Published in final edited form as:
Mutat Res. 2011 November ; 728(3): 118–138. doi:10.1016/j.mrrev.2011.07.003.

Reproductive and Developmental Toxicity of Formaldehyde: A Systematic Review

Anh Duong[a], **Craig Steinmaus**[a,b], **Cliona M. McHale**[a], **Charles P. Vaughan**[c], and **Luoping Zhang**[a,*]

[a]School of Public Health, University of California, Berkeley, CA 94720

[b]Office of Environmental Health Hazard Assessment, California Environmental Protection Agency; Oakland, CA 94612

[c]Global Health Sciences, University of California, San Francisco, CA 94143

Abstract

Formaldehyde, the recently classified carcinogen and ubiquitous environmental contaminant, has long been suspected of causing adverse reproductive and developmental effects, but previous reviews were inconclusive, due in part, to limitations in the design of many of the human population studies. In the current review, we systematically evaluated evidence of an association between formaldehyde exposure and adverse reproductive and developmental effects, in human populations and *in vivo* animal studies, in the peer-reviewed literature. The mostly retrospective human studies provided evidence of an association of maternal exposure with adverse reproductive and developmental effects. Further assessment of this association by meta-analysis revealed an increased risk of spontaneous abortion (1.76, 95% CI 1.20–2.59, p=0.002) and of all adverse pregnancy outcomes combined (1.54, 95% CI 1.27–1.88, p<0.001), in formaldehyde-exposed women, although differential recall, selection bias, or confounding cannot be ruled out. Evaluation of the animal studies including all routes of exposure, doses and dosing regimens studied, suggested positive associations between formaldehyde exposure and reproductive toxicity, mostly in males. Potential mechanisms underlying formaldehyde-induced reproductive and developmental toxicities, including chromosome and DNA damage (genotoxicity), oxidative stress, altered level and/or function of enzymes, hormones and proteins, apoptosis, toxicogenomic and epigenomic effects (such as DNA methylation), were identified. To clarify these associations, well-designed molecular epidemiologic studies, that include quantitative exposure assessment and diminish confounding factors, should examine both reproductive and developmental outcomes associated with exposure in males and females. Together with mechanistic and animal studies, this will allow us to better understand the systemic effect of formaldehyde exposure.

Keywords

formaldehyde; teratogenicity; pregnancy; meta-analysis; human; animal

*Corresponding Author: School of Public Health, University of California at Berkeley, 237 Hildebrand Hall, Berkeley, California 94720-7356, (510) 643-5189, luoping@berkeley.edu.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

1. Introduction

With more than 46 billion pounds produced worldwide annually [1], most of which is widely used in the construction, textile, furniture, medical, chemical, and pharmaceutical industries, formaldehyde heavily impacts the everyday consumer. It is produced endogenously in all living organisms, including humans, but exposure to ubiquitous exogenous sources indoors, outdoors, at work, in residences, in food and medicine, poses a significant threat to public health [2]. Exposed populations include not only adult workers, who are exposed occupationally, but also the elderly, childbearing women, and young children. Emerging evidence supports an association between formaldehyde exposure and multiple adverse health effects [2]. It is increasingly being accepted by IARC [1,3], the US NTP (12^{th} RoC) [4], and the US EPA [5], that formaldehyde is a human carcinogen. Although it has long been suspected, reproductive and developmental toxicity associated with formaldehyde exposure remains inconclusive.

1.1 Previous Reviews of Formaldehyde Reproductive and Developmental Toxicity

Reproductive toxicity broadly refers to the occurrence of biologically adverse effects on the reproductive system that may result from chemical exposure to environmental agents and is characterized by alterations to the female or male reproductive organs, related endocrine system, or pregnancy outcomes [6]. Likewise, *developmental toxicity* (also known as teratogenicity) is the occurrence of adverse effects on the developing organism that may result from chemical exposure prior to conception, during prenatal or postnatal development, and may be detected at any point in the lifespan of an organism. Major manifestations include death of the developing organism, structural abnormality, altered growth, and functional deficiency [6].

Early reviews, of teratogenicity of formaldehyde in animals [7], and teratogenicity and reproductive toxicity of formaldehyde in both animals and humans [8–9], concluded that the evidence was limited and was from a small number of studies, which, in the case of the human studies were often of poor quality, lacking accurate exposure information and statistical power. One limitation identified in many early animal studies was that the effects of formaldehyde were assessed indirectly through its metabolism from hexamethylenetetramine, which is conditional. In its 2006 monograph on formaldehyde, IARC found that existing studies of formaldehyde's reproductive and developmental effects in both humans and animals were inconclusive, noting that most of the epidemiological studies reviewed were not designed to specifically evaluate formaldehyde, and that more exposure-specific follow-up studies were required [1].

The US EPA, in a draft document reviewing formaldehyde inhalation toxicity in animals and humans, suggests that the developing organism and the reproductive system are targets for toxicity following formaldehyde exposure by inhalation, although these findings are subject to revision as part of EPA's ongoing review process [5]. The animal studies examined demonstrated a broad range of adverse outcomes following exposure, while highlighting the inadequacy to assess these outcomes. Since similar outcomes were also observed in human studies, the overall data supported the human relevance of reproductive and developmental toxicity. This review also discussed data gaps in the current literature, such as a lack of assessment of potential reproductive effects in human males [5]. In the most recent review of formaldehyde reproductive toxicity in 2001, Collins *et al.* concluded that the reproductive impact of formaldehyde in humans was unlikely at occupational exposure levels, despite finding evidence of increased risk of spontaneous abortion (SAB) in a meta-analysis of 8 human population studies of formaldehyde exposed workers which reported sufficient data [10]. Further, it was concluded that there was little evidence of

reproductive or developmental toxicity at levels of occupational formaldehyde exposure in the animal studies, although routes of exposure other than inhalation were disregarded.

Here, we conducted the present review to provide a comprehensive, updated assessment of reproductive and developmental toxicity, particularly adverse pregnancy outcomes, associated with formaldehyde exposure.

1.2 Current Approach for this Systematic Review

In this review, most of the published studies in exposed humans and experimental animals are reviewed; findings from a new meta-analysis of human epidemiology studies are reported; additional and relevant evidence from *in vivo* and *ex vivo* animal studies are examined; and potential mechanisms of action of formaldehyde-induced reproductive and developmental toxicity are discussed.

