# OCCUPATIONAL EXPOSURE TO PESTICIDES AND MALE MEDIATED SPONTANEOUS ABORTION: EVIDENCE OF THE LAST TWENTY YEARS


## ABSTRACT

Scientific evidence implicates occupational causes in adverse pregnancy outcomes. This work aims to investigate whether there is an increased risk of spontaneous abortion among wives of men occupationally exposed to pesticides by performing a systematic literature review and a meta-analysis. Epidemiological studies published between 1990 and 2012 concerning spontaneous abortion in relation to occupational exposure to pesticides were identified, selected and analysed under the random effects model. A funnel plot assessed the potential publication bias and a Duval and Tweedie's Trim and Fill analysis was used to re-compute the effect size. The effect size assessed under the random effects model determined a significant relationship between male exposure to pesticide and the risk of spontaneous abortion (OR=1.32; 95% CI: 1.02, 1.71). By contrast, the Duval and Tweedie's Trim and Fill analysis suggested that if all missing studies would have been identified, the true effect would not have been significant. Additionally this study proposes that well-conducted studies tend to report lower odds ratios of male mediated spontaneous abortion.


## 1. BACKGROUND

Spontaneous abortion or miscarriage is defined as fetal loss prior to the 20$^{th}$ week of complete gestation. Although many causes of fetal loss remain unknown, scientific evidence implicates occupational causes in adverse pregnancy outcomes (Paul, 1997). Most of the epidemiological research focuses on maternal or fetal exposure, thereby overlooking the potential role of paternal exposure.

Animal studies on the effects of pesticides on male fertility show evidence of male mediate reproductive toxic effects which may lead to a miscarriage. Pesticide exposure of adult male mice was associated with a decreased number of live fetuses and an increased number of dead and resorption fetuses (Farag et al. 2012). Likewise, epidemiological studies reveal human testicular toxicity after exposure to pesticides. Some significant effects of this exposure are reduced male fertility, altered sperm quality including decreased sperm count per ejaculate, motility and seminal fluid production, increased abnormally shaped sperm, imbalanced sexual hormones concentration and a lower sex ratio of male/female in the offspring of men exposed to pesticides (Tas et al, 1996).

The aim of this work is to investigate whether there is significant evidence of an increased risk of spontaneous abortion among wives of men occupationally exposed to pesticides by performing a systematic literature review and a meta-analysis. In addition we want to test whether the effect size of male mediate spontaneous abortion is consistent among all studies identified. If it is not, we want to identify sources of heterogeneity.

## 2. METHODS

***Data sources.*** Epidemiological studies published between 1990 and 2012 concerning spontaneous abortion in relation to occupational exposure to pesticides were identified by Medline and Embase databases using the following search terms: "Pesticides" and "spontaneous abortion" or "miscarriage" in combination with "male worker", "wives" and "occupational". Cited references were also examined to ensure that all relevant material was included in this review. The search was limited to publications either in English or Spanish. Only articles that offered free full texts were included.

***Study selection***. Inclusion criteria covered articles in which men were occupationally exposed to pesticides, while their female partners were not farmers themselves or they were unemployed. This inclusion criterion allows the isolation of male mediated effects in spontaneous abortion from female mediated effect of exposure to pesticides. Studies focusing on in-vitro fertilization, or those in which females had undergone fertility treatments were excluded from this review. No review papers were included. Consequently the selected papers were analyzed following the tiered approach proposed by Vlaanderen (2011) (Table 1). Inclusion criteria was broadened to be able to incorporate cross-sectional studies.

**Table 1** Overview of the criteria that are used in the three-tiered evaluation of human observational studies for quantitative risk assessment.[a]

| Tier | Evaluation criteria | Outcome | Impact on evaluation | CC[b] | COH[c] | CR[d] |
|---|---|---|---|---|---|---|
| I[e] | 1.1 Is the study design case-control, cohort or cross-sectional? | Yes / no | Selection for QRA[f] | X | X | X |
| I[e] | 1.2 Is exposure expressed on a ratio scale and specific for the agent of interest? | Yes / no | Selection for QRA[f] | X | X | X |
| I[e] | 1.3 Is a detailed description of the statistical analysis provided? | Yes / no | Selection for QRA[f] | X | X | X |
| I[e] | 1.4 Are criteria for inclusion of subjects into the study described with sufficient detail? | Yes / no | Selection for QRA[f] | X | X | X |
| I[e] | 1.5 Is the assessment of the health effect performed according to recognized norms? | Yes / no | Selection for QRA[f] | X | X | X |
| I[e] | 1.6 Are all relevant potential strong confounding factors considered in the study design? | Yes / no | Selection for QRA[f] | X | X | X |
| II[g] | 2.1 Type of study design | Case-control / cohort / cross-sectional | Selection for QRA[f] / study quality ranking[h] | X | X | X |
| III[i] | 3.1 Response rate | Numerical | Selection for QRA[f]/ study quality ranking[h] | X | X | X |
| III[i] | 3.2 Loss to follow-up | Numerical | Selection for QRA[f] / study quality ranking[h] | | X | |
| III[i] | 3.3 Minimum follow-up time | Description | Selection for QRA[f] | | X | |
| III[i] | 3.4 Quality of the exposure measurement methods | Description | Selection for QRA[f] / study quality ranking[h] | X | X | X |
| III[i] | 3.5 Insight into the variability of exposure | Description | study quality ranking[h] | X | X | X |
| III[i] | 3.6 Application of exposure measurements in exposure assessment | Description | Selection for QRA[f] / study quality ranking[h] | X | X | X |
| III[i] | 3.7 Type of exposure metric | Description | Study quality ranking[h] | X | X | X |
| III[i] | 3.8 Specificity of the exposure indicator | Category[j] | Study quality ranking[h] | X | X | X |
| III[i] | 3.9 Blinded exposure assessment | Description | Selection for QRA[f] | X | X | X |
| III[i] | 3.10 Quality of the exposure assignment strategy | Description | Study quality ranking[h] | X | X | |
| III[i] | 3.11 Potential for information bias | Description | Study quality ranking[h] | X | X | X |
| III[i] | 3.12 Blinded health outcome assessment? | Description | Selection for QRA[f] | | X | X |
| III[i] | 3.13 Insight into the potential for systematic error in study results | Description | Study quality ranking[h] | X | X | X |

[a] Evaluation criteria are discussed in detail In Supplemental Material I. [b] Criteria relevant for case-control (CC) study design. [c] Criteria relevant for cohort (COH) study design. [d] Criteria relevant for cross-sectional (CR) study design. [e] Tier I: Initial evaluation. [f] Criteria relevant for selection of HOS for QRA. [g] Tier II: categorization of HOS into the three types of study designs that can potentially be used in QRA. [h] Criteria relevant for ranking of studies based on quality of design, conduct and reporting. [i] Tier III: Specific evaluation of the quality of the design, conduct and reporting of HOS. [j] Categories are constructed based on a combination of: proxy vs. causal exposure and external vs. internal exposure.

