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Meta-analysis of parental occupational exposure to pesticides or agricultural work and congenital heart disease

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Meta-analysis of parental occupational exposure to pesticides or agricultural work and congenital heart disease

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B.A., Rice University, 2011

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Abstract

Meta-analysis of parental occupational exposure to pesticides or agricultural work and congenital heart disease

By Veronica Burkel

Objective: To use systematic review and meta-analysis tools and techniques to identify and synthesize the current literature on occupational exposure to pesticides or agricultural work and congenital heart disease.

Methods: A search on Pub Med for study articles with key terms (See Appendix) resulting in 4,552 articles. Articles were examined for relevance via title, keywords, abstract, and full-text review, resulting in eleven articles for independent abstraction by two reviewers. Pooled effect estimates and 95% confidence intervals (CI) were calculated using random and fixed effects models for maternal and paternal effects on all cardiovascular defects and for ventricular septal defects (VSD).

Results: The results for occupational exposure to pesticides or agricultural work on congenital heart disease were found to be null for maternal and paternal estimates for all pooled results; maternal exposure for all cardiovascular defects (n = 7; OR 0.92; 95% CI 0.78-1.09) maternal exposure with covariate adjustment for all cardiovascular defects (n = 4; OR 0.84; 95% CI 0.69-1.02), maternal exposure on VSD (n = 3; OR 0.90; 95% CI 0.74-1.10), paternal exposure with covariate adjustment for all cardiovascular defects (n = 3; OR: 0.82; 95% CI: 0.68-1.00), and paternal exposure for VSD (n = 3; OR: 1.01; 95% CI: 0.76-1.33). Systematic review of these studies identified study methods in need of improvement for more accurate results, including issues related to exposure measurement and misclassification, precision and consistency of outcome definitions and outcome inclusions, adjustment for covariates, and specificity of the agent.

Conclusions: The current literature on occupational pesticide or agricultural work exposure and congenital heart disease in offspring suggests no association. Due to the challenging nature of data collection, the many avenues of potential bias to the null, and the methodological inconsistencies across studies, we think it is too soon to rule out an effect of exposure on disease. Future studies should attempt to specify an agent, adjust for covariates, enumerate all pesticide exposure sources, measure the dose of the exposure(s), and identify specific ICD-coded birth outcomes.

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Introduction

Pesticides are a group of substances—insecticides, herbicides, fungicides, rodenticides, and nematicides—used to destroy or mitigate pests. The use of pesticides is widespread and has been very effective in controlling disease-bearing vectors as well as preventing economic loss and food deprivation by effectively preventing crop destruction [1, 2]. Although governmental and international bodies regulate pesticide use [1], the adequacy of these regulations has been heavily debated. Occupational exposure to these chemicals is often of higher dose than the typical environmental and dietary exposure and therefore is of key interest. Pesticides are often created, mixed, and diluted with other pesticides and chemicals, and workers may have direct, concentrated exposure with all of these substances [1, 2]. Oftentimes it is not clear what ingredients are, and the extent of their toxicities is unknown [1]. While some research links pesticide exposure with various health effects, such as cancer, fertility issues, and reproductive issues [2], the etiologies of these associations are not clearly defined [3]. Because the workforce is comprised primarily of men and women of reproductive age, it is imperative to research whether or not workplace exposures affect their offspring.

Birth defects are a leading cause of infant mortality and morbidity, and incur substantial costs, both financial and emotional, to families. The prevalence of a birth defect is 3.5% for all live births [4]. Heart defects are one of the most common birth defects, , with 4-6 congenital heart defects per 1,000 births [4]. They are one of the major contributors to birth-defect related infant deaths [5], and incur substantial medical costs for families[6].

Our aim is to evaluate associations of parental occupational exposure to pesticides or agricultural work, before or during pregnancy, with congenital heart defects in offspring by identifying relevant epidemiologic studies and synthesizing these findings in a systematic review and meta-analysis. We investigate if there are adequately similar epidemiologic studies to make a summative conclusion about the relationship between parental occupational pesticide exposure and congenital heart defects. The overall goal is to produce a summary of the current knowledge, describe the challenges and limitations, and suggest future directions in this field of study.

Methods

Data & Analysis

Epidemiologic studies on occupational pesticide exposure and congenital birth defects were identified using a systematic PubMed search. The following exposure terms were used as both key words and MeSH terms: pesticides, occupational exposure, pesticide exposure, maternal occupation, paternal occupation, parental occupation. Outcome terms, search as both keywords and MeSH terms, include: congenital abnormalities, congenital malformations, congenital heart defects, and cardiovascular malformation. See Appendix 1 for the complete PubMed search. The search was limited to publications in English and studies with human populations. We used broad terms for parental occupation to capture those papers that looked at a wide spectrum of jobs, but might provide sufficient pesticide or agriculture-specific data for inclusion in the meta-analysis. We use broad outcome terms to capture studies of birth defects that did not focus specifically on cardiovascular defects but might provide sufficient phenotype-

specific data for inclusion in the meta-analysis. Note that the original search included parental exposure to pesticides, solvents, and heavy metals. Collaborators (VKB and CMR) were unsure of the quantity of relevant literature studying congenital heart defects and pesticides. After narrowing down the relevant literature by title, keywords, and abstract, the remaining studies were categorized and pesticide exposure was singled out for further systematic review and analysis. Pesticide exposure was selected due to the number and breadth of articles found and due to authors' interests.

Publications returned by the search were first reviewed by title, then abstract, and finally full-text review to efficiently exclude off-topic or otherwise ineligible studies (Figure 1). Of 75 studies that underwent full-text review, 64 were excluded due to: analyzing only fetal deaths (n = 4), insufficient information on occupational exposures or using very broad occupational groupings, such as "blue collar" (n = 5); no information on pesticide exposure or agricultural occupation (n = 21); congenital heart defects not evaluated as an outcome or grouped within "any birth defect" (n = 17); cohort studies with no unexposed cases (n = 2); data having been reported elsewhere in the evaluated studies (e.g. in a re-analysis, or pilot study with data included elsewhere in a full report) (n = 5); and not reporting original data, for example in a review or commentary/letter (n = 9). One additional study [7] was excluded based on non-comparability with other included studies, as it looked solely at a non-agricultural pesticide exposure.

From each eligible study, data was abstracted on: study design, period of exposure, case definition, exposure measures, covariates adjusted for, relative risks (RR) or odds ratios (OR), 95% confidence intervals (CI), sample size, case and control

counts/rates, and exposure counts/rates. The information provided by studies varied—some studies reported multiple estimates among different subgroups of exposure or of outcome—but consideration for inclusion in the meta-analysis required, at minimum, either a measure of association with a CI or else sufficient data to calculate such, for either pesticide exposure or agricultural occupation and risk of any congenital heart defect phenotype. Two review authors (VKB and CMR) independently abstracted the data and resolved discrepancies by discussion and consensus. All data were dichotomous (exposed/non-exposed; disease/non-disease) and some studies provided several effect estimates, differing by phenotype or maternal vs paternal exposure. For each association, the natural log of the effect estimate and its variance were calculated from the reported risk ratios and CIs or from the reported raw data. We obtained supplemental tables where available online, or by contacting the authors when the paper indicated that additional calculated data was available on request.