Electronic searches were performed on PubMed using keywords including: formaldehyde, formalin, formol, reproductive toxicity, developmental toxicity, embryotoxicity, teratogenicity, and pregnancy outcomes. Searches included case-control, nested case-control, cross-sectional, and cohort studies in humans, as well as studies conducted via any route of formaldehyde exposure at any dosage on any experimental animal species. We additionally cross-referenced other formaldehyde reviews and books to generate a more complete list of literature. Collaborators from our previous review of formaldehyde in China [2] were able to obtain additional papers from the China National Knowledge Infrastructure, which contains 7,426 Chinese-language journals from 1915 to the present, an otherwise inaccessible source of information. We systematically excluded studies published in languages other than English and Chinese, and studies pre-dating 1980 due to difficulties in acquisition, unless the studies presented human data, for which there were already limited resources. Studies for which full text publications were unobtainable were also excluded. We concentrated on studies with clear and direct formaldehyde exposure, and did not include those in which formaldehyde was a byproduct of exposure to another agent (e.g. formaldehyde-releasing prodrugs and cosmetics, or hexamethylenetetramine and aspartame). The study selection process is detailed in Figure 1.

2. Human Population Studies

We identified 18 human studies reporting on the reproductive effects of formaldehyde-exposed populations. In all but 2 studies, women were chronically and/or occupationally exposed to formaldehyde either before or after conception, and the outcomes examined included menstrual abnormalities, infertility, spontaneous abortions, stillbirths, congenital malformations, premature birth, and birth weight. The remaining 2 studies examined the reproductive effects of paternal exposure to formaldehyde, with one study analyzing sperm morphology in exposed male workers [11], and the other investigating risk of spontaneous aborti on resulting from paternal exposure [12]. Findings from the 18 human studies are summarized in Table 1. Studies are categorized by outcome and listed chronologically for each outcome. Because several studies report multiple reproductive and developmental outcomes, they are cited several times in Table 1 and throughout the text.

2.1 Reproductive Toxicity in Humans

Altered incidences of pregnancies, abnormal menstruation or abnormal sperm may each serve as a potential indicator of reproductive toxicity in humans. In a 1975 Russian cross-sectional study, menstrual disorders were reported 2.5 times more often in women occupationally exposed to formaldehyde than in controls [13]. A later Danish cross-sectional study examined menstrual irregularities in 7 mobile daycare centers in which average indoor formaldehyde concentrations measured 0.43 mg/m^3 or 0.35 parts per million (ppm) due to

the use of urea formaldehyde in their construction [14]. Menstrual irregularities were self-reported in 30 – 40 % of the female exposed workers, compared to none in the matched unexposed control group. The exposed group also experienced greater vaginal irritation and pain during micturition (urination).

A Finnish cohort study investigated the effect of formaldehyde on female fertility as measured by fecundability density ratio (FDR) [15]. An FDR significantly below 1.0 means delayed conception, an indicator of reduced fertility. Exposure to high levels of formaldehyde (mean = 0.33 ppm) was significantly associated with delayed conception; the adjusted FDR was 0.64 with 95% confidence interval (CI) 0.43–0.92 for the high exposed group compared to the control unexposed group. This cohort study also found an increased risk of endometriosis, with an odds ratio (OR) of 4.5 and 95% CI of 1.0–20.0, further suggesting that formaldehyde exposure may have an adverse effect on female reproductive affects.

In contrast to the studies in females, in the only study that examined male reproductive effects, a Finnish cohort study, no adverse effects on sperm production, such as sperm count and sperm morphology, were found to be statistically different between exposed and unexposed groups [11]. However, as acknowledged by the authors, given the small size of the exposure groups (n = 11 in each group) and the large standard errors (SE) in the control group, the study had very low statistical power.

2.2 Developmental Toxicity in Humans

Developmental toxicity describes the ability of a substance to cause adverse effects in the developing organism, with manifestations including spontaneous abortion, stillborn births, congenital malformations and other structural abnormalities, low birth weight and premature births (Table 1).

2.2.1 Spontaneous abortion—Spontaneous abortion (SAB), also known as miscarriage, is defined as a pregnancy that typically ends naturally (not induced) during the first 7 to 28 weeks of gestation, and occurs at a rate of 15–20% in the United States and at lower rates in most developed countries [16]. The majority of studies on SAB associated with formaldehyde exposure examined the effect of maternal exposure, with only a single study examining paternal exposure.

To our knowledge, the earliest study of developmental toxicity in humans was conducted in 1975, in Russia, on occupationally exposed female factory workers [13]. No difference in the rate of abortion between exposed and unexposed workers was found in this cross-sectional study.

A 1982 retrospective cohort study of hospital staff members in Finland found that formaldehyde exposure at concentrations found in Finnish hospital sterilization units (typically 0.03 – 3.5 ppm; not measured in this study specifically) was not associated with an increase in SAB based on analysis of a small number of formaldehyde-exposed women (n = 50 exposed pregnancies) and 1100 unexposed pregnancies [17]. The adjusted rates were 8.3% for unexposed pregnancies and 8.4% for formaldehyde-exposed pregnancies. The same research group conducted a more in-depth case-control study of Finnish hospital nurses and reconfirmed that there was no relation between formaldehyde exposure and SAB [18].

Axelsson *et al.* (1984) interviewed 745 Swedish female university laboratory workers who had a total of 1,160 pregnancies [19]. In this cohort study, there was a slightly elevated relative risk (RR) of miscarriage rate in women exposed to organic solvents during their first

trimester (RR 1.31, 95% CI 0.89–1.91). Among the 10 women specifically exposed to formaldehyde, there were 5 normal births, 2 induced abortions and 3 miscarriages, thus, the miscarriage rate was 30%. The corresponding miscarriage rate for women who did not conduct laboratory work while pregnant was 11.5%. Axelsson *et al.* found that exposure to formaldehyde during pregnancy showed the highest miscarriage rate compared to other volatile organic compounds but the number of cases was too small to conclude a definitive causal relationship.

A study by the National Institute for Occupational Safety and Health (NIOSH) examined the outcomes of 365 pregnancies in a cohort of 407 female textile workers in a facility that fabricated men's work pants in Kentucky and found that the miscarriage rate in those who worked in the facility while pregnant (14%) was similar to the rate of those who worked elsewhere during pregnancy (13%) [20]. Although these rates were similar to the rate of miscarriage in the general population (10–25%), the SAB rate among those textile workers who did not work outside of the home during pregnancy was only 5%.

In a French cohort study examining SAB among hospital nurses handling neoplastic drugs, formaldehyde was assessed as a confounding exposure [21]. Data were collected by interview from May 1985 to May 1986 in three French hospitals and in a large center for cancer treatment. Of 139 pregnancies in nurses occupationally exposed to cytostatic agents (which suppress cell growth and multiplication), the frequency of SAB was 25.9% compared to only 15.1% in the 357 unexposed pregnancies (RR 1.7, 95% CI 1.2–2.5) [21]. When the pregnancies identified as being positive or unknown for previous formaldehyde exposure (n = 113) were excluded, the results concerning cytostatics were not modified. These data indicate that formaldehyde does not interact with cytostatic drugs to cause SABs, but the effect of formaldehyde alone was not analyzed.