**TABLE 1. (Vlaanderen, 2011)**

***Data extraction.*** Reported odds ratios and confidence intervals were extracted. If the authors provided odds ratios adjusted for potential confounders, those were preferred over crude odds ratios. Although the paper by Petrelli et al. (2003) reported adjusted odds ratios, the lack of details regarding the statistical analysis conducted made the crude odds ratios a more reliable measure. In addition, odds ratios went up dramatically after adjustment, probably due to overcorrection considering the small sample size and the large number of potential confounders for which odds ratios were adjusted. A sensitivity analysis was performed to assess the impact of this approach on the overall effect size.

Crude odds ratio was also extracted from the article published by Arbuckle et al. (2001). Rupa et al. (1991) reported number of abortions and pregnancies instead of odds ratio, therefore this was estimated by mean of STATA 12.1.

Considering the big sample size as well and the low frequency of male mediate spontaneous abortion reported by Wolfe et al. (1995) risk ratios were used as approximation of odds ratio in this study. In this case, the odds ratio within the low exposed group was preferred over the high exposed group as it was suspected that the latest group could have been exposed to additional sources of dioxins after their military service. One member of the comparison group with an elevated dioxin level reported occupational exposure to industrial chemicals other than pesticides after his service in Vietnam, therefore using the odds ratio from the highly exposed group is likely to bias to the true effect size (Wolfe et al. 1995).

G Petrelli & Figà-Talamanca, (2001) estimated their odds ratio using a logistic regression and an interaction effects model. Despite it would have been more desirable to use the results of the latest model, the fact that this study was the only one in which an interaction effects model was used made us opt for the first option. A sensitivity analysis was performed to assess the impact of this approach on the overall effect size of male mediated miscarriage.

***Statistical analysis:*** The heterogeneity of those selected studies was assessed by mean of a Q test and squared-I statistic. The Q test showed statistically significant heterogeneity and the squared I statistic determined that 89.3% of this dispersion was attributable to heterogeneity. Considering the observed heterogeneity, a random effects model meta-analysis was conducted to pool the effect size among all selected studies.

In order to detect sources of heterogeneity between studies, a stratified analysis was performed. Since different agricultural tasks may have different degrees of exposure, studies were stratified by job title grouping them into dichotomous variables according to whether workers were sprayer, greenhouse workers or exposed to pesticides due to military service. In a second step, the variation in the effect size among all jobs performed was assessed.

The main sources of variability among studies are usually study design, quality of exposure measurement, and exposure assessment. Studies were grouped into cohort or cross sectional designs, quality of the exposure assessment (studies that assessed exposure by solely self-report or those that used additional methods), and lastly they were classified by whether they considered an exposure windows of at least three months before each pregnancy or not.

Studies were also grouped between those that assessed odds ratios of spontaneous abortion within the first pregnancy of those that considered either any pregnancy, or the last one. Although Bretveld et al. (2008) reported odds ratio for primigravida couples, odds ratio for the most recent pregnancies were preferred to avoid exposure recall bias. In addition, studies that reported possible environmental exposure of wives during gestation were stratified apart from those that did not.

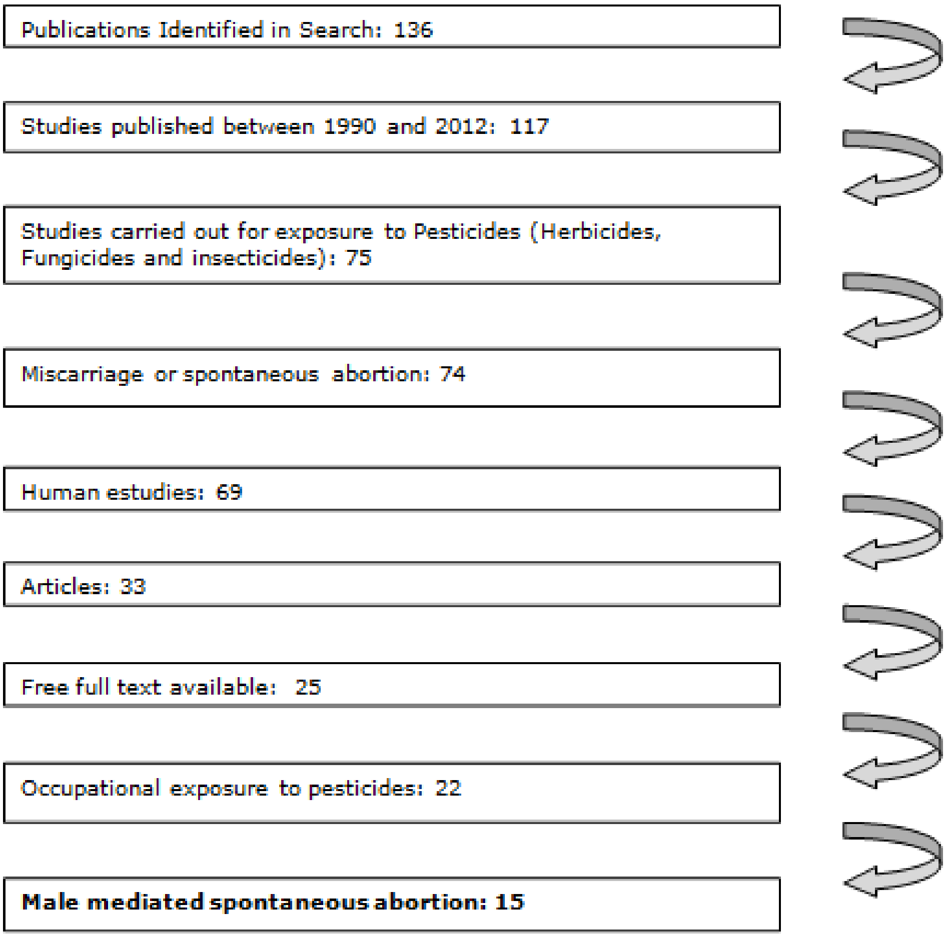
Lastly, regions where those studies were conducted may have differed in regulation, types and use of pesticides (van der Mark et al., 2012). In order to differentiate these regions, they were classified either as developed or developing country. Studied where workers were exposed as a consecuence of their military service were considered as "developing" since those studies reported high levels of exposure (Schnorr et al., 2001) (Wolfe et al., 1995).