Because the available data varied between studies, authors used a hierarchy to choose the highest quality effect estimate. The adjusted effect estimate was used when possible, as this is ostensibly the most accurate measure of association. If adjusted results were not available, we used the raw data over the published OR and CI to increase precision. On a few occasions, our calculated odds ratio varied from the presented odds ratio and could not be explained by rounding errors. In this circumstance, we used the crude count data if this was clearly provided for each cell in a contingency table. If, however, we had to reverse-calculate cell counts from data presented inconsistently in the paper (for example, from a total case and control size presented in the methods, and

percent case and control exposed listed in results), we used the provided crude effect estimate and confidence interval. Reported crude odds ratios were used only when insufficient raw data was provided. Studies had varying exposure periods, although a vast majority had data for the periconceptional time frame, defined as during 1-3 month(s) prior to conception until the end of the third month of pregnancy. One study [8] provided risk estimates for 1) ever/never exposed to pesticides at work and 2) exposed to pesticides during periconceptional period. Since most pesticides have relatively short half-lives, we used the periconceptional measure.

When there were 3 or more studies that provided effect estimates for the same phenotype, a pooled odds ratio was calculated using both the Woolf fixed effects method and the random effect method [9, 10]. Homogeneity between studies was examined to determine if publication bias was likely. Pooled effect estimates were also calculated stratified by study design, exposure periods, type of exposure assessment, heart defect subtype, and adjusted effect estimates where possible.

Results

Study Design and populations

We identified 11 studies with data appropriate for this systematic review and meta-analysis. Most were case-control studies (n=8), although we also identified 2 retrospective cohort studies and 1 cross-sectional study. Cases were most often identified from a birth defects or birth registry. Most studies (n = 8) observed cases among live births only; the other three included both live births and fetal deaths.

Congenital heart defects were not defined consistently across studies. Some studies restricted their case definition to isolated heart defects (individuals with heart defects but no defects in other organ systems), whereas others did not. Some studies did not specify; we presume these studies included cases with either isolated or multiple defects. One study [11] provided data for cases with and without restriction to isolated cases. The article's results between these choices were similar. There are many different types of congenital heart defects. Four [11-14] studies examined heart defects by type, four [8, 15-17] investigated heart defects as grouped, and three [18-20] examined heart defects as a group but also examined some specific types of heart defects.

Studies had varying time frames in which a heart defect could be identified and the infant identified as a case. All controls were live birth infants, but details regarding whether or not they were healthy infants, if they were infants without birth defects, or if they were simply randomly selected infants varied across studies. For study details and facts, see Tables 1 and 2 in the Tables & Figures section of this paper.

No studies had direct measurements of exposure (for example, from personal sampling or workplace sampling) or biomonitoring data for any pesticide. Three studies [14, 19, 20] used occupation in agriculture or as a farmer, based on occupation reported on the child's birth certificate. One study supplemented this with information reported on the agricultural census nearest the child's birth [20]. Six studies relied on self-reported pesticide exposure or job description [8, 11, 13, 15-18, 21]. One study used a job-exposure matrix to infer pesticide exposure from job title [18]. Two studies [11, 17] used expert rater review, with or without guidance from a job-exposure matrix, to assign likely

pesticide exposure based on the job description provided in the questionnaire or interview. Most studies were dichotomous in exposure; studies with three exposure categories—some derivation of likely exposure, possible exposure, and unlikely exposure—ultimately resulted in a dichotomous exposure for analyses. Pesticide exposures can also occur outside work, for example during gardening and lawn care at home. Two studies collected information about potential non-occupational pesticide exposures as well as occupational exposure [13, 21].

Parental exposure varied across studies. One study assumed both parents on family farms were involved in agricultural activities, and consequently pesticide exposure, based on sociologic and demographic data in that country showing that most family farms are actively run by both parents [20], and therefore it is likely that both parents have similar exposures. Four studies assessed maternal agricultural occupation or pesticide exposure only [15-17, 21]. One study exclusively examined paternal occupation [11]. Four examined both maternal and paternal occupational exposure [8, 13, 14, 18]. One study classified exposure based on either parent being classified as pesticide-exposed [19], and was not included in calculations of pooled odds ratios. We decided this based on unknown exposure mechanism—although there is a suggestion of a parent based on the exposure status of the other parent, we are unsure of the mechanism and extent to which this secondary exposure would be comparable with primary exposure.

Most studies reported risk estimates and 95% confidence intervals near unity. Figure 2 is a forest plot displaying the effect estimates and 95% confidence intervals for all cardiovascular defects, separated by paternal and maternal exposure type. A few

elevated but non-significant risk estimates were also reported. The only statistically significant association reported, by Loffredo et al, was for non-TGA (transposition of the great arteries) outflow tract abnormalities associated with maternal occupational exposure to pesticides (OR: 3.84, 95% CI: 1.39, 10.59)[21]. No other studies reported data specific to this heart defect type (Table 3). Given the null results seen across studies, no further assessment of publication bias was made.

Pooled odds ratios were calculated using both the random effects model and the fixed effects model; both models produced similar effect estimates and confidence intervals for each analysis. For the sake of simplicity, we report results using only the random effects model (Table 4). Results from the fixed effects method are available upon request. All analyses are separated by maternal or paternal status. Data was available from at least 3 studies to calculate a pooled odds ratio and confidence interval for: 1) maternal pesticide/agriculture exposure and all cardiovascular defects, 2) maternal pesticide/agriculture exposure and ventricular septal defect (VSD), 3) paternal pesticide/agriculture exposure and all cardiovascular defects, and 4) paternal pesticide/agriculture exposure and VSD (Table 4).

Of the 11 studies, only six provided adjusted results. For the first analysis, seven studies with both crude and adjusted effects resulted in a pooled effect estimate of 0.92 (95% CI: 0.78, 1.09) for maternal pesticide exposure/agricultural occupation and congenital heart defects. Restricting to studies with covariate adjustment (n = 4) resulted in a modestly lowered effect estimate (OR: 0.84, 95% CI: 0.69, 1.02). In the second analysis, the pooled odds ratio for maternal pesticide/agricultural exposure and VSD (n = 3 studies) was near unity (OR: 0.90, 95% CI: 0.74, 1.10). Paternal pesticide/agriculture

exposure was associated with a marginally non-significant reduction in congenital heart defects (OR: 0.82, 95% CI: 0.68, 1.00) based on adjusted results provided by three studies. Paternal pesticide/agricultural exposure was not associated with VSD (OR: 1.01, 95% CI 0.76, 1.33) based on three studies.