A nationwide database of medically diagnosed SAB was used to evaluate the effects of paternal occupation and exposure on SAB risk in Finland. In a cohort of 596 pregnancies, an adjusted OR of 1.0, 95% CI 0.8–1.4 of SAB was found for paternal exposure to moderate or high formaldehyde concentrations [12], and an adjusted OR of 1.1 (95% CI 0.9–1.4) for low formaldehyde exposure, showing no overall excess of SAB in women whose husbands were exposed to formaldehyde. The authors hypothesized that if there had been any male-mediated effects on pregnancy outcome, the only possible damage would be via genetic damage to male germ cells or by secondary maternal exposure. Since individual exposure could not be assessed directly, any conclusions about this study are purely suggestive. The authors recommend that the findings of this study "*need to be confirmed by studies in which individual exposures can be assessed directly*" [12].

To examine the relation between adverse pregnancy outcomes in cosmetologists who are often exposed to a variety of chemicals, including formaldehyde-based disinfectants, a cohort of female cosmetologists from North Carolina were surveyed, and it was found that full-time cosmetologists who used formaldehyde-based disinfectants had a 2.1-fold (95% CI 1.0–4.3) higher risk of SAB than those who did not use formaldehyde-based disinfectants, when adjusted for other chemical exposures and maternal characteristics [22].

In a cohort study of French hospital workers, it was found that the rate of SAB was significantly higher among pregnancies during which women worked in an operating room and were exposed to formol (10% formaldehyde solution), ionizing radiation or anesthetics. Of the 724 total pregnancies, 11.1% of the pregnancies exposed to formol resulted in SAB, compared to only 6.9% in the unexposed group ($p < 0.05$) [23]. However, as discussed by the authors, exclusion of the effects of exposure to chemicals other than formaldehyde in the operating rooms was not possible.

In Finland, a group of scientists identified SAB cases in a nation-wide cohort of women working in laboratory settings. Compared to unexposed women, women who worked in laboratories and were chronically exposed (3–5 days/week) to formalin, a 37% formaldehyde solution, showed an increased risk for SAB (OR 3.5, 95% CI 1.1–11.2) [24]. The same group of researchers also found an increased risk for SAB (OR 3.2, 95% 1.2–8.3) among female wood workers who were chronically exposed to formaldehyde at high levels in a case-control study [15].

2.2.2 Congenital anomalies—Congenital anomalies, or birth defects, are characterized by physical, metabolic or anatomic deviations from the normal pattern of development that are apparent at birth and affect how a baby will look, function, or both. They range from mild to fatal, and affect about 3% of all babies born in the US [25]. Very few studies have examined congenital anomalies and formaldehyde exposure.

Ericson *et al.* reported a higher than expected number of infants who died neonatally and/or had congenital malformations among births in laboratory workers compared to all other births based on data from the 1975 Swedish census and the 1976 Medical Birth Register [26]. In this study, no data was available on formaldehyde exposure and no specific type of laboratory work could be identified to be more common among those with abnormal pregnancy outcomes than the normal controls. In a small case-control study nested within this larger study, no association was seen with formaldehyde exposure in the female laboratory worker mothers of 26 infant singletons who had died or had malformations compared with 50 randomly selected, age-matched controls. In this nested case-control study, qualitative exposure data was obtained by questionnaire (i.e. subjects were asked to list harmful substances to which they were exposed) and was therefore subject to possible recall bias.

In the aforementioned Hemminki *et al.* (1985) case-control study, while SAB risk was not increased, risk for congenital malformations was increased among children born to female hospital nurses with formaldehyde exposure. Among the 34 cases of malformed children three (8.8%) were born to women who reported formaldehyde exposure during their first trimester. Among controls, 5 of 95 (5.3%) working women reported formaldehyde exposure [18]. The authors noted that because their study had low statistical power, only very potent effects could have been identified.

In addition to spontaneous abortion, the French cohort study conducted by Saurel-Cubizolles also investigated birth defects in babies born to female hospital nurses with and without formaldehyde exposure. Of 641 total pregnancies, there was a greater frequency of birth defects in the pregnancies exposed to formol (5.2 %) than those who were unexposed (2.2 %) [23]. It was also found that formol caused the highest frequency of birth defects among all the exposure agents, such as anesthetics and ionizing radiation, investigated in this study. In a newer study conducted in Lithuania, it was found that residence in an area with ambient formaldehyde > 2.4 $\mu g/m^3$ was associated with increased congenital heart malformation by 24% (OR 1.24, 95% CI 0.81–2.07) [27]. A Danish cohort study found that the adjusted hazard ratio of high formaldehyde exposure and ‘major’ malformations was 1.5 (95% CI 0.8–2.9) [28].

Taskinen *et al.* also found no increase in ORs for congenital malformations due to maternal exposure to solvents in general through work in a laboratory, but did not examine formaldehyde exposure specifically [24].

2.2.3 Low birth weight—A population-based non-occupational study in Lithuania compared low birth weight (< 2500 g) rates among women residing in areas with high or

low concentrations of formaldehyde in ambient air [29]. In this cross-sectional study, the crude risk ratio of low birth weight babies among women residing in high formaldehyde exposure areas (> 4.67 μg/m^3) was 1.68 with 95% CI 1.24–2.27 compared with women residing in low exposure areas (> 4.67 μg/m^3). Once adjusted for potential confounders, the OR was 1.37 (95% CI 0.90–2.09) for exposures > 3.5 ug/m^3 compared to <3.5 ug/m^3. Increasing levels of formaldehyde exposure resulted in increased incidence of low birth weight, with 48.3 per 1000, 49.5 and 81.1, in low, moderate (> 3.48 μg/m^3) and high-exposure areas, respectively. The same research group conducted a follow-up study of all newborns born in 1998 in the city of Kaunas, Lithuania [30]. Residential exposure levels were monitored at 12 municipal monitoring sites, one in each residential district, and logistic regression was used to estimate the effect of pollutants on reproductive outcomes. The adjusted OR for low birth weight (< 2500 g) at the highest ambient formaldehyde level was OR of 2.09 (95% CI, 1.03–4.26). This OR was adjusted for parity, maternal age, marital status, education, season, smoking, and gestational age. The unadjusted OR was lower and not statistically significant (1.39; 95% CI, 0.91–2.12). In a Danish study, the adjusted OR for low birth weight for mothers who were laboratory technicians with high formaldehyde exposure was 1.2 (95% CI 0.6–2.2) [28].

2.2.4 Premature birth—Shumilina *et al.* found that the rate of premature water breaking was 37.23±2.41% in Russian female factory workers occupationally exposed to formaldehyde, compared to 23.63±1.23% in the unexposed group, but no information on significance was provided. Additionally, the threat of intra-uterine fetal asphyxiation, a condition in which there is an extreme decrease in oxygen supply to fetuses, was more than 2 times higher in the exposed group than in the control though no actual data on oxygen levels were reported [13]. Premature birth rates did not differ between exposed and unexposed groups.