The true heterogeneity explained by each stratification was assessed under the random effects model as well as applying a meta-regression. In order to determine the presence of small study effects, a funnel plot was visually assessed to evaluate potential publication biases among studies (Sterne & Egger, 2001). Additionally, a Duval and Tweedie's Trim and Fill analysis was performed to remove the most extreme small studies from the positive side of the funnel plot, fill the funnel plot with missing studies and re-compute the effect size (Duval & Tweedie, 2000).

All analyses were performed with STATA 12.1 and the commands "metan", "metareg", "metafunnel", "metabias" and "metatrim" were used.

## 3. RESULT

The Literature review identified 136 articles. After the selection process, which was summarized in Flow Chart 1, 15 publications remained that met the inclusion criteria. The Hjollund et al. (2004) study was excluded as wives had undergone in-vitro fertilization. A second article (Zhu J.L. et al. 2006) was excluded because the control population was selected from a population of workers exposed to pesticides. Additionally, the paper by Cocco et al. (2005) was rejected since the author did not provide any information about the total number of pregnancies requiring us to assume that each worker reported a single pregnancy event. Finally an overall meta-analysis for the remaining twelve articles was performed.




Flow chart 1: Study selection

Appendix I gives an overview of the studies characteristics following the tiered approach suggested by Vlaanderen (2011). Seven of these articles were classified as a cohort, and five were cross-sectional studies. Adjustment for potential confounders included smoking and drinking habits, caffeine consumption, vitamin use, socioeconomic status and oral contra conceptive treatment among others. Exposure was assessed solely by self-report in eight out of twelve articles.

Figure 1 depicts effect sizes for each of the studies and its 95% confidence intervals. The size of the squares reflects the statistical weight of the study attached by the random effect model. Even though the sensitivity analysis performed to assess the effect of the approach followed to extract data from the article by G Petrelli & Figà-Talamanca (2001, 2003) showed a reduction of the funnel plot asymmetry, the low weight assigned to these studies by the random effects model resulted in this approach not affecting the overall effect size.

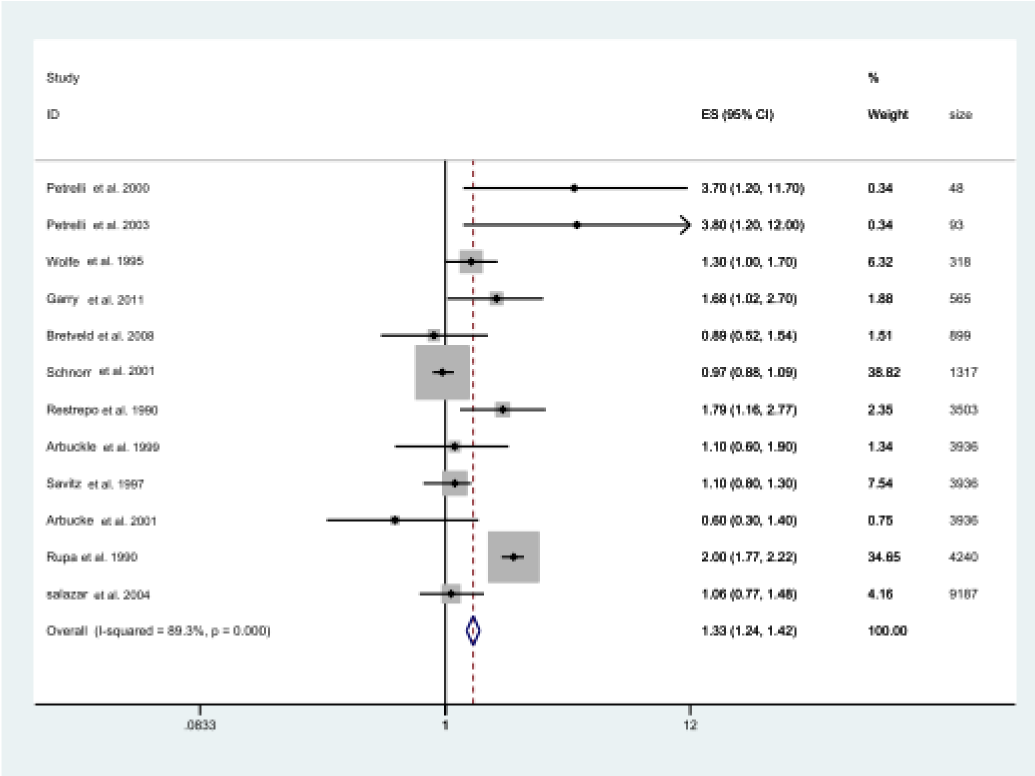



**FIGURE 1** Forest plot for male mediate spontaneous abortion and occupational exposure to pesticides. This plot depicts effect sizes for each of the studies and its 95% confidence intervals. The size of the squares reflects the statistical weight of the study in the attached by random effect model.

The effect size assessed under the random effects model determines a significant relationship between male exposure to pesticide and the risk of spontaneous abortion (OR=1.32; 95% CI: 1.02, 1.71). By contrast, the Duval and Tweedie's Trim and Fill analysis suggests that if all missing studies would have been identified, the true effect would not have been significant.

Figure 2 presents subgroups according to whether an exposure window of at least three months before pregnancy was considered or no exposure window was considered. 97.45% of between study variance is explained by means of this stratified analysis (I-squared = 9.2% P-value: 0,000).

