the risk is 40%. Revise sentence accordingly. P 6, L 23/24: Revise sentence "In case…" L 33 should read: Drug exposure interval P 6; L 46: make clearer what is meant with "but gaps…were not permitted in one period" P 8, L 28 must read >39 weeks (instead of >40) L 35 must read: including 45… and 6 pregnancies conceived within… P 9, L 11 and figure 2: were figures in 2007 lower than in 2006 because the study period was limited to September? If so, add a note. P 9, L 31 must read 95% CI 3.54… P 12: Shorten the paragraph "Description of cases" because information is scarce, i.e. exposure has not been confirmed in particular in cases of non-sequential prescriptions, no details on fetal death (spontaneous or ETOP for personal reasons or after prenatal diagnostics), no details on birth defects. Again: fetal deaths and congenital anomalies should be evaluated separately. Years of conception do not add important information. L 43/44 delete sentence "in the majority..." because it is repeated in L 56 P 13, L 1: add recommended before maximum L 43: explain the meaning of "…contribute…system." P 14, L 9: delete the sentence "Nevertheless, …" because it adds no specific information in this context. L 15 must read septal defect L21-25: Move sentence to next paragraph because this is a major weakness of the study. L 28-50: Weaknesses of study design are insufficiently discussed. The authors should underline that isotretinoin exposure cannot be confirmed using dispensing data, neither in general nor for specific time intervals. Therefore, accurate "exposure" rates cannot be concluded from these data. Revise 1st bullet point page 3 accordingly. L 44: I recommend deleting the statement that "…they confirm the undisputed adverse reaction", because of the rather low power of the study and the weakness in case description. Make clear whether cases <17 weeks (as stated on P 6, L 36) or <16 weeks (P 14, L 49) are excluded. L 42: In spite of prospective procedure data may be biased due to

	the well-known risk (e.g. detection bias based on more careful diagnostics in exposed pregnancies/newborns). P 15, L41: add “additional” before regulatory. Titles of figure 1 and 2: Replace per calendar year by “by calendar year” Skip Table S1. It does not add specific information.

REVIEWER	Christina Chambers University of California San Diego, La Jolla, CA USA
REVIEW RETURNED	18-Jul-2014

GENERAL COMMENTS	The authors address an important and continuing worldwide problem for the few medications that such as isotretinoin that carry a known high risk of teratogenicity. The major emphasis and value of this paper is to demonstrate that despite pregnant prevention programs that are quite stringent, it appears that a substantial number of exposed pregnancies are still occurring. However, the main limitation in the presentation of results is that this study cannot propose to address the issue of congenital anomalies. The vast majority of congenital anomalies occurring in these pregnancies would be occurring in pregnancies not ascertained in this type of analysis (and thus the lower than expected rate) - and no congenital anomalies that are consistent with the known embryopathy. I would suggest that the authors focus on stillbirth, which is an important and unique finding, and not lump fetal deaths with congenital anomalies (although there appears to be one case categorized in both). The authors should also make clear whether any of the 2 sets of twins resulted in fetal death. Other minor points that should be addressed are the improper use of the wording regarding exposure timing. The authors cannot know if drug dispensed was actually taken as prescribed, so the exposure itself as well as exposure timing is being estimated. Finally, it would be very helpful to include in this paper the number of women of reproductive age during this time period who were prescribed isotretinoin to put into perspective what percent of potentially pregnant women did not get pregnant.

REVIEWER	Edward J. Lammer, MD UCSF Benioff Children's Hospital Oakland Oakland, CA USA
REVIEW RETURNED	28-Jul-2014

GENERAL COMMENTS	This MS reports results of a Dutch population-based cohort study of isotretinoin use in the month before, and after, conception, using data from linked Dutch registries of pharmaceutical dispensing and pregnancy outcomes. The MS provides useful information showing very poor compliance with the PPP for isotretinoin in Nederlands. The number of “exposed” pregnancies, however, is too small to add any new information to what is already known about the magnitude of risks for either fetal death or major malformations. Appropriately, the conclusions focus on the PPP program and apparent poor compliance with its features, rather than risks for adverse outcomes of pregnancy, for which this dataset lacks adequate numbers of postconceptionally exposed pregnancies and adequate