Maroziene and Grazuleviciene studied the effects of ambient formaldehyde and premature birth in a cross-sectional study of 3,988 births and found that at high ambient formaldehyde levels, the risk of premature birth was 1.37 (95% CI 0.91–2.05) [30].

The most recent epidemiology study on formaldehyde exposure and pregnancy outcomes identified and surveyed a cohort of female laboratory workers [28]. A reduced risk of pre-term birth, OR 0.7 (95% CI 0.3–1.7), was found for those who reported laboratory work involving frequent and/or high formaldehyde exposure.

2.2.5 NIOSH analysis of combined adverse birth outcomes—In 1987, NIOSH was requested to conduct a health evaluation at Rockcastle Manufacturing, a textile plant that fabricated men's work pants in Kentucky [20]. Employees were complaining of headaches, nausea, vomiting, fainting, and adverse reproductive effects at the facility. Formaldehyde air sampling results ranged from 0.32–0.70 ppm in 13 air area samples obtained throughout the plant, lower than the current US Occupational Safety and Health Administration (OSHA) occupational exposure limit of 0.75 ppm [31]. Additionally, fabric samples that the company produced released 163–1430 micrograms of formaldehyde per every gram of fabric (μg/g). Past and present employees were surveyed for health information and reproductive health data (including miscarriage, birth defects, premature births and stillbirths) were assessed. The response rates were 98% for current employees and only 18% for past employees. A total of 365 pregnancies were divided into 3 categories: (1) pregnancies that occurred while the woman was employed at Rockcastle; (2) pregnancies that occurred while the woman worked elsewhere; (3) and pregnancies that occurred while the woman was not working outside the home. The rates of birth defects, stillbirths and premature births combined among workers in categories (1), (2) and (3) were 42 %, 5 % and 6 %, respectively. The RR of having any of these adverse pregnancy outcomes in category (1) compared to those

pregnancies in categories (2) and (3) combined was 6.9 (95% CI, 3.6–13.1, $p < 0.001$) [20]. The rates of miscarriage in groups 1, 2, and 3 were 14%, 13%, and 5%, respectively. The authors noted that the rate of miscarriage in group 3 (those not working outside the home) were 2–3 times below national averages. This low rate, combined with the lack of details regarding the methods used, and the use of exposed women as their own unexposed comparison group, makes the results of this study difficult to interpret.

2.3 Limitations of the Human Studies

Among the 18 human studies identified, there were more developmental studies than reproductive toxicity studies, likely because developmental toxicity has greater and more obvious physical manifestations, whereas reproductive toxicity effects are more difficult to detect and determine. The studies suffer from limited design and scope, and thus do not conclusively determine whether formaldehyde exposure causes human reproductive and developmental toxicities. Many of the older studies relied on self-reported data, and may suffer from reporting, recall, and selection biases. As they were predominantly retrospective epidemiological studies, few provided levels of formaldehyde exposure because they were not specifically designed to evaluate this. In addition, the results obtained may have been confounded by other co-exposures. None of the studies offer a plausible biological mechanism by which reproductive and developmental toxicity could occur.

There is an overwhelmingly larger portion of human studies examining reproductive outcomes associated with female exposures and thus a dearth of studies assessing potential effects of formaldehyde exposure in males. More human studies of reproductive effects resulting from exposure in males are needed, in order to understand male initiated mechanisms.

In summary, despite study design limitations, this brief evaluation of the previous human studies provides at least some evidence that formaldehyde exposure may be associated with reproductive and developmental toxicity, whether impacting one or multiple reproductive outcomes. To further evaluate the association between formaldehyde association and these outcomes, we conducted an updated meta-analysis.

3. Updated Meta-Analysis

3.1 Previous Results from Collins *et al*

To date, only one other meta-analysis has examined the relationship between formaldehyde exposure and adverse pregnancy outcome [10]. This meta-analysis, by Collins *et al.*, included 8 epidemiology studies with data on occupational formaldehyde exposure and spontaneous abortion. Collins *et al.* reported a summary RR of 1.4 (95% CI 0.9–2.1), which the authors stated “showed some evidence of increased risk”. However, the authors also identified evidence of publication bias (i.e. the tendency of researchers and journals not to publish smaller studies with negative or null results). Evidence of publication bias was seen in the funnel plot of the study’s effect sizes (e.g. the OR) versus their sample sizes, in Beggs test, and in their subgroup analyses based on study size (that is, the summary RR in the studies with 40 or more expected cases was 0.7 (95% CI 0.5–1.0) although the analysis of the smaller studies only included two studies. In addition, part of the reason these tests may have shown some indication of publication bias was the inclusion of the large negative study of paternal exposures by Lindbolm *et al*. [12]. The authors also raised the possibility of recall bias and performed subgroup analyses based on whether formaldehyde exposure was determined using self-reported data. The summary RR for the 5 studies using self-reported exposure data was 2.0 (95% CI, 1.4–2.8). In contrast, the summary RR for studies using other methods of exposure assessment was lower (summary RR = 0.7; 95% CI, 0.5–1.0),

although there were only two studies in this group. Based on the possibility of recall and publication bias, the authors interpreted their results as negative and concluded that occupational exposure to formaldehyde did not increase risk of spontaneous abortion. The results were further interpreted to conclude that formaldehyde is unlikely to cause any adverse pregnancy outcomes at occupational exposure levels.

3.2 Unique Approach in the Present Analysis

The present meta-analysis differs from the previous Collins *et al.* meta-analysis in several ways. First, we performed separate meta-analyses for SAB and for all developmental outcomes combined, whereas Collins *et al.* only presented results for SAB. We grouped these outcomes together for analysis to increase the power to detect developmental outcomes generally and because they may potentially result from effects of exposure on similar targets or pathways during the critical preconception window, e.g. genotoxic damage to germ cells. Second, Collins *et al.* combined studies of maternal and paternal exposure in their main analyses, while our main analyses only included studies of maternal formaldehyde exposures. Third, we included the study of operating room nurses by Saurel-Cubizolles *et al.* (1994), which identified a statistically significant increase of SAB in formol-exposed nurses (11.1% vs 6.9%, $p < 0.005$; calculated COR =1.68, 95% CI 1.01–2.82). This paper, however, was not mentioned in Collins *et al.* Fourth, when relative risks were given for several different levels of exposure (e.g. low, medium, and high), we used the relative risk for the highest exposure level. In contrast, Collins *et al.* used the RR for all exposure levels combined. If true associations exist, higher exposure groups are generally associated with greater statistical power and less likelihood of important confounding [32]. Importantly, this difference only applied to one study: Taskinen *et al.*, 1999 [15]. Fifth, we excluded the study of SAB and antineoplastic drugs by Stucker *et al.*, 1990 [21], which was included in Collins meta-analysis, because of the large number of people for whom formaldehyde exposure was unknown (50 people had known formaldehyde exposure, whereas for 63 people the formaldehyde exposure was unknown). Finally, there were several mostly minor differences in the methods used to calculate crude ORs and confidence intervals when only raw 2 × 2 table data were provided.