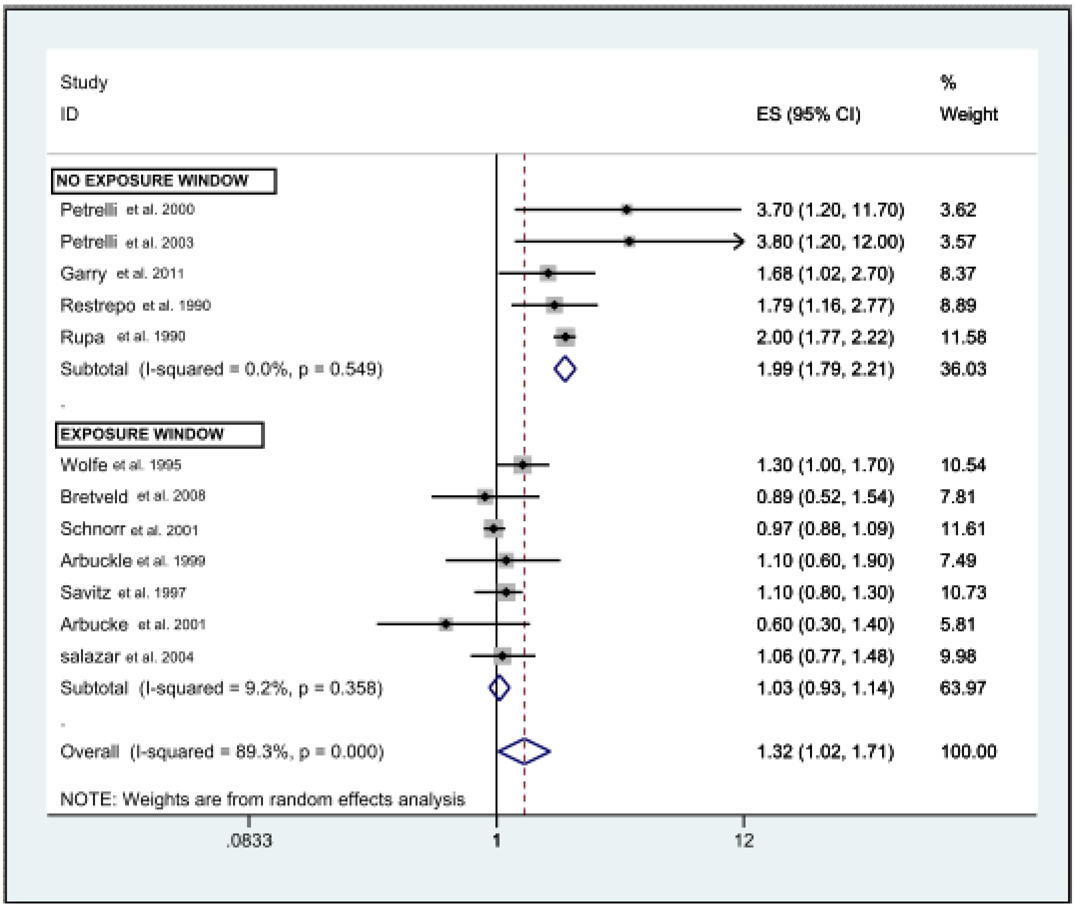



**FIGURE 2** Forest plot for male mediate spontaneous abortion and occupational exposure to pesticides. The studies are stratified by studies that did or did not consider an exposure window of at least three month before each pregnancy. Studies were pooled with the random effects method.

Stratified analysis by possible environmental exposure during gestation explains 16.03% of heterogeneity between studies although results submit a higher risk of spontaneous abortion for those studies that did not report possible environmental exposure during gestation (OR: 1.47; 95% CI: 1.07, 2.17) (Fig. 3).

There was no evidence for any statistically significant contribution to the heterogeneity by the remaining stratified analysis. The stratified analysis by socioeconomic status of the country where the index population was selected (Fig.4) shows a slightly increased risk of male-mediated spontaneous abortion in developing countries ($OR_{\text{developed countries}}$: 1.26; 95% CI: 0.89, 1.77)($OR_{\text{developing countries:}}$ 1.36; 95%CI: 0.92, 2.01). Stratified analysis based on whether studies assessed exposure by solely self-report or by using additional methods addresses an increased effect size for the first method ($OR_{\text{self-report}}$: 1.414; 95% CI: 1.00 – 2.00)($OR_{\text{additional methods:}}$ 1.150; 95% CI: 0.92, 1.43) (Fig.5). Lastly, Figure 6 depicts the stratified analysis by study design ($OR_{\text{cross-sectional studies}}$: 1.45; 95% CI: 0.99 – 2.141) ($OR_{\text{cohort studies:}}$ 1.24; 95% CI: 0.91, 1.69).

Stratified analysis by whether the index population works in greenhouses or outdoor present increased odds ratios for greenhouse workers ($OR_{\text{Greenhouse}}$: 1.59, 95% CI 0.82, 3.11) ($OR_{\text{Outdoor}}$: 1.27 95% CI: 0.94, 1.71). Stratified analysis by whether workers were sprayers or not does not display any difference in odds ratio assessed for these two groups.

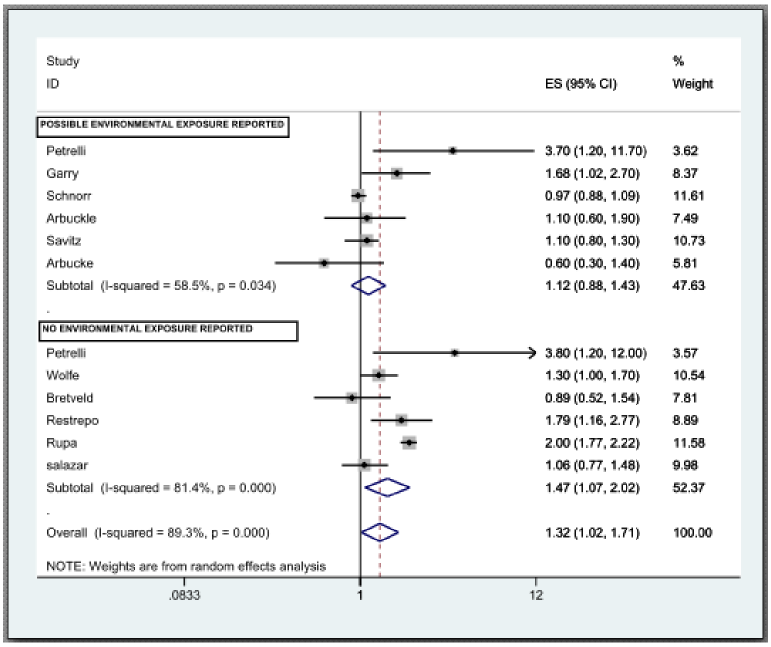
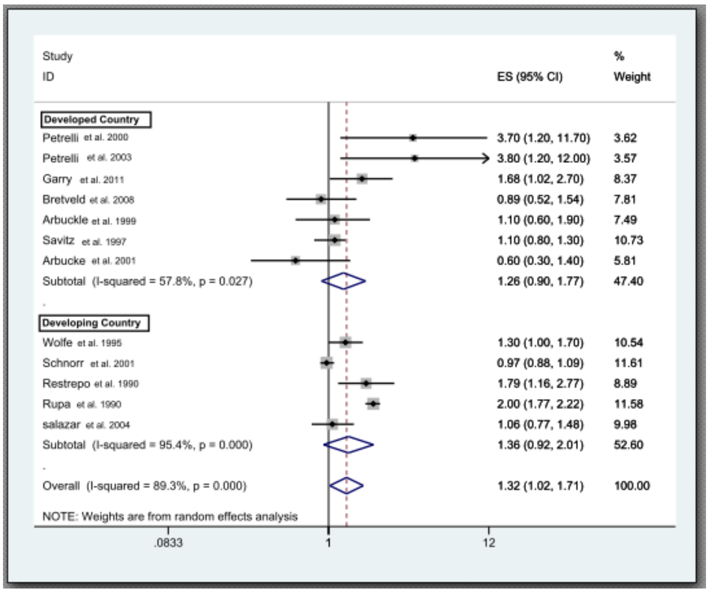