	assessments for malformations (which is lacking for all of the fetal deaths that were identified). Comments: Introduction 1. The use of “during the first 10 weeks of pregnancy” is confusing because it is not clear if the authors mean 10 weeks after the LMP or 10 weeks after conception. 2. Recommend deleting the sentence that starts with “Exposure beyond the first 10 weeks … in approximately 40% of live births” -- because this is a confusing statement. Do the authors mean exposures that start beyond 10 weeks? Or do the authors mean exposures that start before 10 weeks and continue beyond 10 weeks? Either way, the statement is not supported in reference #3 or 4. 3. The data in reference #4 is not independent of the data in reference #3 and most of the data in reference #4 was provided by the authors of reference #3. Recommend deleting reference #4 from the MS. 4. Risk for spontaneous abortion is dependent on the gestational age at which pregnancies are ascertained. If pregnancies are not identified early, the risk for abortion will appear incorrectly low because most of them will have occurred before ascertainment. The correct number for the risk for spontaneous abortion is 40%, for pregnancies that are ascertained before GA week 13. In our studies, every spontaneous abortion associated with isotretinoin exposure occurred before 15 weeks GA, so none of these adverse outcomes would be found >16 weeks. The comment on page14 line 47 could be changed so that it is clear that the authors’ results definitely underestimate risk for spontaneous abortion, because in our experience, these outcomes have all occurred before 16 weeks GA. Methods 1. Regarding use of isotretinoin that ends before conception, there is no evidence that such exposures cause adverse outcomes of pregnancy. In fact, there is no demonstrated risk for mothers who take isotretinoin after conception but stop before 15 days after conception. So, it is not recommended to perform analyses that combine preconceptional exposures with postconceptional exposures. It would be helpful in the Discussion, to mention the half life of isotretinoin and the relevance of this information for considering the extremely unlikely chance that there might be risk from preconceptional exposures. 2. Page 7 lines 1-2. The definition of fetal death should include consideration of gestational age. Many countries do not label deaths between 17 and 20 weeks as fetal deaths. Results 1. page 12 line 22. It would be more informative to know which day after conception that isotretinoin exposure began, rather than that it was used for 19 days in the first trimester. That information is not useful without knowing which 19 days within the first trimester during which the drug was taken. 2. I am aware of one infant with spina bifida associated with isotretinoin exposure, but that mother took the drug after conception. It is highly unlikely that the case observed by the authors in which isotretinoin was used only preconceptionally is related to the drug exposure. Discussion

	1. The authors' conclusion is that "the PPP is not completely effective" is an understatement. Recommend language that is much stronger. The compliance looks terrible. 2. Page 14 line 15. Change "symptom" to "feature" and recommend deleting "such as ventricular septal defect". 3. Page 15 line 10. "reasons for PPP failure cannot be identified'. I do not understand this conclusion. If prescribing physicians performed a pregnancy test on every female who was prescribed isotretinoin, 60% of the exposures (women who were already pregnant) would have been avoided. Such exposures are probably the easiest to prevent.

VERSION 1 – AUTHOR RESPONSE

Comments from reviewer 1:

Christof Schaefer, Institution and Country Pharmakovigilanzzentrum Embryonaltoxikologie, Charité-Universitätsmedizin Berlin, Spandauer Damm, Germany.

General comments:

1. The authors deal with the important issue of failure of pregnancy prevention programs (PPP) in context with the established teratogen isotretinoin. To conclude, the submitted paper is worth to be published in a shorter, succinct format to illustrate the failure of the isotretinoin PPP. Data on pregnancy outcome should be presented as secondary study goals in a much more comprehensive way addressing the critical aspects discussed below including separate evaluation of birth defects and spontaneous fetal deaths.

Author's response: The main message of the manuscript is the failure of the PPP and we agree with the reviewer that the emphasis of the manuscript should be on this issue. The analysis of adverse fetal outcomes is addressed as a secondary objective of this study, to show that despite the implemented PPP adverse fetal outcome still occur in isotretinoin exposed pregnancies that were potentially preventable. The information on adverse fetal outcomes is presented in a concise way, and placed in the context of the limitations of the data that are used. The manuscript has been adapted accordingly.

2. The study approach using nationwide prescription/dispensing data may allow an estimate of the extent of failure of the PPP. For assessing embryotoxic or teratogenic risks these data are not really suitable. Essential information is lacking, such as precise time of exposure, infant's diagnosis, early pregnancy loss until week 16, maternal characteristics potentially relevant for pregnancy outcome. In addition, the no. of 28/45 women exposed during the first trimester is too low to reach adequate power. The results are based on only 45 exposed pregnancies documented between 1999 and 2007. Why has the study period been limited to 2007?

Author's response: The comments of the reviewer are acknowledged and the data currently available may not be adequate to study teratogenic risk of isotretinoin. However, the aim of our study was not to confirm the teratogenic effect of isotretinoin, but to show that isotretinoin exposure during pregnancy still occurs, and that this still leads to an increased risk of adverse fetal outcomes. The limitations of the data sources have been addressed in the Discussion section, and better described in the first bullet point.