3.3 Meta-Analysis Methods

From the 18 human studies identified, certain studies were excluded from the meta-analysis if RRs or estimates of variance were not provided or could not be estimated [11,13–14], or if the study did not include an independent group of unexposed controls [20], or did not provide formaldehyde exposures for the majority of exposed subjects [21]. The excluded studies and reasons for their exclusion are summarized in Table 2. As discussed, if different RRs were presented for different levels of exposure, the RR for the highest exposure category was used in the meta-analysis [15,29–30].

Meta-analyses were done for two outcomes categories: SAB and all reproductive and developmental outcomes combined, which include SAB, birth defects or malformations, and low birth weight. SAB was the only individual outcome with an adequate number of studies (n=8; Table 1) to perform a meta-analysis. Several studies provided data for more than one outcome. In order to help assure independence across studies, in the meta-analysis of all outcomes combined, a relative risk for a single outcome was selected from each study in the following order: SAB, birth defects/malformation, and low birth weight. SAB and birth defects/malformations were chosen first and second because these were the first and second most common individual outcomes assessed. All but one selected study [12] assessed formaldehyde exposure in the mother. Separate analyses were done with and without this study to assess its impact on overall results.

Microsoft Excel 2008 and STATA version 11 (College Station, Texas) were used for all calculations. Summary RR estimates were calculated using both the fixed effects inverse variance weighting method and the random effects method [33–34]. Heterogeneity was evaluated using the general variance-based method [35]. If heterogeneity is present, the random effects model incorporates between-study variation into the summary variance estimate and confidence intervals. Some authors have suggested that the random effects model may be more conservative [35]. However, unlike the fixed effects model, where weights are directly proportional to study precision, the random effects model weighs studies based on a highly complex and non-intuitive mix of study precision, RR, and meta-analysis size (i.e. the number of studies included) [33]. As a consequence, this model assigns greater weight to smaller studies than the fixed effects model, and therefore may actually be less conservative [36]. To avoid this problem, we used the method presented by Shore *et al*. [37] and used in several subsequent meta-analyses [38–42]. In this method, the summary RR estimate is calculated by directly weighing individual studies by their precision, while between-study heterogeneity is only incorporated into the summary RR's variance (i.e. the 95% CI). Funnel plots and Egger's and Begg's tests were used to evaluate publication bias [43–44]. Missing confidence intervals in cohort studies were calculated using Byars approximation [45]. All *p*-values are one-sided since there was a clear *a priori* hypothesis that formaldehyde would increase, not decrease, reproductive and developmental outcomes.

3.4 Results

Seven studies with data on maternal formaldehyde exposure and SAB and 12 studies with data on all combined developmental outcomes were used in this meta-analysis. In addition, one study of SAB and paternal formaldehyde exposure was identified [12], and this was included with the maternal exposure studies in separate analyses. Table 1 shows the data from all human studies, while Table 2 shows those studies excluded from the meta-analysis and reasons for exclusion. Figure 2a and 2b show the Forest plots for the analyses of SAB and combined pregnancy outcomes for maternal formaldehyde exposures, respectively. Of the studies of maternal formaldehyde exposure, 5 of the 7 (71%) in the SAB analysis (Figure 2a) and 9 of the 12 (75%) in the all outcomes analysis (Figure 2b), had relative risks ≥ 1.01.

The results of the meta-analyses are shown in Table 3. In the meta-analysis of SAB and maternal formaldehyde exposure, the summary relative risk was 1.76 (95% CI, 1.20–2.59). The summary relative risks for all outcomes combined for maternal formaldehyde exposure was 1.54 (95% CI, 1.27–1.88). In analyses limited only to those studies that assessed formaldehyde exposure by methods other than direct self-reports, the summary relative risks for SAB and all outcomes combined were 1.29 (95% CI, 0.52–3.21) and 1.40 (95% CI, 1.11–1.78), respectively. In analyses limited to studies using direct self-reported formaldehyde exposure information, the corresponding summary relative risks were higher (SAB: RR = 2.04 (95% CI, 1.40–2.97); all outcomes: RR = 1.95 (95% CI, 1.35–2.81)).

When the Lindbohm *et al*. study of paternal exposure was added to the studies of maternal exposure the summary relative risks for SAB and all outcomes combined were 1.29 (95% CI, 0.94–1.76) and 1.34 (95% CI, 1.10–1.62), respectively.

Figure 3 shows the funnel plots for publication bias for the meta-analyses of SAB (Figure 3a) and all outcomes combined (Figure 3b). Without publication bias, a funnel shape is expected in these plots since RRs from larger studies, which have smaller standard errors (SEs), are expected to have less dispersion due to random chance than the RRs from smaller studies (which have larger SEs) [44]. For maternal formaldehyde exposures and SAB, obvious publication bias was not seen in the funnel plot (Figure 3a), or in Eggers (p=0.65) or Beggs (p= 0.45) tests. Similarly, no clear evidence of publication bias was seen in the meta-

analysis of all outcomes combined in the funnel plot (Figure 3b) or in Eggers (p=0.25) or Beggs tests (p=0.34).

3.5 Discussion

As a whole, the results of this meta-analysis provide some evidence that maternal formaldehyde exposure is associated with SAB and possibly other reproductive outcomes. Summary RRs were elevated in analyses of SAB (RR=1.76; 95% CI, 1.20–2.59) and all reproductive outcomes combined (RR=1.54; 95% CI, 1.27–1.88). The low p-values associated with these summary RRs (p=0.002 and < 0.0001 for SAB and all outcomes combined, respectively) show that the excess relative risks are unlikely due to chance. For maternal exposure, statistically significant heterogeneity was not seen in the meta-analysis of SAB (X^2 = 8.99, p=0.17) or all outcomes combined (X^2 = 11.2, p=0.43). In addition, the fact that greater than 70% of the individual RRs in both analyses were above 1.0, provides some indication of that the positive results were fairly consistent across studies. Summary RRs decreased somewhat for both SAB and all outcomes combined when the large Lindbolm *et al.* study of paternal formaldehyde exposure was included, though no definite conclusion can be made based on only one paternal study.