**Fig. 3** Stratified analysis by studies that did or did not reported possible environmental exposure during gestation. Studies were pooled with the random effects method.



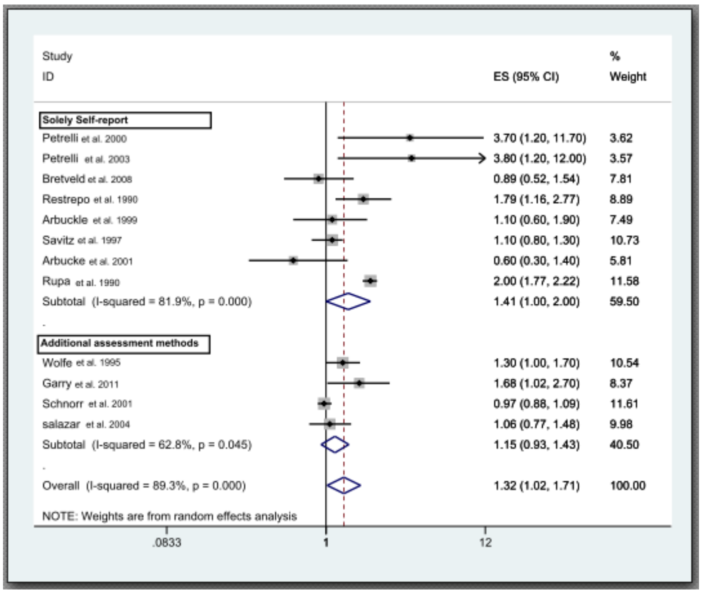
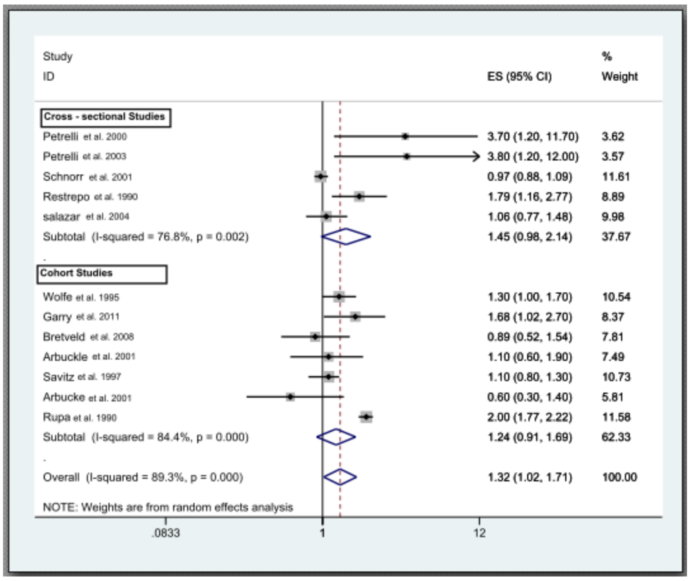




**Fig. 4** Stratified analysis by socio economic status of the country where index population was selected (OR $_{\text{developed countries}}$: 1.26; 95% CI: 0.89 – 1.77)(OR $_{\text{developing countries:}}$ 1.36; 95%CI: 0.92, 2.01).

**Fig. 5** Stratified analysis according whether studies assessed exposure by solely self-report or using additional methods (OR $_{\text{self-report}}$: 1.414; 95% CI: 1.00 – 2.00)(OR $_{\text{developing countries:}}$ 1.150; 95% CI: 0.92, 1.43).

Figure 7 shows the funnel plot displayed for small study effect. The x-axis contains the OR and the y-axis contains the standard error of this OR. Egger test estimates the presence of significant small study effect (P=0.043)



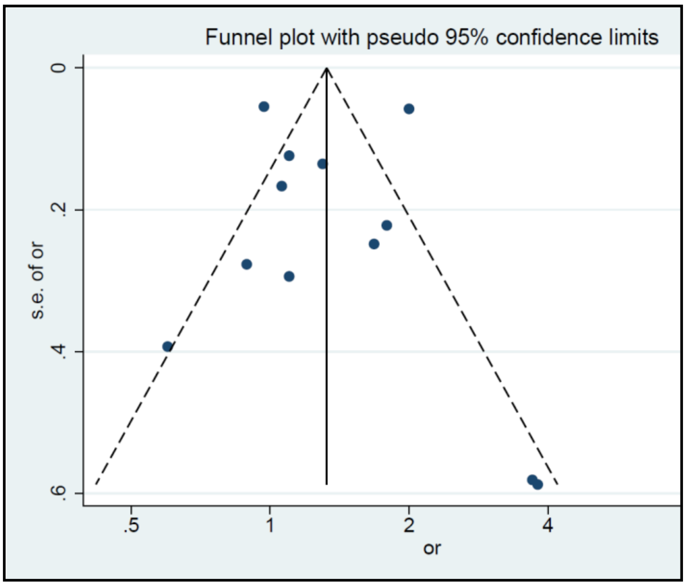




**FIGURE 6** Stratified analysis by study design. Random Effects Model assess an OR $_{\text{cross-sectional studies}}$: 1.45; 95% CI: 0.99 – 2.141 and OR $_{\text{cohort studies:}}$ 1.24; 95% CI: 0.91, 1.69.