Unfortunately, the study period is limited to 2007 since the linkage between the PRN database and the PHARMO Database Network is only available until 2007.

3. It should be critically discussed that congenital anomaly rates were only adjusted for maternal age and not for additional contributing factors such as smoking, alcohol and concomitant medication.

Author's response: This is a very good point indeed although it should be emphasized that the paper did not intend to prove that isotretinoin may cause fetal death and congenital malformations but that – despite national PPP – such events still happen. Indeed, it is known that apart from maternal age there are other important confounders that should be taken into account when studying congenital anomalies. Unfortunately, many potential confounders are not registered in the linked databases. Moreover, since the absolute numbers in our study are small including a low number of cases, we could only adjust for one factor. The need to consider the other important confounding factors is addressed in the discussion section.

4. There is no rationale to combine fetal deaths and birth defects in one outcome variable. Even if spontaneous abortions and birth defects are both considered as "embryotoxic effects", evaluation should be performed separately. Furthermore, evaluation of fetal loss rates should consider spontaneous abortions/fetal deaths and ETOPs as competing risks.

Author's response: The reviewer addresses a relevant comment regarding the competing risks of spontaneous abortions, ETOPs and fetal deaths. Unfortunately, we were not able to perform separate analyses for spontaneous abortions since those prior to gestational age of 16 weeks are not covered in the PRN database. Elective termination of pregnancies (ETOPs) are also not included in the cohort since these occurred before a gestational age of 16 weeks. Consequently, they are not included in the PRN database and elective abortions can only be performed in the specialised abortion centers and those people/centers do not report pregnancies to the PRN and midwifes are generally not involved. Therefore, we could not consider spontaneous abortions and elective abortions in our study, which is now addressed in the discussion section.

However, the primary aim of this study is not to estimate the teratogenic risk of isotretinoin, but to show that isotretinoin exposed pregnancies still occur and that some of these result in an adverse fetal outcome. Also considering the missing information on spontaneous abortions and ETOPs during the first trimester estimating the teratogenic risks using the available data would be biased. Since risk estimates for specific embryotoxic effects cannot be provided with the data available, we decided to only present combined analysis of adverse fetal and neonatal events (including all intrauterine deaths ≥ 16 weeks of gestation and congenital malformations) and removed the separated analysis of fetal death and congenital anomalies from the manuscript to focus completely on the main message, i.e. that despite a nationwide PPP adverse isotretinoin-associated fetal outcomes still occur.

5. Assessing the PPP failure rate, exclusion of pregnancies until week 16 may substantially under-estimate the real failure rate. Other studies have shown that up to or even more than 70% of pregnancies with isotretinoin exposure were lost either spontaneously or by elective termination. Most of the ETOPs were performed until week 16. Since some of the ETOPs followed diagnostics of birth defects, the exclusion of pregnancies <17 weeks may also result in an underestimation of the risk of birth defects.

Author's response: We agree with this comment of the reviewer, and now addressed it in the discussion section. Both the spontaneous abortions and elective termination of pregnancies (ETOPs) that occurred until week 16 are not covered in our data. ETOPs performed after week 16 are not likely to be included in the database. This is an important limitation with regard to the estimated number of isotretinoin-exposed pregnancies and the observed adverse fetal events, which might both be underestimated. This is emphasized in the discussion section. Of course, the main message remains, i.e. that despite the PPP, such isotretinoin exposure during pregnancy and adverse fetal events still happen [and probably even at a higher rate].

6. With the exception of roughly estimating the occurrence of PPP failure, the author's statement is not correct that their data are superior to data from teratology information services (TIS). In contrary, TIS data on maternal characteristics and pregnancy outcome are much more detailed since TIS have the possibility to check the quality and plausibility of the clinical information due to their position being involved in the treatment. In addition, exposure data including concomitant medication are collected by TIS close to the real exposure time making recall and documentation bias less likely. Their concept of comparison cohorts including disease comparison cohorts ascertained with similar procedures may even allow controlling for (confounding) disease effects on pregnancy outcome.

Author's response. The point that we intended to raise was that when using a large cohort of pregnant women, the isotretinoin exposure rate can be estimated on a population based level, which may be more difficult using TIS data since not all pregnancies are included in the TIS system but only those that were reported for a specific reason. However, as our objective was not to study and compare both approaches, the statement has been removed from the manuscript.

Specific comments:

7. P 4, L 10: a potential is no effect, replace by "…is an important characteristic of isotretinoin."

Author's response: The text has been adapted as suggested.