Only two studies provided data specific to other congenital heart defect types, including conotruncal defects, Tetralogy of Fallot (TOF), transposition of the great arteries (TGA), and coarctation of the aorta (COA). Although we did not calculate pooled odds ratio and confidence intervals unless there were at least 3 studies with results suitable for pooling, the individual studies all reported effect estimates near unity for these defect subtypes. Data on conotruncal outcome [11, 13] found OR 1.0, 95% CI 0.6-1.9 and the other OR 0.9, 95% CI 0.6-1.9. Papers [11, 18] provided results for TOF [OR 1.46 (0.38-5.69) and 1.0 (0.7-1.3) respectively]; for TGA [1.00 (0.23-4.35) and 0.9 (0.6-1.2)], and for COA [1.19 (0.25-5.64) and 0.9 (0.6-1.2) respectively].

Discussion

Pooled effect estimates

Overall we observed no associations between parental occupational exposure to pesticides as a group and either congenital heart defects as a group or ventricular septal defects. Based on the substantial limitations in the evaluated studies, however, we cannot conclude that pesticides, or specific types of pesticides, are associated with congenital heart defects as a whole or with specific types of congenital heart defects. Exposure misclassification and non-specificity of exposure measures are likely to have biased

results towards the null. Inadequate control of confounding and issues in outcome classification, as well, may have biased results.

Publication bias is a major concern in meta-analysis, commonly dealt with qualitatively by visualizing the distribution of effect measures plotted against sample size in a funnel plot. Publication bias occurs when negative or null studies are less likely to be published than studies showing a positive effect when an exposure is considered possibly harmful. In a funnel plot, this would create the appearance of part of the 'funnel shape' being missing. Since only 1 study [21] evaluated in this meta-analysis showed an elevated effect measure, and that paper presented null findings for other outcomes, we considered publication bias to be highly unlikely.

Exposure assessment issues

Because heart defects are a relatively uncommon outcome, occupational cohort studies or prospective cohorts generally had insufficient sample size to examine congenital heart defects as an outcome. These studies, while often having better occupational exposure assessments, generally were limited to examining all birth defects as a group. Consequently, they were excluded from this analysis. In general, the study designs that are most efficient for rare outcomes, such as retrospective cohort, case-control, and cross sectional studies face difficulties in accurately reconstructing past occupational exposures.

The exposure for each article was whether parents were occupationally exposed to pesticides or agricultural work; agricultural work was often used as a proxy for pesticide exposure. Other types of workers may also be exposed to pesticides, however. Some

people in the non-agricultural reference group may be misclassified as to pesticide exposure. Agricultural workers may share other exposures in addition to pesticides, such as fertilizers or diesel fuel, that may be related to the outcome.

While examining pesticide exposure across occupations reduces these issues, direct measurement of exposure is typically not possible in retrospective studies. The evaluated studies relied on mostly dichotomous exposure statuses gathered from either self-reported information from interviews or questionnaires, or on inferred exposure from birth certificates, job titles, and profession censuses. Two studies [13, 17] utilized expert raters to quantify the job descriptions or exposures reported into exposure categories. The job categories considered exposed include farming, agricultural work, or pesticide application. One study used a job exposure matrix (JEM) to categorize participants into categories of probable, possible, and unlikely exposure. Inferring exposure based on job category is imperfect, because individual jobs within the same title or category will have varying tasks.

Self-reported exposure is vulnerable to interviewer bias or leading questions, recall errors (due to both lapses in memory or the participant not knowing what they were exposed to), and reporting biases . It is possible that response may differ by job type, job history, or that some may not know the extent to which they are using pesticides. Literature suggests that the validity of self-reported occupational exposures can be very low, although it varies depending on individual and job characteristics [22]. Six of the 11 studies relied on self-reported pesticide exposure or job description although two of these

6 utilize expert raters to determine exposure; expert raters are considered to have the highest validity out of all retrospective exposure assessment methods [22].

While assessing occupational pesticide exposure retrospectively would always face these challenges, this issue was compounded for investigators of the studies evaluated by unclear mechanisms by which parental pesticide exposure might cause congenital heart defects—and therefore, which parent's exposures were relevant. Maternal exposure around the time of conception and during pregnancy or paternal exposure during spermatogenesis are often cited as possible methods, but maternal exposure to take-home exposures from pesticides carried home by the father (or from those pesticides known to concentrate in semen) has also been proposed as a route of relevant exposure. Additionally, because pesticide use may vary seasonally, the season of conception may also effect whether relevant exposures occurred in the narrow critical windows for fetal development. The critical periods may vary by cardiac phenotype.

Due to these exposure assessment issues, the potential for misclassification in these studies seems quite high. In general, this would tend to bias the results to the null, except for situations in which the biases may be differential by disease status—in particular, studies in which parents of case children (intentionally or unintentionally) differentially mis-report pesticide exposure compared to parents of control children. Information on job history, job title, company, and duties are believed to be less susceptible to recall bias than self-reported exposure or frequency of exposure. However, false positives are known to be a large source of bias in these study designs [23]. With

false negatives being less of a concern, one method to rectifying this issue is to reassess exposure in those first assessed as exposed [23].

Studies in this systematic review tended to not consider pesticide exposure outside of occupation, with the exception of two—articles by Shaw and Loffredo [13, 21]—such as home gardening, weed control, treating a home for insect pests, or flea and tick preventatives and treatments for pets. Often occupational exposures occur at much higher intensities than residential or environmental exposures, but in the case of pesticides, home uses can incur very high exposures. It is possible that home exposures are acute in nature compared to daily or more frequent occupational exposures. These other sources of pesticide contact have potential to outweigh occupational exposure. At the very least, they would contribute to an individual's overall pesticide exposure, which could contribute to overall misclassification of exposure. These exposures, when not measured and adjusted for in the analysis, could dilute any effect we may see for occupational exposure on disease.

Exposure time periods in the included studies range from pre-pregnancy and the first trimester, the entire pregnancy, and exposure status at time of birth. For our purposes, we did not consider these inconsistent "critical periods" of exposure to be a major source of bias or an impediment to pooling studies, because occupation tends to be fairly stable over relatively short periods of time. These studies all used a dichotomous measure (ever/never), rather than trying to construct variables based on days of exposure in the critical period. It would be interesting to note, however, if a woman stopped

working or modified her job duties during pregnancy; this might impact her dose during relevant periods of fetal development.

Another important issue with the exposure metrics used in these studies is that of pesticide type. Agricultural workers handle a variety of pesticides, depending on the types of crops or livestock they handle. Even among those studies using a JEM or expert rater to assess exposure, pesticides were assessed as a group. Only one study [20] reported a pesticide type although the study also includes unknown pesticides. Pesticides can differ by purpose (herbicide, rodenticide, insecticide, fungicide), properties (solid, liquid, vaporized), active ingredients, inactive ingredients, and hazard information [1]. Pesticides may have varying toxicities; grouping all pesticides could dilute a strong effect that one type of pesticide has on congenital heart defects.