**FIGURE 7** Funnel plots of studies included in the meta- analysis. Egger's test *p*-values is 0.043 indicating the presence of small study effect.

## 4. DISCUSSION

This study suggests that well-conducted studies report lower odds ratios than those in which a cross-sectional design was selected, the sample size was not statistically representative or did not define an exposure window of at least three month before pregnancy.

The literature review identified twelve relevant studies on male mediate spontaneous abortion as a consequence of occupational exposure to pesticides. A number of previous studies have examined the relation between maternal exposure to pesticides and the risk of spontaneous abortion, yet only a few studies have examined whether parental exposure influences the risk.

There is the possibility that some studies on male-mediated spontaneous abortion have not been published, since the frequency of the outcome studied is low and those studies that present statistically significant results are more likely to be published (Michael Borens t ein, L. V. Hedges, 2009). Additionally, not all relevant studies on male-mediated spontaneous abortion that have been published were considered as only those that offer free full texts were selected for this meta-analysis. The Duval and Tweedie trim and fill analysis suggests that if all relevant studies on male mediated spontaneous abortion and occupational exposure to pesticides would have been identified, the overall effect size would not have shown a significant relationship between the risk of spontaneous abortion and parental exposure to pesticides, therefore it can be assumed that the lack of studies selected may have biased the effect size toward the positivity. Supporting this finding, the funnel plot shows an asymmetry toward the right side of the graph as a consequence of the small study effect that has biased the meta-analysis.

The stratified analysis by studies that have or have not considered an exposure window of at least three months before pregnancy explains 97,45% of the variation between studies. The I-squared value (9,2%) indicates that this variation is due to significant heterogeneity rather than random chance and results are consistent within the two subgroups. Here, the loss of power within these subgroups ($P_{\text{no exposure window}}$: 0.548; $P_{\text{exposure window}}$: 0.358) may be due to the small number of studies since the overall P value assessed by meta-regression (P: 0,000) shows that the heterogeneity explained by this stratification is significant.

The initial theory was that those studies that assessed preconceptional exposure would report a higher risk of spontaneous abortion than those in which paternal exposure occurred at any time over the reproductive history. The random effect model, on the contrary, assessed $OR_{\text{no exposure window}}$ 1.99, (95% CI: 1.79, 2.21) and $OR_{\text{exposure window}}$ 1.03, (95% CI: 0.93, 1.14) suggesting that better conducted studies are less likely to detect a significant relationship between occupational exposure to pesticides and male-mediated spontaneous abortion.

Even though no all studies that defined an exposure window are cohort studies, those that chose a cross-sectional design within this group used additional methods apart from self-report to assess exposure. An example is the study by Schnorr et al. (2001) in which exposure was assessed combining the company's work-history records and (modeled) individual serum TCDD level at the time of each conception. Likewise, the cross-sectional study by Salazar-García et al. (2004) used p,p′-DDE concentration in fat tissue as subrogate of DDT exposure. Each measured concentration was modeled afterwards to assess DDT exposure at the time of pregnancy.

Different stratified analyses by job title do not explain significant variability among strata. When stratifying by whether index populations work inside a greenhouse or outdoors, a slightly increased risk of spontaneous abortion can be observed within the greenhouse workers and sprayers. Nevertheless the lack of information about determinant factors of exposure such a reentry activities, greenhouses ventilation system (Methner & Fenske, 1996) or crop height, set a limit for further analysis. Likewise, stratified analysis by whether workers performed spraying tasks or not is unable to provide any accurate result as far as information regarding exposure determinants like spraying method and pressure used (Methner & Fenske, 1996), the use or personal protective equipment (Marquart, 2003), amount of pesticides used (Goede, 2003) and worker training (Methner & Fenske, 1994) and hygienic behavior (Marquart, 2003) among others, is missing.

One important weakness of this study is that no one study designed a prospective cohort, thus exposure was assessed after the spontaneous abortion occurred. Studies that attempt to assess exposure retrospectively are apt to introduce differential recall bias (Coughlin, 1990). In this case exposure was assessed by solely self-reporting in many selected studies in which workers were asked to report exposure over a period up to 20 years (Rupa et al., 1991) and a time interval between exposure and interview up to 26 years (Wolfe et al., 1995).

In addition to the weak exposure assessment, studies of spontaneous abortion are likely to introduce recall bias. Wilcox and Horney found that spontaneous abortion recall was determined by the time of pregnancy with early abortions less often remembered (WILCOX & HORNEY, 1984), noteworthy considering that the aim of this study was to test male-mediated spontaneous abortion during the first and early second trimester of pregnancy. Even though the number of self-reported spontaneous abortions was validated with clinical records solely in the study by Wolfe et al. (1995), questionnaire report of spontaneous abortion can be accepted without verification from medical records (Axelsson, 1990)

As expected, stratified analysis by socioeconomic status of the country where the index population was selected showed higher risk of male-mediated spontaneous abortion in developing countries, but it does not explain significant heterogeneity among studies. Pesticides regulations, formulations and uses vary from country to country since environmental regulations tend to be laxer in developing countries and workers are more likely to misuse pesticides (Ecobichon, 2001), thereby increasing chances of exposure.

Lastly, there was an attempt to stratify the selected studies by intensity, frequency and duration of exposure, however just four out of twelve studies associated length of exposure with reproductive history. Similarly, an attempt to stratify by pesticides family or active ingredient was done but the small sample of articles not allow for any accurate meta-analysis to be performed with this characteristic.

To conclude, even when the random effect model shows a significant relationship between occupational exposure to pesticides and male mediated spontaneous abortion, this finding should be carefully read since well-conducted studies are less likely to find a strong relationship between paternal exposure to pesticides and miscarriage. Further researches should focus on prospectively assessing exposure by using more personal sampling techniques and combining these measurements with toxicokinetics models to get to a more reliable and better detailed explanation of witnessed heterogeniety of results, and ultimately a better understanding of pesticide-associated pregnancy outcomes.

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# APPENDIX I

## EVALUATION OF STUDIES SELECTED

1. **(a)** Arbuckle Tye E. et al. Exposure to Phenoxy Herbicide and the Risk of Spontaneous Abortion. Epidemiology, Vol. 10, No. 6 (pp 752 – 760) (1999).

   **(b)** Arbuckle Tye E. et al. An explanatory Analysis of the Effect of Pesticide Exposure on the Risk of Spontaneous Abortion in an Ontario Farm Population. Environmental Health Perspectives, Vol. 109, No. 8 (2001).