8. L 22-23: It is debatable whether only exposure beyond week 10 leads to CNS defects and whether the risk is 40%. Revise sentence accordingly.

Author's response: The statement on the developmental and other CNS effects after isotretinoin exposure beyond the first 10 weeks of pregnancy has been removed from the manuscript as suggested by the other reviewer.

9. P 6, L 23/24: Revise sentence "In case…"

Author's response: The sentence has been revised and now reads: "In case isotretinoin dispensings that were pooled together had different lengths, the length of the single dispensing with the longest duration was used."

10. L 33 should read: Drug exposure interval

Author's response: The text has been adapted as suggested.

11. P 6; L 46: make clearer what is meant with "but gaps…were not permitted in one period"

Author's response: Gaps, isotretinoin free periods between two isotretinoin dispensings, were not permitted meaning that an isotretinoin exposure period ends once an isotretinoin free period was identified. The sentence has been revised explaining what is meant with gaps and how we dealt with gaps between two isotretinoin dispensings.

12. P 8, L 28 must read >39 weeks (instead of >40)

Author's response: The text has been adapted as suggested.

13. L 35 must read: including 45… and 6 pregnancies conceived within…

Author's response: The text has been adapted as suggested.

14. P 9, L 11 and figure 2: were figures in 2007 lower than in 2006 because the study period was limited to September? If so, add a note.

Author's response: No this is not the case. Instead of presenting absolute numbers of pregnancies per year, we calculated the number of pregnancies per 10,000 pregnancies for which the shorter period in 2007 could be taken into account.

15. P 9, L 31 must read 95% CI 3.54…

Author's response: The text has been adapted accordingly.

16. P 12: Shorten the paragraph "Description of cases" because information is scarce, i.e. exposure has not been confirmed in particular in cases of non-sequential prescriptions, no details on fetal death (spontaneous or ETOP for personal reasons or after prenatal diagnostics), no details on birth defects. Again: fetal deaths and congenital anomalies should be evaluated separately. Years of conception do not add important information.

Author's response: The paragraph that described the cases of adverse fetal events that were potentially exposed to isotretinoin shortly before or during pregnancy has been removed from the manuscript. The information available has been presented in the footnotes of table 3.

To emphasize that isotretinoin exposure cannot be ascertained based on only drug dispensing data, we used the wording "potential isotretinoin exposure" throughout the manuscript and also addressed this again in the discussion section.

17. L 43/44 delete sentence "in the majority..." because it is repeated in L 56

Author's response: The sentence has been deleted as suggested by the reviewer.

18. P 13, L 1: add recommended before maximum

Author's response: The text has been adapted accordingly.

19. L 43: explain the meaning of "…contribute…system."

Author's response: iPLEDGE is a pregnancy prevention programme in the US which consists of strict requirements for all parties in the distribution chain (isotretinoin prescribers, pharmacies that dispense isotretinoin, and wholesalers that distribute isotretinoin) and all patients (male and female) receiving prescriptions. All these parties have to register in the iPLEDGE database and regularly update and check information on the teratogenic knowledge, pregnancy status and the contraceptive measures that are used prior to isotretinoin dispensing. This basically means that they have to do this on a monthly basis. Considering the fact that added value of these requirements has not yet been demonstrated, these requirements and tasks may add unnecessary burden to the healthcare system. The corresponding sentence in the manuscript has been updated and addresses this issue now more clearly.

20. P 14, L 9: delete the sentence "Nevertheless, …" because it adds no specific information in this context.

Author's response: We agree with the reviewer and the sentence has been removed.

21. L 15 must read septal defect

Author's response: The text has been adapted accordingly.

22. L21-25: Move sentence to next paragraph because this is a major weakness of the study.

Author's response: The paragraph on strengths and weaknesses of the study has been rewritten including the sentence of the previous paragraph.

23. L 28-50: Weaknesses of study design are insufficiently discussed. The authors should underline that isotretinoin exposure cannot be confirmed using dispensing data, neither in general nor for specific time intervals. Therefore, accurate "exposure" rates cannot be concluded from these data. Revise 1st bullet point page 3 accordingly.

Author's response: It should be emphasized that we have individualized [anonymous] data showing that those women who filled prescriptions for isotretinoin came back for refills. In our experience, people coming back for refills, really use the drug. Nevertheless, the limitations of the study with regard to drug exposure and pregnancy outcome have been more strongly emphasized in the paragraph on study limitations and better described in the first bullet point.

24. L 44: I recommend deleting the statement that "…they confirm the undisputed adverse reaction", because of the rather low power of the study and the weakness in case description.