Similarly, using a dichotomous exposure could be problematic. None of the studies included in this paper measured exposure in any dose categories. As previously mentioned, two studies identified sources of exposure in addition to occupational exposure. One [13] reported results by increased sources of exposure—from "no to all" to "yes to all five" possible exposure sources—which suggests an increase of dose with each source, though it is not a precise measure of dose. Often populations tend to have a pyramidal pattern of exposure— there is a small, yet highly exposed group at the top of the pyramid and at the bottom a larger group that is less exposed. Combining these extremes may dilute any strong effect pesticides have in the highly exposed group. It may be possible to use job categories as a basis of dose exposure: a pesticide applicator, for

example, may have a higher exposure than a grocery store clerk who stocks produce. In this way, with a sufficiently-sized population, epidemiologic studies may be able to separate out the highly dosed group and, if there is truly a proportional dose response, results may more accurately depict this.

Outcome classification

The definitions or specific diseases included as *congenital heart disease, cardiac defects*, or *cardiovascular malformations* may vary across the years or across study locations. While most studies reported using an international coding standard (most often ICD-9 or BPA) to identify and categorize outcomes, many did not specify which codes and criteria were included in their cases, or define heart defect subtypes.

To improve the ability of researchers to compare results, we recommend that authors carefully define their case groups in publications, including providing the actual eligible codes and identifying their coding scheme. As scientific journals have expanded their online presence, they have expanded the ability of authors to provide supplemental online data. Careful case group definitions could be provided in such supplemental material. Likewise, expanded results can now be provided as supplements; we encourage authors to take advantage of this capacity. Attempts to contact corresponding authors are more difficult as time passes.

We excluded several studies in which cardiac defects were not reported separately. Often these studies looked at “all birth defects” or “all major malformations”

as a group, or create a category such as “cardiovascular and respiratory defects”. Likewise, we excluded several studies that grouped exposure as “all chemicals” or one of many different classes of chemical. While these groupings were sometimes necessary due to small sample sizes, different birth defect phenotypes (or different chemical classes) are unlikely to share the same mechanisms. Grouping multiple homogeneous outcomes, or homogeneous exposures, is likely to dilute results by mixing effects.

Another issue with study outcome is in collecting all the true cases of congenital heart disease in the study population. Several studies took cases and controls from only *live births* that were diagnosed as having a congenital heart defect. This criterion represents birth prevalence, rather than true incidence of congenital heart defects. It overlooks infants whose defects may have been so severe that they did not survive, or very severe malformations (particularly those that would have resulted in severe disability or death for the child) that were prenatally diagnosed and led to therapeutic abortions. Looking only at live births introduces survivor bias; it can artificially create the appearance of a protective effect if those with the highest exposures and highest susceptibility have already died and been removed from the population at the point in time when prevalent cases are assessed. Even studies examining fetal death certificates (for late pregnancy losses, or stillbirths) and records of therapeutic abortions may not identify all incident cases. Early pregnancy losses (miscarriage) due to congenital defects cannot typically be identified.

Among live births and fetal deaths, however, outcome misclassification may be less of a concern for some defects. All of the studies included in this meta-analysis had a system in place to confirm, verify, or even re-diagnose a birth defect case as having the birth defect reported. With heart defects as such a critical and specific area of research, the specificity of studies in identifying and confirming cases is a benefit. However, studies had differing times after birth during which a child could be diagnosed as having a defect. Children with defects that are less severe or less noticeable may not all be enumerated and are therefore missing from the study or are included in the study as a non-case.

Other study limitations

Some studies did not adjust for important (or any) potential confounders. In the case of occupational exposures this is particularly important because occupation is tightly linked to education and income. While education and income are unlikely to have a direct causal link to birth defects, they do have direct causal links to many health behaviors, the home environment, and household exposures. Seniority or job experience also effect exposures and are also associated with age—a well-known risk factor for many birth defects. A careful evaluation of confounding is therefore important in studies of occupational exposure.

As it stands, despite null findings, we cannot conclude that pesticides do not cause congenital heart defects. We can conclude that the studies to date have not observed an effect, but there were substantial limitations in the data evaluated. Overall, many study

characteristics could bias results to the null. We must consider that an effect may not have been observed due to exposure misclassification or mixing effects from multiple exposures, along with additional issues mentioned in this paper. Future studies should focus on overcoming these substantial sources of bias.

Although a single observational study cannot be considered conclusive, a strong study would be able to accurately classify exposure, accurately classify outcome, and control for all relevant confounders. Accurate exposure classification includes evaluating exposure to a specific agent (what type of pesticide and what active or inactive ingredients is it comprised of) rather than a large, heterogeneous group of agents; accounting for both occupational and non-occupational sources of exposure to the agent; and measurement of exposure dose. Accurate classification of outcome would require ascertainment of most or all incident cases, rather than prevalent cases; specific ICD-coded birth defect phenotypes; exclusion of heart defects that occur as part of known chromosomal disorders or syndromes of known etiology; and stratification by whether the defect is isolated or co-occurred with other congenital defects in other organ systems. This type of study has not been conducted to evaluate associations between any specific pesticide and congenital heart defects.

Conclusion

This meta-analysis includes eleven studies that estimated the effects of occupational exposure to pesticides or agricultural work on congenital heart disease.

Overall we did not find an association between pesticide or agricultural work and congenital heart disease; however, there are significant limitations to the studies including exposure misclassification, inconsistent definitions of outcomes, grouping pesticides into a single heterogeneous group, inadequate evaluation of specific types of congenital heart defects (which have varying mechanisms), and lack of covariate adjustment. Future studies should attempt to specify an agent, adjust for covariates, enumerate all pesticide exposure sources, measure the dose of the exposure(s), and identify specific ICD-coded birth outcomes.