An analysis of dose-response can be an important part of assessing causal inference, although it is not a *sine qua non*, and in some instances where a true association exists, a clear dose-response relationship may not be present [34]. Six studies did provide some dose-response data [12,15,24,28–30]. In the Lindbolm et al. study of paternal exposures, ORs for SAB were near 1.0 in both the high (OR = 1.0) and low exposure groups (OR = 1.1). In several studies, ORs were elevated in the highest exposure group, but not in the lower exposure groups [24,29]. For example, Grazuleviciene *et al.* measured low birth weight risk in 3 regions with different formaldehyde concentrations, and crude RR increased with higher ambient concentration of formaldehyde from 1.0 (reference group) to 1.02 (95% CI, 0.76–1.38) to 1.68 (95% CI, 1.24–2.27) for exposure groups of < 1.94 μg/m3, 1.94–3.5 μg/m3, and > 3.5 μg/m3, respectively. In other studies, ORs were higher in the exposed groups than in the unexposed controls, but a clear monotonic dose-response relationship was not seen [15,30]. For example, in Taskinen et al., 1999, SAB ORs were 1.0, 2.4 (1.2–4.8), 1.8 (0.8–4.0), and 3.2 (1.2–8.3) in the unexposed (reference), low, medium and high exposure groups, respectively. Overall, few studies exhibited clear dose-response relationships. However, the wide confidence intervals for many of the ORs reported in these studies raises the possibility that dose-response trends might not have been evident because of small sample sizes and insufficient statistical power to assess risks in low to moderate exposure categories.

Confounding could be responsible for some of the elevated excess RR identified in the meta-analyses. Many of the studies involved women who were exposed to agents other than formaldehyde, which may be linked to reproductive or developmental effects, and these other exposures were not adjusted or controlled for in most of the relative risk estimates we used in this meta-analysis. For example, for the Saurel-Cubizolles *et al.* study, we calculated a crude odds ratio between formol exposure and SAB of 1.68 (95% CI, 1.01–2.82), since an adjusted OR was not provided in the article. However, many of the operating room nurses were also exposed to anesthetic gases and ionizing radiation, two other agents linked to SAB risk in this study. In fact, the authors reported that 52% were simultaneously exposed to all three (formol, anesthetic gases, and ionizing radiation). The fact that these two other agents were fairly strongly related to both the exposure (formaldehyde) and outcome (SAB) of interest raises the concern that they may have caused important confounding. Solvents (in laboratory workers or wood workers), chemotherapy agents (in nurses), or other agents might have caused confounding in other studies. As a whole, few studies provided formaldehyde relative risk estimates that were adjusted for these other agents.

Differential recall is another bias that could falsely elevate RRs if women with reproductive outcomes have a greater tendency to recall past exposures than women without these outcomes. The results of our subgroup analyses, where summary relative risks were lower in studies which did not use self-reported information on formaldehyde exposure compared to studies that did, provide evidence that this bias is a major concern in the overall meta-analysis results.

The results of this meta-analysis are somewhat similar to those of a previous meta-analysis by Collins *et al.*, which reported a summary RR of 1.4 (95% CI 0.9–2.1) for 8 studies of SAB. The differences between this meta-analysis and Collins *et al.* are shown in Table 4. As seen, a major difference between our meta-analysis and that of Collins *et al.* is our use of the 1994 Saurel-Cubizolles *et al.* study [23], which was not used by Collins *et al.*. Another major difference was that Collins *et al.* used data from the study of chemotherapy agents by Stucker *et al.* and the study of paternal formaldehyde exposure by Lindbohm *et al.* in their main analysis, whereas we did not. We could not determine the source of the RR of 1.0 (95% CI 0.5–2.0) used by Collins *et al.* for the Stucker *et al.* study. In addition, Stucker *et al.* did not specifically report a relative risk in a formaldehyde exposed group or the raw data to estimate it. Importantly, both our meta-analysis and that of Collins *et al.* found that the summary relative risks were lower in the studies that did not rely on self-reported exposure data compared to the summary relative risk in those studies that did.

In summary, the elevated RRs identified in this analysis, combined with the consistency indicated by the positive findings (RRs >1.0) seen in the large majority of the individual studies, all provide evidence that maternal formaldehyde exposure may be associated with SAB and possibly other reproductive outcomes. However, recall bias and confounding cannot be ruled out at this time and may have caused at least some of the elevated RRs seen in this meta-analysis. Further research is needed to assess these biases and confirm the findings presented here.

To date, there have been very limited human studies on the effects of formaldehyde and reproductive/developmental toxicity. And because it is difficult to devise ethically acceptable experiments to test formaldehyde's reproductive toxicity in humans, animal toxicity studies provide the next best models to study these effects.

4. Experimental Animal Studies

We examined the findings on reproductive and developmental toxicity associated with formaldehyde exposure in experimental animal studies for comparison with the human findings. As the most recent review of formaldehyde's reproductive and developmental toxicity was performed nearly a decade ago [10], our review focuses on more recent studies, a majority of which find reproductive, developmental, and post-natal toxicity associated with formaldehyde exposure. These studies, summarized in Table 5, have been performed in a range of animal species via different exposure routes at various formaldehyde exposure levels to test formaldehyde's toxicity. The findings are organized by study type (reproductive toxicity, developmental toxicity, *ex vivo* and in vitro), study animal, type of exposure (inhalation, injection, oral).

4.1 Exposure Routes Relevant to Human

Though it has been argued that only inhalation exposure studies are relevant to humans [10], all routes of human exposure, including inhalation, topical, oral and injection, require consideration, given the increasing exposure via these routes. In the past, humans were typically exposed to formaldehyde occupationally, particularly in professions involving embalming, laboratory work, and plastic and wood manufacturing. In recent years, human

exposure by environmental pollution or through off-gassing in buildings has become increasingly more common [2]. In many cases, without knowing it, people are exposed to furniture and fabrics contaminated with formaldehyde, and consumed foods, particularly fruits, vegetables and seafood that have been illegally preserved with a diluted form of formaldehyde called formalin, a widespread problem in China [2]. The widely used artificial sweetener aspartame could be also a potential exposure source as it is metabolized to formaldehyde, and accumulates in tissues, at least in rats, following oral exposure [46]. Even in infancy, children are exposed by injection to formaldehyde present in polio and diphtheria vaccines preparations as a result of the manufacturing process [47]. Several therapeutics used to treat malignancy are formulated with formaldehyde which is required for drug activation [48], or release formaldehyde [49]. Thus, the multiple routes of formaldehyde exposure examined in the animal studies discussed below may be relevant and applicable to humans.

4.2 Reproductive Toxicity

Animal studies have examined the effects of formaldehyde on adult animals and their reproductive organs and systems. The major endpoints include reproductive organ malformation or dysfunction, as well as other physical anomalies that hinder or prevent successful mating and copulation. Reproductive studies have been conducted only on mammalian and avian species, and are organized by animal type and exposure source.

4.2.1 Rats—In formaldehyde inhalation studies in rats, decreased or damaged seminiferous tubules were consistently observed [50–52]. Reduced or damaged testicular tissues [52–53] and decreased testosterone levels [51] were also reported. Among the adverse effects observed in formaldehyde injection studies (all intraperitoneal) in male rats were: Leydig cell impairment [54]; decreased testicular weight and levels of serum testosterone [54–55]; decline in sperm count [55], motility [55–56] and viability [56]; increased phenotypic sperm abnormalities, lethal mutations and reduced number of successful matings [57]; and decreased DNA and protein content in the male testis, prostate and epididymis [56]. The only reproductive study to orally administer formaldehyde to male rats found sperm head abnormalities in the exposed group compared to the control group [58].