Author's response: The sentence has been rephrased as: "….are in line with the undisputed embryotoxicity of isotretinoin and indicate that such events still occur despite the implemented PPP." because the causal relationship between isotretinoin is generally considered as certain and our findings are in line with that, even if the risk estimator is imprecise.

25. Make clear whether cases <17 weeks (as stated on P 6, L 36) or <16 weeks (P 14, L 49) are excluded.

Author's response: Pregnancies with gestational age <16 weeks were not included in the cohort. The sentence on page 6 has been adapted accordingly.

26. L 42: In spite of prospective procedure data may be biased due to the well-known risk (e.g. detection bias based on more careful diagnostics in exposed pregnancies/newborns).

Author's response: The potential for detection bias is addressed in the amended section on strengths and limitations of the study and data sources.

27. P 15, L41: add "additional" before regulatory.

Author's response: The text has been adapted accordingly.

28. Titles of figure 1 and 2: Replace per calendar year by "by calendar year"

Author's response: The titles of the figures have been adapted as suggested.

29. Skip Table S1. It does not add specific information.

Author's response: The table SI (supplementary information) presented a description of the classification system for congenital anomalies used in our study has been removed, as well as the reference to this table in the Methods section.

Reviewer 2:

Christina Chambers, Institution and Country University of California San Diego, La Jolla, CA, USA

General comments:

The authors address an important and continuing worldwide problem for the few medications that such as isotretinoin that carry a known high risk of teratogenicity.

1. The major emphasis and value of this paper is to demonstrate that despite pregnant prevention programs that are quite stringent, it appears that a substantial number of exposed pregnancies are still occurring. However, the main limitation in the presentation of results is that this study cannot propose to address the issue of congenital anomalies. The vast majority of congenital anomalies occurring in these pregnancies would be occurring in pregnancies not ascertained in this type of analysis (and thus the lower than expected rate) - and no congenital anomalies that are consistent with the known embryopathy. I would suggest that the authors focus on stillbirth, which is an important and unique finding, and not lump fetal deaths with congenital anomalies (although there appears to be one case categorized in both). The authors should also make clear whether any of the 2 sets of twins resulted in fetal death.

Author's response: We thank the reviewer for her comments. It is agreed that the main findings of our study are the dispensing of isotretinoin to pregnant women despite the pregnancy prevention programme. In the revised version of the manuscript we focused on these findings. Because spontaneous abortions and elective abortions until the 16th week of gestation are not included in our cohort and detailed information on the adverse fetal cases is lacking, we could not adequately study specific teratogenic risks of isotretinoin, which we addressed in the section on strengths and weaknesses of our study. However, this study was not intended to estimate the teratogenic risk of isotretinoin, but to show that exposure during pregnancy still occurs, and that some of these pregnancies even result in adverse fetal outcomes. The separated analyses are therefore removed from the manuscript and the adverse fetal outcomes are presented in a more concise way.

The cases that were potentially exposed to isotretinoin in the 30 days before and during pregnancy did not concern multiple births. This has been added to the manuscript.

2. Other minor points that should be addressed are the improper use of the wording regarding exposure timing. The authors cannot know if drug dispensed was actually taken as prescribed, so the exposure itself as well as exposure timing is being estimated.

Author's response: A well known limitation of using drug dispensing data is that it cannot be ascertained that the isotretinoin has been taken as prescribed. It should be emphasized that we have individualized [anonymous] data showing that those women who filled prescriptions for isotretinoin came back for refills (32 of the 40 women that received isotretinoin during pregnancy received it more than once). In our experience, such people really use the drug. Nevertheless, the fact that we are not sure whether the dispensed isotretinoin exposure is taken is also addressed as a study limitation. We adapted the terminology used to describe the "potential" isotretinoin exposure as we only use the dispensing of drugs and have no confirmation on the actual use of the isotretinoin. The methods, results and discussion section have been updated accordingly.

3. Finally, it would be very helpful to include in this paper the number of women of reproductive age during this time period who were prescribed isotretinoin to put into perspective what percent of potentially pregnant women did not get pregnant.

Author's response: Earlier, we studied how many women of childbearing age used isotretinoin without concomitant contraception and found that the use of concomitant contraception during isotretinoin use was limited (59%), indicating that pregnancies may occur. [Teichert M, Visser LE, Dufour M,

Rodenburg E, Straus SM, De Smet PA, Stricker BH. Isotretinoin use and compliance with the Dutch Pregnancy Prevention Programme: a retrospective cohort study in females of reproductive age using pharmacy dispensing data. Drug Saf 2010;33:315-26]. The current study demonstrates that pregnancies indeed occur in female isotretinoin users.
The proportion of female isotretinoin users "at risk of getting pregnant" which does not get pregnant is an interesting outcome to further estimate the effectiveness of the PPP in the Netherlands. Unfortunately, we were not able to study this since the cohort of pregnant women does not provide information on women of reproductive age who received isotretinoin and did not get pregnant. Furthermore, estimating the number women who did not become pregnant remains uncertain since pregnancies that ended before 16th week of gestation are not captured in the PRN database.