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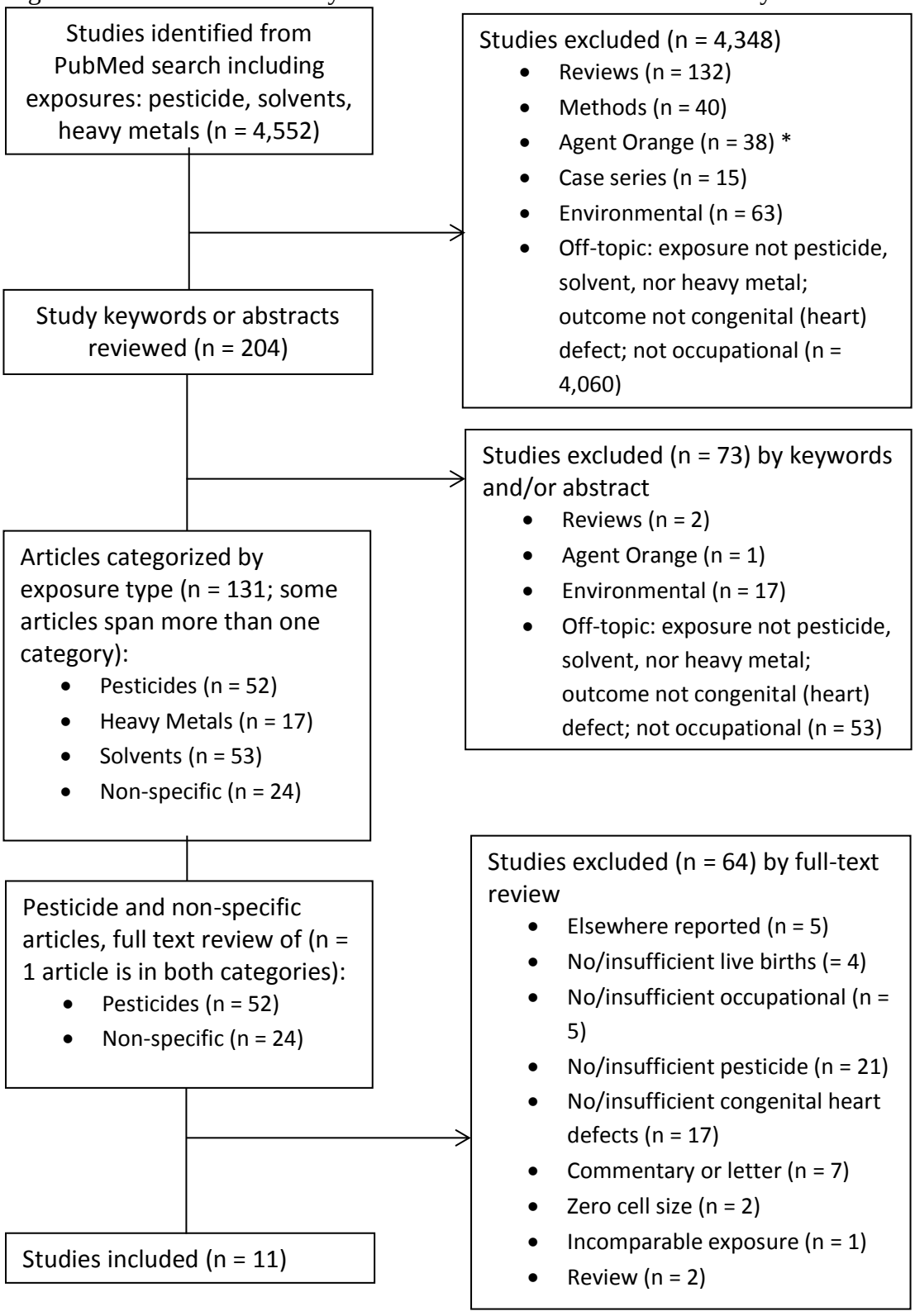
Tables & Figures

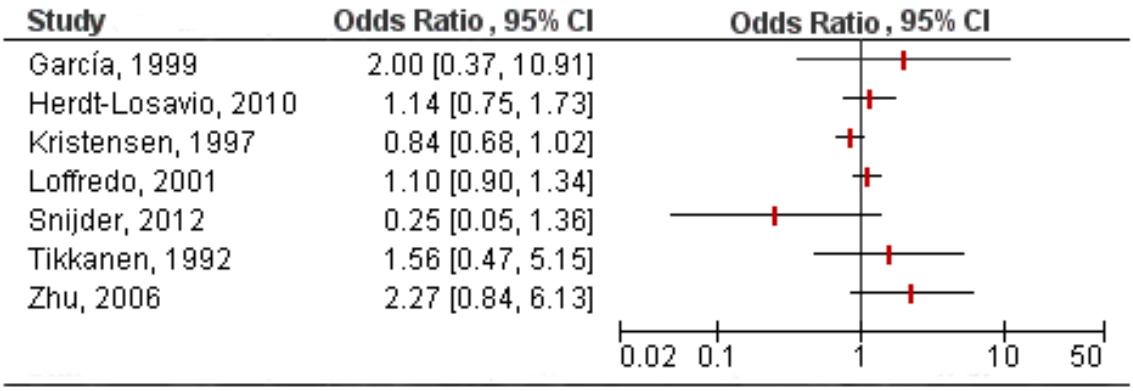
Figure 1. Flow scheme of study selection for inclusion in the meta-analysis



*Studies pertaining to Agent Orange in Vietnam were excluded due to this being an extraordinary and atypical exposure event.

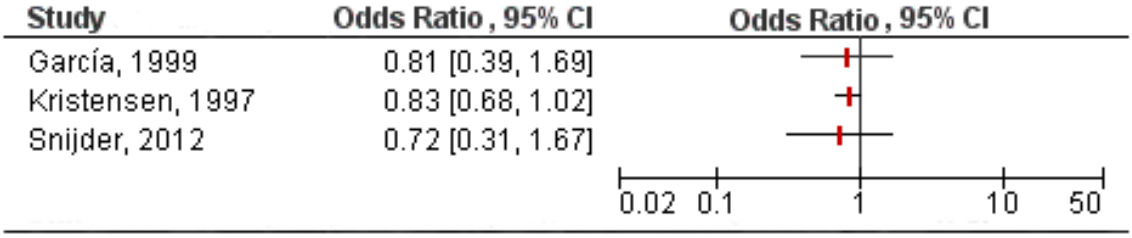
Figure 2. Forest plot of all effect estimates and confidence intervals, calculated or extracted from study articles, separately for maternal and paternal exposure to pesticides or agricultural work and all cardiovascular defects

Figure 2.1 All effect estimates and confidence intervals, calculated or extracted from study articles*, for maternal exposure to pesticides or agricultural work and all cardiovascular defects



*see Table 3 in *Tables & Figures* for complete details of studies with crude estimates, adjusted estimates, covariate adjustment, and other study data.

Figure 2.2 All effect estimates and confidence intervals, calculated or extracted from study articles*, for paternal exposure to pesticides or agricultural work and all cardiovascular defects



*see Table 3 in *Tables & Figures* for complete details of studies with crude estimates, adjusted estimates, covariate adjustment, and other study data.

Table 1. Basic characteristics of studies for the association between parental occupational exposure to pesticides and congenital heart defects

Lead author, year	Study location; time period	Relevant Exposures	Exposure Type	Exposure Levels	Exposure Period	Pesticide Type	Occupational Relevance	Relevant Birth Defects
Cohort Studies								
Kristensen, 1997 [20]	Norway; 1967 – 1991	Farming (pesticide)	Parental	1. Farmer (horticulture, animal husbandry, grain farmer, etc) 2. Non-farmer	Current farmer	Xenoestrogenic organochlorine compunds; others unknown	Occupational cohort compared to base population	VSD, other cardiovascular defects
Zhu, 2006 [16]	Denmark, Jun 1997 – Feb 2003	Farming (pesticide)	Maternal	1. Farmer 2. Non-farmer	3 mo. before & throughout pregnancy	N/A	Selected based on job before/during pregnancy	Heart malformation, other cardiovascular defects
Case-control studies								
Tikkanen, 1992 [15]	Finland; 1982 – 1983	Pesticide	Maternal	1. Regular exposure (approx. every day) 2. Occasional (less than regular) 3. No exposure	During first 3 mo. of pregnancy	N/A	Exposures to chemicals at work	VSD, PDA, COA, ASD, TGA, LHHS, TOF, ECD, TAC, other heart
García, 1999 [8]	Comunidad Valenciana, Spain; Jan 1993 – Dec 1994	Agricultural work (pesticide)	Paternal, Maternal	1. Exposed (agricultural work, pesticide application) 2. Non-exposed	1. Any exposure 2. Acute risk period exposure	N/A	Exposures to pesticides at work	Cardiovascular defects, excluding unspecified anomalies of the heart
Shaw, 1999	California, USA; Jan	Pesticide	Paternal, Maternal	1. Likely exposed 2. Maybe exposed	1 month before & first	N/A	Exposures to pesticides at	Conotruncal heart defects