4.2.2 Mice—Four inhalation studies were conducted in male mice, in which damage to seminiferous tubules [59], decreased number of sperm [60–61], decreased sperm survival rate [61], increased deformity rate [61–62] and increased micronuclei frequency in early spermatids [60], were reported. In addition, male mice exposed through inhalation displayed decreased levels of serum testosterone and lactate dehydrogenase (LDH) [62], glutathione peroxidase (GSH-Px) [59], glucose-6-phosphate dehydrogenase (G-6PD) and succinate dehydrogenase (SDH) [61–62]. Exposure of female mice by inhalation resulted in hypoplasia of the uterus and ovaries after 13 weeks of exposure at 40 ppm [63].

In the mice studies, mostly male mice were exposed through intraperitoneal (i.p.), intravenous (i.v.), intramuscular (i.m.) and intragastric (i.g.) injection, as detailed in Table 5. One such study found a linear relationship between sperm head DNA alkylation and administered dosages of formaldehyde by injection (i.p. and i.v.) in male CF-1 strain mice [64]. Several studies reported decreased sperm counts and increased rates of deformed sperm cells [60,65–67]. DNA-protein crosslinking (DPC) was observed in the testicular cells of formaldehyde-injected males in two studies [68–69], and one of these studies also reported DNA breakage [68–69]. The only injection study of female mice found irregular estrous cycles, damaged and smaller oocytes and fewer mitochondria and fibrosis in reproductive tissue [70]. In the only oral study of formaldehyde-exposed mice, there was a small but non-significant increase in abnormal sperm cells in male mice [11].

4.2.3 Other animals—Reproductive toxicity studies were conducted on three bird species. During the avian flu epidemic in 2008–2009, a study was conducted to test the effectiveness of formalin-based avian influenza inactivated vaccines. It was found that vaccine preparations containing 0.81% formalin injected intramuscularly significantly reduced egg production in hens, lowered estradiol and hemaglutination inhibition antibody levels and caused a degenerative change in ovarian follicles and the uterus [71].

Male Japanese quails that were fed formalin showed depression, lower food consumption and body weight, as well as decreased testes weight and seminiferous tubule diameter [72]. Lowered testes volume and seminiferous tubule diameter was also observed in cockerels to which formalin was orally administered at higher doses [73].

4.3 Developmental Toxicity

Several animal studies have focused on the effects of formaldehyde on fetal development and health. In these studies, pregnant animals or embryos were exposed to formaldehyde and the developing fetuses were observed for anomalies. These studies are summarized in Table 5, and organized by animal type then exposure source and outcome.

4.3.1 Rats—In 2001, Thrasher and Kilburn found that exposure of pregnant rats to formaldehyde concentrations between 0–1.5 mg/m^3 via inhalation resulted in damaged blastomeres, increased rate of embryo degeneration, chromosome aberrations and aneuploidy, involution of lymphoid tissues, and hypertrophy of Kupffer's cells in the fetus [74]. The most recent inhalation study found a decrease in placenta and corpus luteum size, increased fetal abnormalities, and shorter than average limbs in the newborn pups [75]. In contrast, an earlier study found that the corpus luteum, which produces important hormones that maintain pregnancy, and fetal weights were unaffected [76]. Saillenfait *et al.* concluded that formaldehyde may be slightly toxic to the fetus based on reduced fetal weight [77].

In their 2001 study, Thrasher and Kilburn also examined the effects of exposure through injection and oral exposure. They found that pre- and post-implantation deaths increased twofold following exposure by i.g. injection [74]. Results following prenatal oral exposure were inconclusive, though physical deformities were observed in the rat pups of exposed mothers [74].

4.3.2 Mice—To the best of our knowledge, no studies of developmental toxicity in mice following formaldehyde exposure by inhalation were conducted.

Several studies examined developmental toxicity following injection. As well as examining the effects of formaldehyde on rat fetal development described above, in the same study, Thrasher *et al*. also injected the tail veins of pregnant adult mice with 0.05 ml of 1% formalin containing 3.5 mg of ^{14}C-labled formaldehyde. The animals were killed at intervals from 5 min to 48 hrs, and radioactive formaldehyde incorporation was followed by frozen section autoradiography and liquid scintillation detection. In the first 5 minutes, more rapid uptake of radioactive formaldehyde was observed in uterus, placenta and fetal tissues, compared with other maternal organs. Incorporation of the labeled isotope was found to be greater in fetal brain than the maternal brain and elimination of formaldehyde from fetal tissues was slower than in maternal tissues [74]. Formaldehyde elimination was also shown to be slower in fetal tissue than in maternal tissue following maternal exposure by injection, also in the tail vein, in another study [78]. A Chinese study injected (i.g.) pregnant mice with various concentrations of formaldehyde and found evidence of DNA breakage and damage and DPC, with more severe effects in the fetus than in the mother [79]. Pre- and post-implantation deaths increased significantly with paternal exposure by intraperitoneal injection [80–81]. In a study of 34 pregnant mice who were orally exposed to formaldehyde,

22 died before analysis was performed; however, teratogenic effects were not observed in the fetuses of the 12 survivors [82].

4.3.3 Other animals—Exposure of pregnant rabbits to 12 ppm formaldehyde by inhalation throughout the gestation period, resulted in abnormalities in the newborns including meromelia (lacking limbs, 6.8%), oligodactyly (missing fingers or toes, 4.1%), encephalocele (cranium bifidum, 6.1%), and umbilical hernia (3.4%) [83]. In two chicken studies, embryotoxicity was examined in whole eggs exposed to formaldehyde vapor. Margas exposed eggs at an early stage of development, between the second and fourth days of incubation, to formaldehyde vapor for 1 hr every 12 hours, between one and six times [84]. Intact eggs, and eggs in which a small hole was drilled in the air chamber, were tested. Although the intact eggs did not show any particular abnormalities after exposure to formaldehyde vapors, the perforated eggs were affected at a rate of 29:100. These embryotoxic effects were mainly early and late prenatal deaths, extensive and limited congenital anomalies as well as reduction deformities. Hayretdag and Kolankaya applied pre-incubation formaldehyde fumigation to 1-day old embryos at two different concentrations for 20 or 40 minutes and examined the effects on tracheal epithelia [85]. Transmission electron microscopy revealed shortening and loss of cilia, vascuolisation and swelling of mitochondria and spoiling of cristae. These effects were increased with exposure duration.

A study of topically exposed pregnant hamsters did not find significant effects of formaldehyde on fetal weight, length or malformation, possibly due to the confounding effects of anesthetic administration [86].