Reviewer 3:
Edward J. Lammer, MD, Institution and Country UCSF Benioff Children's Hospital Oakland, Oakland, CA, USA.

General comments:

This manuscript reports results of a population-based cohort study of isotretinoin use just before, and after, conception, using data from linked Dutch registries of pharmaceutical dispensing and pregnancy outcomes.

1. The results are mainly about frequency of likely isotretinoin use during pregnancy and utilization, or lack thereof, of the pregnancy prevention program. Results show rather poor compliance with the prevention program – a phenomenum that has been demonstrated repeatedly in several countries. The number of exposed pregnancies is small and does not allow inferences about the magnitude of risk for malformations or fetal death, since no malformed liveborn infants were identified. The research has value as far as demonstrating the poor compliance with the prevention program. It does not contribute any new information about magnitude of risk for adverse outcomes of pregnancy. The authors appropriately focus on the former in the Discussion section. Recommend acceptance with revisions.

Author's response: It is agreed that the main findings of our study are the dispensing of the well-known teratogen isotretinoin during pregnancy despite a pregnancy prevention program. In the revised version of the manuscript we focused on these findings, and presented information on adverse fetal outcomes in a more concise way. With the data available we are not able to study the specific teratogenic risks of isotretinoin, which we also addressed in the section on strengths and limitations of our study. However, the aim of our study was not to confirm the teratogenic effect of isotretinoin, but to show that isotretinoin exposure during pregnancy still occurs, and that this still leads to an increased risk of adverse fetal outcomes.

2. This manuscript reports results of a Dutch population-based cohort study of isotretinoin use in the month before, and after, conception, using data from linked Dutch registries of pharmaceutical dispensing and pregnancy outcomes. The manuscript provides useful information showing very poor compliance with the PPP for isotretinoin in Nederlands. The number of "exposed" pregnancies, however, is too small to add any new information to what is already known about the magnitude of risks for either fetal death or major malformations. Appropriately, the conclusions focus on the PPP program and apparent poor compliance with its features, rather than risks for adverse outcomes of pregnancy, for which this dataset lacks adequate numbers of postconceptionally exposed pregnancies and adequate assessments for malformations (which is lacking for all of the fetal deaths that were identified).

Author's response: The limitations of the data and the low numbers of potential isotretinoin-exposed pregnancies (45) and three of these who report fetal death or a congenital anomaly are too low to further characterize or confirm the known teratogenic risk of isotretinoin. However, our message is

that such events still occur rather than providing a precise risk estimate. After all, the causal relationship between isotretinoin and malformations is generally considered as certain.

Specific comments:

Introduction

3. The use of "during the first 10 weeks of pregnancy" is confusing because it is not clear if the authors mean 10 weeks after the LMP or 10 weeks after conception.

Author's response: We meant 10 weeks after conception. The description of the adverse fetal effects of isotretinoin exposure during pregnancy is now clarified.

4. Recommend deleting the sentence that starts with "Exposure beyond the first 10 weeks … in approximately 40% of live births" -- because this is a confusing statement. Do the authors mean exposures that start beyond 10 weeks? Or do the authors mean exposures that start before 10 weeks and continue beyond 10 weeks? Either way, the statement is not supported in reference #3 or 4.

Author's response: We agree that the sentence was unclear and as suggested by the reviewer the sentence has been deleted.

5. The data in reference #4 is not independent of the data in reference #3 and most of the data in reference #4 was provided by the authors of reference #3. Recommend deleting reference #4 from the manuscript.

Author's response: Since the data included in the study of Dai et al. (1992) (reference #4) is overlapping with the data analyzed in the study of Lammer et al.(1985) (reference #3), we decided to delete reference number 4 from the manuscript.

6. Risk for spontaneous abortion is dependent on the gestational age at which pregnancies are ascertained. If pregnancies are not identified early, the risk for abortion will appear incorrectly low because most of them will have occurred before ascertainment. The correct number for the risk for spontaneous abortion is 40%, for pregnancies that are ascertained before gestational age week 13. In our studies, every spontaneous abortion associated with isotretinoin exposure occurred before 15 weeks gestational age, so none of these adverse outcomes would be found >16 weeks. The comment on page14 line 47 could be changed so that it is clear that the authors' results definitely underestimate risk for spontaneous abortion, because in our experience, these outcomes have all occurred before 16 weeks gestational age.