[13]	1987 – Dec 1988			3. No pesticide exposure 4. Didn't work	3 months during pregnancy		work	
Loffredo, 2001 [21]	Maryland, D.C., & adjacent counties of Virginia, USA; 1981 – 1989	Pesticide	Maternal	1. Exposed (pesticide exposure during critical time frame) 2. Non-exposed	During 3 mo. before pregnancy and/or first 3 mo. of pregnancy	N/A	Jobsite as one of several potential places of exposure	All cardiac, TGA, non-TGA outflow tract defects, endocardial cushion defect, left-side obstructive lesion, VSD, pulmonic stenosis, ASD
Batra, 2007 [14]	Washington, USA; Jan 1987 – Dec 2003	Agricultural work (pesticide)	Paternal, Maternal	1. Agricultural worker (farmer, orchardist, horticulturalist, produce picker or inspector, etc) 2. Non-agricultural worker 3. Unemployed	At time of birth	N/A	Exposure based on job type	VSD
Herdt-Losavio, 2010 [17]	10 states in the USA (AK, CA, GA, IA, MA, NJ, NY, NC, TX, UT); Oct 1997 –	Agricultural work	Maternal	1. Farm worker 2. Non-farm worker	During 1 mo. before pregnancy and first 3 mo. of pregnancy	N/A	Exposure based on job type	Cardiovascular birth defects

	Dec 2003							
Desrosiers, 2012 [11]	10 states in the USA (AK, CA, GA, IA, MA, NJ, NY, NC, TX, UT); Oct 1997 – Dec 2004	Agricultural work	Paternal	1. Farm worker 2. Non-farm worker	During 3 mo. before pregnancy and first 1 mo. of pregnancy	N/A	Exposure based on job type	Conotruncal defects, TOF, TGA, COA, ASD
Snijder, 2012 [18]	(Western) Netherlands; June 2003 – Jan 2010	Pesticides	Paternal, Maternal	1. Probable exposure 2. Possible exposure 3. Unlikely exposure	N/A	N/A	Exposure based on job type and JEM estimation	pVSD, TOF, AVSD, COA, TGA, HLHS, aortic valve stenosis, pulmonary valve stenosis, miscellaneous
Cross-sectional studies								
Schwartz, 1986 [19]	Imperial County, California, USA; Jan 1975 – Dec 1978	Agricultural work (pesticide)	Parental	1. One or both parents classified as agricultural worker (farm owner, farm laborer, pesticide applicator, etc) 2. Neither parent an agricultural worker	Current occupation	N/A	Exposure based on job type	cardiovascular system

VSD – ventricular septal defect
pVSD – perimembranous ventricular septal defect

AVSD – atrioventricular septal defect
PDA – patent ductus arteriosus
COA – coarctation of the aorta
ASD – atrial septal defect
TGA – transposition of the great arteries
LHHS – hypoplastic left ventricle
TOF – tetralogy of Fallot
ECD – endocardial cushion defect
TAC – truncus arteriosus

Table 2. Detailed characteristics of study methods for the association between parental occupational exposure to pesticides and congenital heart defects

Lead author, year	Subjects		Exposure Assessment	Outcome Assessment
	Births	Selection		
Cohort Studies				
Kristensen, 1997 [20]	Farmers' births (n = 192 417) Reference population births (n = 61 351)	Agricultural census in 1969, 1979, and 1989; and horticultural censuses in 1974 and 1985 conducted by Statistics Norway (mandatory for governmental subsidies) provide list of farm holders. Reference population comprises all births to non-farmers in the agricultural municipalities	Information from the agricultural and horticultural censuses for farm holders and the basis for their exposure are the following indicators: money spent on pesticides in the 1968 census; amount of tractor pesticide spraying equipment on the farm in 1979 census	Agricultural and horticultural results linked with the Central Population Register (to identify spouses) and then with the Medical Birth Register of Norway to identify births. Reference population was also linked with the Medical Birth Registry, which records birth data up to three defects
Zhu, 2006 [16]	Farmers (n = 210) Other workers (n = 60 022)	Data from the National Birth Cohort in Denmark, selecting farmers' pregnancies to compare to the pregnancies of other workers	Computer-assisted telephone interviews (2 during pregnancy 2 after pregnancy) to measure occupational pesticide exposure 3 mo. before and during pregnancy. Asked questions about frequency of pesticide contact, kinds of pesticides, and use of protective measures	Link the National Birth Cohort data with the National Hospital Register and the Medical Birth Register using Denmark personal identification numbers. Information regarding malformation diagnosis (ICD-10) recorded here
Case-control studies				
Tikkanen, 1992 [15]	Cases (n = 406) Controls (n = 755)	Infants selected from all children born in Finland from 1982 – 1983.	Maternal interview by a midwife at a maternity welfare center or directly after delivery. Interview asked about occupational education and working conditions; various work exposures categorized into regular exposure or	Cases are born with a cardiovascular malformation as identified through the Finnish Register of Congenital Malformations or the Children's Cardiac Register. The defect must be detected within 1 year of birth by

			occasional exposure	diagnosis and at least one other technique (cardiac catheterization, surgery, autopsy, or echocardiography). Anomalies are coded by a pediatric cardiologist. Controls are randomly selected hospital deliveries.
García, 1999 [8]	Cases (n = 261) Controls (n = 261)	Infants born between January 1, 1993 and December 31, 1994 whose family lived outside of major towns and went to any of 8 public hospitals in the area for the birth.	Telephone or face-to-face interview with a gender-specific questionnaire for the parents asking about agricultural work, pesticide application, etc. and information on when, if direct handling, and how the compounds were handled. Relevant exposure periods were calculated: "acute risk" for fathers is exposure 3 mo. before pregnancy and/or during first 3 mo. of pregnancy; for mothers is exposure 1 mo. before pregnancy and/or during first 3 mo.	Cases are live-born and diagnosed with one of the chosen malformations within 1 year of life in the study period. Information acquired from hospital discharge letters. Controls are infants born closest to the date of birth of each case at the same hospital, without any diagnosis of birth defect during the study period.
Shaw, 1999 [13]	Cases (n = 207) Controls (n = 972)	Infants were selected from all live-born and fetal deaths delivered during the time frame in select California counties.	Maternal telephone interview after delivery (average time was 3.75 years after birth) asking about occupational pesticide exposures, use at home or with pets, residential proximity to cropland, and about father's occupational exposure (3 mo. before and first 3 mo. of pregnancy). From this mothers were grouped into categories of exposure.	Cases are live-born and fetal deaths identified from the California Birth Defects Monitoring Program from medical records reviewed at all hospitals and genetic centers in the area. Eligibility determined by medical geneticists and diagnostic information confirmed by echocardiography, cardiac catheterization, surgery, or autopsy. Controls are selected from all healthy live births in the same area with no major malformation diagnosis