   **(c)** Savitz David A. et al. Male Pesticide Exposure and Pregnancy Outcome. American Journal of Epidemiology Vol. 146 No. 12 (1997).

**T.I.1** A cohort design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Sufficient details were provided regarding the performed statistical analysis
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. Farm couples belonging to The Ontario Farm Family Health Study were choose for these studies.
**T.I.5** Miscarriage was assessed by self-reported spontaneous abortion of less than 20 weeks of gestation
**T.III.1** The response rate of these studies was 64 -75%
**T.III.3** The follow up time in these studies varies from 12 month (including pregnancy time and two exposure windows) to five years
**T.III.4** Quality of exposure assessment was discussed, the exposure assessment being the main limitation as it was assessed by self-report. Time weighted average was not quantified.
**T.III.5** Variability in the degree of exposure was poorly assessed. Pesticide formulation, application conditions, handling practices and interindividual toxicokinetics response were not available.
**T.III.6** The pesticide history file for each farm was constructed on the basis of the calendar month and year that each specific pesticide was use.
**T.III.8** Pesticide were classified according to their active ingredients
**T.III.10** Exposure was assigned by means merging reproductive and pesticide exposure history data to create pesticide unit variable for month preceding and during each pregnancy.
**T.III.13** A sensitivity analysis was performed to assess the influence of reference group in reported risk.

2. Bretveld Reini W. et al. Reproductive Disorders Among Male and Female Greenhouse workers. Reproductive toxicology (Elmsford, N.Y.) Vol.28 (pp 107-14) (2008).

**T.I.1** A retrospective cohort design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Sufficient details were provided regarding the performed statistical analysis (Logistic regression).
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. Population was selected from a trade union and Chamber of Commerce databases.
**T.I.5** Miscarriage was assessed by self-reported spontaneous abortion of less than 20 weeks gestation
**T.I.6** Relevant potential confounders were considered in this study.

**T.III.1** The response rate of this study was below 52%. Causes of this low response rate were discussed.
**T.III.3** The follow up time in this study was 18 month including pregnancy time and exposure to relevant substance within the previous year.
**T.III.4** Exposure was assess by self-report, therefore there is the probability of non-differential misclassification.
**T.III.5** Variability in exposure measurement and the potential for measurement error was not provided.
**T.III.6** Exposure was qualified by job tittle reported for the 6-month prior to the last pregnancy.
**T.III.8** No distinction was made in amounts and kind of pesticides used, what might lead on risk underestimation.
**T.III.11** As control group was selected from another group of worker with similar socio-economic characteristics it is assumed that there was limited potential for information bias.
**T.III.13** No sensitivity analysis was performed.

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**3.** Garry Vincent F. et al. Reproductive Outcomes in the Women of the Red River Valley of The North. I. The spouses of Pesticide Applicators: Pregnancy Loss, Age at Menarche and Exposure to Pesticides. Journal of Toxicology and Environmental Health, Part A: Current Issues (pp 769-786) (2011).

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**T.I.1** A retrospective cohort design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Sufficient details were provided regarding the performed statistical analysis (Logistic regression).
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. Population was selected from Minnesota Department of Agriculture and the Minnesota Extension Service databases.
**T.I.5** Miscarriage was assessed by self-reported first and second-trimester miscarriage.
**T.III.3** The follow up time in this study was five years.
**T.III.4** Exposure was assessed by self-report. Limitations of exposure assessment were not discussed.
**T.III.5** Although personal exposure history was reconstructed, there was limited discussion on the variability in exposure measurement.
**T.III.6** Exposure was historically and currently assessed. When possible, specific pesticides exposure and uses were identified.
**T.III.8** Pesticides were classified according to classes and uses. In addition, the risk of using combinations was assessed.
**T.III.10** Exposure was qualified by means of pesticide name, number of days per year applied, acreage treated, and type of crop and use of personal protective gear.
**T.III.11** Since the control group was selected from the same group of worker, it is assumed that there was limited potential for information bias.
**T.III.13** No sensitivity analysis was performed.

**4.** Petrelli G. et al. Male-Mediated Risk: Spontaneous Abortion among Wives of Pesticide Applicators. European Journal of Epidemiology, Vol. 16, No.4, (pp 391 – 393) (2000)

**T.I.1** A cross-sectional study design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Insufficient details were provided regarding the performed statistical analysis.
**T.I.4** Criteria for inclusion of subjects were described with sufficient details.
**T.I.6** Relevant potential confounders were considered in this study.
**T.III.1** The response rate of this study was 100%
**T.III.4** The quality criteria for exposure measurement were discussed. Although personal exposure history was reconstructed, the small sample size reduces the precision of the estimated risk
**T.III.5**. Variability in exposure measurement and the potential for measurement error was not discussed.
**T.III.6** Exposure was indirectly reconstructed from information given by each worker based on the substances used in a certain time span.
**T.III.8** Pesticides were classified according to their active ingredients, nevertheless no specific exposure-response assessment was provided.
**T.III.10** Reproductive history and self-reported exposure were merged for each worker.
**T.III.11** As control group was selected from another group of worker with similar socio-economic characteristics it is assumed that there was limited potential for information bias.
**T.III.13** No sensitivity analysis was performed.

**5.** Petrelli G. et al. Spontaneous Abortion in Spouses of Greenhouse Workers Exposed to Pesticides. Environmental health and preventive Medicine 8, (pp 77 – 81) (2003).

**T.I.1** A cross-sectional study design was used in this study*****
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Insufficient details were provided regarding the performed statistical analysis.
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. The study population is whether greenhouse workers or sprayers involved in a stable relationship with at least one pregnancy event.
**T.I.6** Relevant potential confounders were considered in this study.
**T.III.1** The response rate of this study was 100%
**T.III.4** The quality criteria for exposure measurement were discussed. Although personal exposure history was reconstructed, the small sample size reduces the precision of the estimated risk
**T.III.5**. Variability in exposure measurement and the potential for measurement error was not discussed.
**T.III.6** Exposure was indirectly reconstructed from information given by each worker based on the substances used in a certain time span.
**T.III.8** Pesticides were classified according to their active ingredients, nevertheless no specific exposure-response assessment was provided.
**T.III.11** As control group was selected from another group of worker with similar socio-economic characteristics it is assumed that there was limited potential for information bias.
**T.III.13** No sensitivity analysis was performed.