Author's response: The information on elective termination of pregnancy and the occurrence of spontaneous abortion has been updated and rephrased. Based on the data presented by Lammer et al. (1985) and Dai et al. (1992), which contain overlapping data, show that for more than 50% of all isotretinoin exposed pregnancies an elective abortion was decided. Of the remaining isotretinoin exposed pregnancies, around 20% resulted in spontaneous abortion (18.3% in Dai et al. and 20% in Lammer et al.) In the section on limitations of our study we address the fact that spontaneous abortions prior to week 16 were not included in our study and that we therefore were not able to adequately study the risk on spontaneous abortions with the current data available.

Methods

7. Regarding use of isotretinoin that ends before conception, there is no evidence that such exposures cause adverse outcomes of pregnancy. In fact, there is no demonstrated risk for

mothers who take isotretinoin after conception but stop before 15 days after conception. So, it is not recommended to perform analyses that combine preconceptional exposures with postconceptional exposures. It would be helpful in the Discussion, to mention the half life of isotretinoin and the relevance of this information for considering the extremely unlikely chance that there might be risk from preconceptional exposures.

Author's response: According to the PPP women should not get pregnant within 30 days after isotretinoin discontinuation. This study was not intended to prove the teratogenic risk of isotretinoin pre- or postconceptionally, but to show that despite the PPP potential exposure to isotretinoin in the 30 days before conception and during pregnancy still occurs. Furthermore, some of these pregnancies even result in adverse fetal outcomes [and probably even at a higher rate than we measured]. Because the 30-day period is so clearly mentioned in the PPP, we think that it is important that the information on isotretinoin exposure in the 30 days before conception remains included in the manuscript.

We are aware of the relatively short elimination half life of isotretinoin (mean value of 19 hours) and its main metabolite 4-oxo-isotretinion (mean value of 29 hours). Although effects of preconceptional use of isotretinoin may be unlikely considering the half life times, the regulatory authorities may have had other reasons that led to the recommendation not to get pregnant and use contraceptive measures until 30 days after isotretinoin discontinuation. For instance, the possibility that the actual period that a patient uses a drug can be longer than prescribed because patients sometimes miss a dose and take it the other day.

8. Page 7 lines 1-2. The definition of fetal death should include consideration of gestational age. Many countries do not label deaths between 17 and 20 weeks as fetal deaths.

Author's response: In the revised version of the manuscript we defined the "adverse fetal and neonatal outcomes" more clearly in the Method section. The adverse fetal and neonatal outcomes include all intrauterine deaths ≥ 16 week of gestation and live born infants with major congenital anomalies. Unfortunately, we were not able to perform a separate analysis for spontaneous abortions in our study since those prior to gestational age of 16 weeks are not covered in the PRN database. Furthermore, detailed information on congenital malformations is missing. Therefore, it was not possible to estimate specific teratogenic risks of isotretinoin. In order to take into account the spontaneous abortions that are reported in our database (those that occurred ≥ 16 weeks), as well as all deaths with a gestational age of at least 22 weeks completed (fetal deaths according to the WHO definition) we decided to analyse "adverse fetal and neonatal outcome" in general. After all, this study was not intended to estimate the teratogenic risk of isotretinoin, but to show that exposure during pregnancy still occurs, and that some of these pregnancy even result in adverse fetal outcomes. Since risk estimates for specific embryotoxic effects cannot be provided with the data available, we decided to only present combined analysis of adverse fetal events (including all intrauterine deaths ≥ 16 weeks of gestation and congenital malformations) and removed the separate analysis of fetal death and congenital anomalies from the manuscript.

Results

9. Page 12 line 22. It would be more informative to know which day after conception that isotretinoin exposure began, rather than that it was used for 19 days in the first trimester. That information is not useful without knowing which 19 days within the first trimester during which the drug was taken.

Author's response: The description of cases has been removed and incorporated in table 3.The estimated exposure timing based on the drug dispensing data has now been more clearly addressed.

10. I am aware of one infant with spina bifida associated with isotretinoin exposure, but that mother took the drug after conception. It is highly unlikely that the case observed by the authors in which isotretinoin was used only preconceptionally is related to the drug exposure.

Author's response: As discussed above, the aim of this study is not to prove the teratogenic risk of either pre- or postconceptional isotretinoin exposure but to show that isotretinoin exposure still occurs as well adverse fetal outcomes despite the PPP. This has been emphasized in the Discussion section. The paragraph that described the cases of adverse fetal events that were potentially exposed to isotretinoin shortly before or during pregnancy has been removed from the manuscript. The information available has been presented in the footnotes of table 3.