				before 1 year.
Loffredo, 2001 [21]	Cases (n = 1 832) Controls (n = 771)	Data from the Baltimore-Washington Infant Study collected from 1981 – 1989 with collection from all 6 pediatric cardiology centers in the study area and home interviews with parents. Researchers used a subset of this study data.	Home interviews, the majority of which took place within 1 year after birth. Mothers asked about exposure to chemicals 3 mo. prior to pregnancy through pregnancy. Exposure information includes: type, mode of use, place where exposure occurred, frequency of exposure, and time of exposure, by trimester. From this mothers were grouped into categories of exposure.	Cases are live-born births to parents living in the study area with a structural heart disease confirmed within 1 year of life by a pediatric cardiologist OR by death certificates. Controls are live-born births in the same region, randomly selected and without CHD.
Batra, 2007 [14]	Cases (n = 3 489) Controls (n = 13 290)	Base population of any births occurring at nonfederal hospitals participating in the Comprehensive Hospital Abstract Reporting System between January 1987 – December 2003 in Washington. Researchers linked hospital discharge data with birth certificate data	Occupations of parents acquired from the birth certificates, in part with an open-description of work done in the past year. This was used to categorize participants into agricultural employment (farmer, orchardist, horticulturalist, farm laborer, migrant farm worker, vegetable/fruit picker, vegetable/fruit inspector, etc), non-agricultural employment, and unemployed.	Cases are live-born singletons identified within 2 years of life as having VSD as recorded in the Comprehensive Hospital Abstract Reporting System using ICD-9. Controls from the same system; excluded if diagnosed with any types of congenital heart disease other than patent ductus arteriosus. Frequency matched for year of birth.
Herdt-Losavio, 2010 [17]	Cases (n = 42) Controls (n = 3 383)	Data from the National Birth Defects Prevention Study (NBDPS); cases identified from birth defect surveillance at 10 centers across the United States. Controls identified via hospital records or birth certificates.	Maternal interview via telephone 6-24 weeks after estimated delivery date asking about maternal job title and industry during the exposure period. Jobs coded by two occupational epidemiologists and an industrial hygienist using the 2000	Cases are live births, fetal deaths, or prenatally diagnosed elective terminations from birth defect surveillance in 10 different states. Clinical geneticists at each center review case and coding. Cases with major defects are reviewed again by

			Standard Occupational Classification Manual and the 1997 the North American Industry Classification System.	NBDPS clinician to confirm, classify, and group by primary organ affected; only non-syndromic cases are included. Controls are randomly selected live births from hospital records and birth certificates without major defects.
Desrosiers, 2012 [11]	566 farmers & farm workers compared to 2,643 managers, administrator and salesworkers within a larger population-based case-control study of >60 birth defects	Data from the National Birth Defects Prevention Study (NBDPS); cases identified from birth defect surveillance at 10 centers across the United States. Controls identified via hospital records or birth certificates.	Maternal interview via telephone 6-24 weeks after estimated delivery date asking, in part, about paternal occupation during the exposure period. Jobs coded by two occupational epidemiologists and an industrial hygienist using the 2000 Standard Occupational Classification Manual and the 1997 the North American Industry Classification System.	Cases are live births, fetal deaths, or prenatally diagnosed elective terminations from birth defect surveillance in 10 different states. Clinical geneticists at each center review case and coding. Cases with major defects are reviewed again by NBDPS clinician to confirm, classify, and group by primary organ affected; only non-syndromic cases are included. Controls are randomly selected live births from hospital records and birth certificates without major defects.
Snijder, 2012 [18]	Cases (n = 424) Controls (n = 480)	Data from the HAVEN study; cases, recruited from 4 medical university centers found to be diagnosed with CHD in the hospital registry, are diagnosed with CHD within 15 mo. of birth	Self-reported job status and description ~15 mo. after birth, via questionnaire. Descriptions are coded into titles by the Dutch Standard Classification of Occupations and linked to a job exposure matrix based on exposure to 7 categories of chemicals.	Cases identified by hospital CHD registry; diagnosis by a pediatric cardiologist within 15 mo. of birth and confirmed with echocardiography, cardiac catheterization, or surgery.
Cross-sectional studies				
Schwart	Agricultural	Infants selected from all singleton	Review of maternal charts provided	Review of birth records and infant

z, 1986 [19]	workers (n = 990) Non-agricultural workers (n = 1 365) Unknown (n = 108)	births from one of three major hospitals in the agricultural community of Imperial County, California called Pioneer Hospital.	by Pioneer Hospital that listed maternal and paternal occupations. Categorized occupations into agricultural and non-agricultural employment. If at least one parent had an agricultural job, they are categorized together as exposed.	charts provided by the Pioneer Hospital.

CHD – congenital heart defect
ICD-9 – International Classification of Diseases, 9th edition
ICD-10 – International Classification of Diseases, 10th edition
VSD – ventricular septal defect

Table 3. All effect estimates and confidence intervals, calculated or extracted from study articles									
Lead author, year	**Population**	**Sample Size**	**Outcome data collection**	**Exposure Type**	**Phenotype**	**Estimate Type**	**Results**		
							Estimate	**95% CI**	
Cohort Studies									
Kristensen, 1997	Norway, 1967 – 1991	192,417 births	Record linkage between national registries	Parental occupation of "farmer" vs. all other occupations	VSD	Adjusted[1]	0.83	0.68	1.02
					All cardiac		0.76	0.50	1.16
Zhu, 2006	Denmark, June 1997 – Feb 2003	210 farmers 60,022 non-farmers	Record linkage between national registries	Maternal "farmer" vs. all other occupations	Heart	Crude	2.27	0.84	6.13
					Other circulatory system		1.22	0.17	8.71
Case-control studies									
Tikkanen, 1992	Finland, 1982 – 1983	406 cases, 755 controls	Cases identified via national surveillance; controls selected randomly from hospital births	Maternal occupational has "regular" vs. "no" pesticide exposure	All cardiac	Crude	1.56	0.47	5.14
García, 1999	Comunidad Valenciana, Spain; Jan 1993 – Dec 1994	261 cases, 261 controls	Hospital-based cases and controls	Maternal agricultural workers: Ever	All cardiac	Matched case-control[2] (1:1)	1.11	0.58	2.10
				Maternal agricultural workers: 6 month risk period			2.00	0.37	10.92
				Paternal agricultural workers: Ever			1.18	0.67	2.09
				Paternal agricultural workers: 6 month risk period			0.81	0.39	1.69
Shaw,	California,	127 cases,	Birth defects	Maternal "likely"	Conotruncal	Adjusted[3]	0.3	0.04	2.7