**6.** Restrepo M. et al. Prevalence of Adverse Reproductive Outcome in a Population Occupationally Exposed to Pesticides in Colombia. Scandinavian Journal of work, Environmental health. Vol 16 No. 4 (pp 232 – 238) (1990)

**T.I.1** A cross – sectional design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Insufficient details were provided regarding the performed statistical analysis
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. Based population was selected from 58 companies affiliated with the Colombian Association of Flower Growers.
**T.I.5** Miscarriage was assessed by self-reported fetal loss at less than 20 weeks of gestation.
**T.I.6** Sources of bias was discussed.
**T.III.1** The response rate of this study was 95%
**T.III.4** Exposure was assessed by self-report. The possibility of misclassification was discussed.
**T.III.5** There was discussion on the variability of exposure and recall bias that may have lead on underreporting of the outcome.
**T.III.6** Exposure was assessed in two stages. In a first stage, based population was characterized into a binomial classification. In a second stage, population was classified into different degrees of exposure.
**T.III.8** Air levels of Captan were used as indicator of exposure.
**T.III.10** Exposure was qualified by means of personal job history reconstruction, size of company, amount of pesticides used and length of exposure
**T.III.11** Control group was selected from the same non exposed population, therefore it is assumed that there was limited potential for information bias.
**T.III.13** No sensitivity analysis was performed.

**7.** Rupa D. S. et al. Reproductive Performance in Population Exposed to Pesticides in Cotton Fields in India. Environmental Research Vol 55 (pp 123 – 128) (1991)

**T.I.1** A retrospective cohort design was used in this study
**T.I.3** Insufficient details were provided regarding the performed statistical analysis
**T.I.4** Criteria for inclusion of subjects were described. Male sprayers were included in this study
**T.I.5** Miscarriage was assessed by self-reported fetal loss at less than 20 weeks of gestation.
**T.I.6** Risk was adjusted for smoking.
**T.III.4** The quality criteria for exposure measurement were not included.
**T.III.5** There was no discussion on the variability of exposure measurement and the potential for measurement error.
**T.III.6** Job time period was used to assess exposure.
**T.III.11** Control group was selected from a similar non-exposed population, therefore it is assumed that there was limited potential for information bias.
**T.III.13** No sensitivity analysis was performed.

**8.** Salazar-Garcia F. et al. Reproductive Effect of Occupational DDT Exposure among Male Malaria Control Workers. Environmental Health Perspectives vol.112 No. 5 (2004)

**T.I.1** A cross sectional design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Sufficient details were provided regarding the performed statistical analysis.
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. Subjects were selected from a cohort assembled by the Ministry of Health of Mexico
**T.I.5** Miscarriage was defined as fetal loss at less than 20 weeks of gestation.
**T.I.6** Relevant potential confounders were considered in this study. Sources if bias were discussed.
**T.III.4**. Quality of the exposure measurement was discussed. Exposure was self-reported therefore misclassification was likely to occur due to recall bias. In addition biomarkers of DDT exposure were obtained and modeled.
**T.III.5** Three different approaches were used to assess the sensitivity of differences in exposure
**T.III.8** p,p'-DDE concentration in fat tissue was used as subrogate of DDT exposure.
**T.III.10** To assess the metabolite concentration in fat for each pregnancy INDEXPO model based on an index of occupational exposure was used. In addition Exposure to other pesticides was considered.
**T.III.11** The same group of workers acted as control group by comparing spontaneous abortion ratio before and after exposure. Limited potential for information bias is assumed.
**T.III.13** A sensitivity analyses was performed to test the influence of two extreme scenarios.

**9.** Schnorr Teresa M. Abortion, Sex Ration and Paternal Occupational Exposure to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. Environmental Health Perspectives, Vol. 109, No 11 pp(1127-1132) (2001)

**T.I.1** A cross sectional design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** Sufficient details were provided regarding the performed statistical analysis. Univariate analyses using SAS GENMOD and a model that correlates different pregnancies for the same woman was used.
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. Male chemical workers who were exposed to TCDD in New Jersey and Missouri were selected.
**T.I.5** Miscarriage was assessed by self-reported fetal loss at less than 20 weeks of gestation.
**T.I.6** Relevant potential confounders were considered in this study.
**T.III.1** The response rate of this study varied from 70% to 94.2% among groups
**T.III.4**. Quality of the exposure measurement was discussed.

**T.III.5** There was insight into the variability of exposure as individual biomarker levels were measured. In addition the model used account variation on individual body burden over time.
**T.III.6**. Exposure was assessed by company work-history record and paternal serum TCDD level
**T.III.8** Serum TDCC level was used as biomarker of exposure
**T.III.10** TCDD level at the time of each conception was estimated using a pharmacokinetic model. Background level exposure in the referent population was measured using a random sample of this population.
**T.III.11** Control group was selected from the same non exposed population, therefore it is assumed that there was limited potential for information bias.
**T.III.13** No sensitivity analysis was performed.

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**10.** Wolfe William H. et al. Paternal Serum Dioxin and Reproductive Outcomes among Veterans of Operation Ranch Hand. Epidemiology Vol. 6 No. 1 (pp17-22) (1995)

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**T.I.1** A cohort design was used in this study
**T.I.2** Exposure was expressed on a ratio scale
**T.I.3** insufficient details were provided regarding the performed statistical analysis.
**T.I.4** Criteria for inclusion of subjects were described with sufficient detail. Veterans of the unit responsible for aerial spraying of herbicides in Vietnam from 1962 to 1971 were selected.
**T.I.5** Miscarriage was assessed by medically confirmed fetal loss at less than 20 weeks of gestation.
**T.I.6** Relevant potential confounders were considered in this study but miscarriage odd ratio was not adjusted.
**T.III.1** The response rate of this study was 93%
**T.III.4**.Quality of the exposure measurement was discussed. Exposure was self-reported and dioxin concentration in serum was measured.
**T.III.5** There was insight into the variability of exposure. Individual dioxin level was estimated using a first order decay rate model where the time of exposure was also considered.
**T.III.8** Serum TDCC level was used as biomarker of exposure
**T.III.10** Parental serum dioxin level was merged with their reproductive history.
**T.III.11** Control group was selected from a similar non exposed population, therefore it is assumed that there was limited potential for information bias.
**T.III.12** Miscarriage assessment was blinded to the father's exposure status
**T.III.13** No sensitivity analysis was performed.