Discussion

11. The authors' conclusion is that "the PPP is not completely effective" is an understatement. Recommend language that is much stronger. The compliance looks terrible.

Author's response: The conclusions of our findings have been rephrased and strengthened.

12. Page 14 line 15. Change "symptom" to "feature" and recommend deleting "such as ventricular septal defect".

Author's response: The text has been adapted as recommended.

13. Page 15 line 10. "reasons for PPP failure cannot be identified'. I do not understand this conclusion. If prescribing physicians performed a pregnancy test on every female who was prescribed isotretinoin, 60% of the exposures (women who were already pregnant) would have been avoided. Such exposures are probably the easiest to prevent.

Author's response: What we actually meant was that not all elements of the PPP were adequately evaluated in our study such as the performance of pregnancy tests, the use of contraceptives and the extent of counseling and knowledge on teratogenic risks among prescribers, pharmacists and female isotretinoin users with reproductive age. However, the findings of our study that 60% of the women were already pregnant at time of first isotretinoin dispensing suggest that exclusion of pregnancy at time of isotretinoin dispensing was not complied with, and that isotretinoin exposure should have been avoided. This has been highlighted in the discussion section of the paper.

VERSION 2 – REVIEW

REVIEWER	Christina Chambers University of California, San Diego, La Jolla, CA USA
REVIEW RETURNED	31-Aug-2014

GENERAL COMMENTS	As raised by the other reviewers and myself, the revised manuscript still spends inordinate amount of text focusing on the combined "adverse outcomes" following isotretinoin exposure. With the revised manuscript providing more detail on these outcomes, they consist of 3 fetal losses (1 SAB by U.S. criteria and 2 stillbirths) and 3 not well specified isolated defects, all 3 of which appear to be associated with pre-conception or all-or-none exposures (to the extent that timing of exposure can be estimated) that are not known from

	substantial previously published evidence to be due to isotretinoin. The conclusion that adverse outcomes still occur with exposure even after earlier SAB and ETOP have taken place doesn't make much sense, as adverse outcomes occur in pregnancy anyway. The main point of the paper is that ~50 pregnancies occurred that shouldn't have if the prevention program was working, and that there is striking evidence that refills for isotretinoin took place or that new prescriptions were written quite late in some pregnancies. The "adverse outcomes" are very secondary in this paper and add little to the literature, other than to describe them.

REVIEWER	Edward Lammer, MD UCSF Benioff Children's Hospital Oakland Oakland, California USA Previously, Dr. Lammer received research funds from the manufacturer (Roche) to investigate human developmental toxicity of isotretinoin.
REVIEW RETURNED	26-Aug-2014

GENERAL COMMENTS	The authors have satisfactorily responded to the comments of the three reviewers. This is a much improved, more focused MS.

VERSION 2 – AUTHOR RESPONSE

The primary aim of the study was to estimate the isotretinoin exposure in pregnant women despite the implemented PPP and to explore whether adverse fetal outcomes occur, rather than quantifying the teratogenic risk of isotretinoin.

Since there is uncertainty regarding the actual exposure periods and sometimes limited details on the fetal outcomes available, as well as on other factors that could have played a role in the development of the fetal events, we were not able to fully assess the aetiology of the adverse fetal and neonatal outcomes. This means that at individual case level, the potential effect of isotretinoin on the development of the adverse outcome cannot be confirmed but neither be excluded. However, it is noted that all three intrauterine deaths were potentially exposed in early pregnancy and two also later during pregnancy which is worrisome. With regard to the two cases of malformation, based on data dispensing data these cases were only exposed pre-conceptionally, but according to the PPP the pregnancy should not have occurred. Although the logistic regression does not aim to quantify a specific teratogenic effect of isotretinoin, because the spontaneous and elective abortions are not included, the results point towards an increased risk suggesting that such events occur at a higher rate among the potentially exposed pregnancies compared to the unexposed. It is fully agreed that, given the uncertainties in the data and small numbers involved, the fetal outcomes are considered secondary results that require careful interpretation. During previous revision, we shortened and made major changes to the information on adverse fetal outcomes in both the results and discussion section. To emphasize that the potential consequences of isotretinoin dispensed for use in the 30 days before or during pregnancy can be very serious, we highly recommend that the adverse fetal outcomes remain included in the manuscript. However, we have further adapted the manuscript to better emphasize that the potential isotretinoin exposure is not necessarily the aetiology of the adverse outcomes of the individual cases and rephrased the conclusions of the study.