1999	USA; Jan 1993 – Dec 1994	259 controls		monitoring program cases and healthy live birth controls	occupational pesticide exposure vs. no pesticide exposure	heart defects			
		186 cases, 410 controls			Paternal occupational pesticide exposure vs. no pesticide exposure		1.0	0.6	1.9
Loffredo, 2001	Maryland, D.C., & adjacent counties of Virginia, USA; 1981 – 1989	1,001	771 controls	Cases from 6 pediatric cardiology centers, controls randomly selected live births without any heart defect	Maternal pesticide exposure from any source (job, home, other, multiple)	All cardiac	Crude	1.1	0.9	1.3
		31				Laterality and looping		0.8	0.3	1.8
		66				TGA		2.0	1.2	3.3
		114 cases				Non-TGA outflow tract defects		1.0	0.6	1.5
		87				Endocardial cushion defect		1.5	0.9	2.4
		147				Left-sided obstructive lesions		0.9	0.6	1.4
		80				Pulmonic stenosis		1.2	0.7	2.0
		373				VSD		1.0	0.9	1.5
		103				ASD		1.3	0.8	2.1
		66			Maternal pesticide exposure, job only	TGA		3.29	0.89	12.10
		114				Non-TGA outflow tract defects		3.84	1.39	10.59

Batra, 2007	Washington, USA; Dec 1987 – Dec 2003	1,936 cases 7,326 controls	Cases and controls via private hospital reporting system; controls excluded if any CHD except for patent ductus arteriosus	Maternal "agricultural" occupation vs. all other occupations	VSD	Crude	0.78	0.48	1.26
		2,653 cases 10,450 controls		Paternal "agricultural" occupation vs. all other occupations			1.12	0.91	1.38
Herdt-Losavio, 2010	10 states (AK, CA, GA, IA, MA, NJ, NY, NC, TX, UT); Oct 1997 – Dec 2003	42 cases, 3,383 controls	Birth defect surveillance. Cases reviewed by clinicians; controls are live births without major defects	Maternal occupation of "farm worker" vs. all other occupations	Cardiovascular	Adjusted[4]	1.14	0.75	1.73
Desrosiers[5], 2012	10 states (AK, CA, GA, IA, MA, NJ, NY, NC, TX, UT); Oct 1997 – Dec 2003	566 farmers and farm workers, 2,643 managers administrator & salesworkers within larger case-control study of >60 birth defects	Birth defect surveillance. Cases reviewed by clinicians; controls are live births without major defects	Paternal occupation of "farmer or farm worker" vs. occupational grouping of "managers, administrators" and "salesworkers"	Conotruncal heart defects	Adjusted[6]	0.9	0.6	1.9
					TOF		1.0	0.7	1.3
					d-TGA		0.9	0.6	1.2
					COA		0.9	0.6	1.2
					ASD, secundum or NOS		0.9	0.7	1.2
Snijder, 2012	Western Netherlands; June 2003 – Jan	424 cases, 480 controls	Hospital birth defect registry and healthcare centers	Maternal occupational pesticide exposure	All cardiac	Adjusted[78]	0.25	0.05	1.36
							1.00	0.23	4.35
		421 cases,		Paternal	All cardiac		0.72	0.31	1.67

	2010	477 controls		occupational pesticide exposure					
		113 cases			pVSD		1.35	0.44	4.18
		52 cases			TOF		1.46	0.38	5.69
		44 cases			AVSD		0.38	0.04	3.59
		44 cases			CoA		1.19	0.25	5.64
		63 cases			TGA		1.00	0.23	4.35
Cross-sectional studies									
Schwartz, 1986	Imperial County, California; Jan 1975 – Dec 1978	16 cases	2,257 controls	Record linkage between national registries; randomly selected controls from hospital births	Either parent occupation of "agricultural worker" vs. all other occupations	All cardiac	Crude	0.83	0.68	1.02
		6				VSD		0.69	0.13	3.76
		6				PDA		0.69	0.13	3.76
		4				Multiple phenotype		1.38	0.19	9.78

[1]Adjusted for year of birth, maternal age, geographical region, and parental consanguinity
[2]Matched on hospital and infant born closest to the date of birth of the case
[3]Adjusted for maternal periconceptional vitamin use, cigarette smoking, education level, and race/ethnicity
[4]Adjusted for study center, folic acid use, maternal age at delivery, maternal pre-pregnancy body mass index, maternal race/ethnicity, maternal education, parity, maternal smoking and maternal alcohol during the first trimester
[5]Data for this study was made available upon request to the corresponding author. Results used are for isolated and multiple phenotypes
[6]Adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, periconceptional smoking, periconceptional alcohol use, periconceptional vitamin/supplement intake, and center (ie. residence at delivery)
[7]Maternal data adjusted for maternal age, education level, ethnicity, parity, CHD in family, periconception alcohol use, periconception medication use, periconception folic acid use, and urban density
[8]Paternal data adjusted for paternal age, education level, ethnicity, and urban density

Table 4. Results from exposure-specific meta-analyses using the random effects method								
	All Studies				Studies with covariate adjustment			
	N	OR	95% CI		N	OR	95% CI	
Maternal								
All cardiovascular defects	7	0.92	0.78	1.09	4[a]	0.84	0.69	1.02
VSD	3	0.90	0.74	1.10	NC[b]	NC[b]	NC[b]	NC[b]
Paternal								
All cardiovascular defects	NC[c]	NC[c]	NC[c]	NC[c]	3	0.82	0.68	1.00
VSD	3	1.01	0.76	1.33	NC[b]	NC[b]	NC[b]	NC[b]

[a]References [15], [21], and [16] excluded due to lack of covariate adjustment
[b]Insufficient number of studies to calculate a pooled risk estimate
[c]All articles had covariate adjustment
VSD – ventricular septal defect

Appendix

Appendix 1. The complete PubMed Search:

((((((((((((("pesticides"[MeSH Terms] OR "polycyclic hydrocarbons, aromatic"[MeSH Terms]) OR "metals, heavy"[MeSH Terms]) OR "occupational exposure"[MeSH Terms]) OR "pesticide exposure"[All Fields]) OR ("pesticides"[Pharmacological Action] OR "pesticides"[MeSH Terms] OR "pesticides"[All Fields] OR "pesticide"[All Fields])) OR "occupational exposure"[All Fields]) OR "maternal occupation"[All Fields]) OR "paternal occupation"[All Fields]) OR "parental occupation"[All Fields]) OR "polycyclic aromatic hydrocarbons"[All Fields]) OR ("polycyclic hydrocarbons, aromatic"[MeSH Terms] OR ("polycyclic"[All Fields] AND "hydrocarbons"[All Fields] AND "aromatic"[All Fields]) OR "aromatic polycyclic hydrocarbons"[All Fields] OR "pahs"[All Fields])) OR ("solvents"[Pharmacological Action] OR "solvents"[MeSH Terms] OR "solvents"[All Fields])) OR "heavy metals"[All Fields]) AND "humans"[MeSH Terms] AND English[lang]) NOT ("warfarin"[MeSH Terms] OR "warfarin"[All Fields])

AND

(((((("congenital abnormalities"[MeSH Terms] OR "congenital abnormalities"[MeSH Terms]) OR "heart defects, congenital"[MeSH Terms]) OR "cardiovascular malformation"[All Fields]) OR "congenital heart defects"[All Fields]) OR "birth defects"[All Fields]) OR "congenital malformations"[All Fields]) AND "humans"[MeSH Terms] AND English[lang])