4.3.4 Post-natal exposure and developmental toxicity—The studies discussed above examined developmental toxicity associated with prenatal formaldehyde exposure. Several studies examined the effects of postnatal formaldehyde exposure on development in rats. The lungs of rat pups exposed to formaldehyde by inhalation for 30 days showed decreased tissue superoxide dismutase (SOD) activity, copper and iron levels were decreased, and increased zinc levels, suggestive of oxidative damage in lung tissue [87]. Increased heat shock protein 70kDa (hsp70) synthesis and damaged neurons were detected in the hippocampus of rat pups after 30 day exposure by inhalation [88]. These effects had diminished or disappeared by by 30–60 days after cessation of exposure, suggesting that the changes were reversible. A study that observed behavioral responses of rat pups exposed to 1 or 2.5% formalin by subcutaneous injection, found specific and non-specific pain responses in neonatal rats, which decreased in intensity and varied by type, with age [89].

4.5 *Ex vivo* and *in vitro* Animal Studies

Ex vivo studies, examining the effects of formaldehyde exposure on rat and mouse embryos in culture, were conducted. Harris *et al*. exposed mouse whole-embryos (gestation day 10–12) to formaldehyde in culture medium and found that formaldehyde had deleterious effects on embryo growth and viability and produced a depletion of glutathione (GSH) in the visceral yolk sac and embryo [90]. Neuropore closure, crown-rump length and somite number were reduced by formaldehyde. Further, GSH depletion was shown to potentiate formaldehyde toxicity. Hansen and colleagues exposed mouse and rat embryos in culture to formaldehyde by direct addition to the culture medium and by microinjection [91]. They observed a dose-dependent loss in viability and significant increases in incomplete axial rotation and neural tube closure following both exposure routes in mice but microinjection induced these effects at the lowest concentration range tested (0.003 – 0.5 μg). Ten to 15-fold higher concentrations were required to elicit the same decrease in viability and increase in incomplete axial rotation in exposed rat embryos. These findings show that the visceral

yolk sac serves a general protective role against toxicity and inherent differences in the embryonic metabolism of formaldehyde may determine species sensitivity.

In a study designed to develop an *in vitro* system for testing teratogenicity, blastocyst-stage mice embryos were removed from the uteri and the inner cell mass was isolated and cultivated. The cell cultures were then exposed to various chemicals and a cytotoxic range was determined for each chemical. At 440 and 690 μM, 10% and 50%, respectively, of the cells were affected, though the researchers concluded that formaldehyde was not a teratogenic agent [92]. An *in vitro* study found that directly washing ram sperm with 0.005 % formaldehyde (in phosphate buffered saline) reversibly inhibited sperm motility, while at 0.04% the effect was irreversible [93].

4.6 Key Findings

After reviewing the literature on reproductive and developmental toxicity associated with formaldehyde exposure in animals, a few observations are noteworthy. Unlike the human studies, reproductive toxicity was examined more frequently than developmental toxicity in animals, and within reproductive toxicity, there were more male than female exposure studies. This skewing towards male studies may be because effects on male reproduction are more readily observable and require fewer invasive procedures. Despite variability in study design and size, choice of animal types, and exposure routes, levels and durations, overall, the studies found associations between formaldehyde exposure and reproductive toxicity in males. Regarding route of exposure, many studies examined the effects of exposure by inhalation and injection but few examined the effects of oral exposure to formaldehyde. In order to improve our current understanding of reproductive toxicity associated with formaldehyde exposure in animals, studies assessing (1) female reproductive toxicity with particular attention to organ and tissue function, and (2) multigenerational reproductive toxicity due to formaldehyde exposure, are crucially needed.

Developmental toxicity studies are relatively easy to conduct and the physical endpoints are easily detected. Overall, as shown in Table 5, the majority of the published reproductive and developmental toxicity studies conducted in animals, as well as the *ex vivo* and *in vitro* studies, found adverse outcomes associated with formaldehyde exposure. These findings improve and enhance our current understanding of formaldehyde and its relationship with reproductive and developmental toxicity. However, it is possible that studies with negative findings were unreported.

5. Potential Mechanisms of Action

Despite the fact that formaldehyde exposure may cause reproductive and developmental toxicity, as suggested by evidence from both human and experimental animal studies, our current understanding of the likely mechanisms of action (MOA) is very limited. To date, few human studies have been designed to investigate possible formaldehyde MOAs, though hypotheses have been generated from limited preliminary results obtained in recent animal studies. Currently, the mechanisms by which formaldehyde is proposed to induce reproductive and developmental toxicity include genotoxicity, oxidative stress, disruption of the activity of proteins, enzymes and hormones important for the maturation of the male reproductive system, apoptosis and DNA methylation. It should be noted that most of the proposed mechanisms are hypothetical and require validation, particularly in reproductive systems.

5.1 Chromosomal Damage and DNA Lesions

Formaldehyde is genotoxic, inducing chromosomal aberrations (CAs), micronuclei (MN), sister chromatid exchanges (SCEs), DNA breakage, and DNA-protein crosslinks (DPCs) in

nasopharyngeal and buccal cells, and possibly in blood and bone marrow cells (though this is controversial), following inhalation in humans and rodents [1,94]. Thus, it is plausible that formaldehyde could cause similar chromosomal damage and DNA lesions at reproductive sites. Indeed, Lindbohm (1991) hypothesized that the MOA for SAB was genetic damage to germ cells following paternal exposure to chemicals [12]. Evidence of such genetic damage has been reported and was briefly described above in the animal study section. When male mice were exposed to formaldehyde (0.2, 2.0, 20.0 mg/kg by i.p. for 5 days), increased frequencies of MN and SCEs was observed in early spermatogenic cells [95]. A Dutch *in vitro* study showed that when Chinese hamster ovary cells were treated with varying concentrations of formaldehyde for 2 hrs, frequencies of CAs and SCEs increased with increasing dose. All types of CAs (gaps, breaks, exchanges) were induced by formaldehyde, and, since all of the aberrations were chromatid-type, an S-dependent mode of action was indicated [96].

The induction of DPCs by formaldehyde is well known to occur in many cell types, including reproductive tissues and cells. Peng and colleagues detected DPCs in the testicular cells of Kunming mice (at 20.0 mg/kg by abdominal injection) between 6 and 18 hrs after exposure, suggesting that formaldehyde may be responsible for reproductive damage in these male mice [68]. After 24 hr the DPC levels were similar to those of un-exposed mice, indicating activation of a DPC repair process between 18 hr and 24 hr treatment. The biochemical pathways underlying DPC repair were largely uncharacterized until a recent study demonstrated, using a yeast gene deletion screening system, a differential pathway response to chronic versus acute formaldehyde exposure involving homologous recombination (chronic exposure) and nucleotide-excision repair (acute exposure) [97]. Wang and colleagues detected DNA strand breakage by comet assay (also called single cell gel electrophoresis) in testicular cells isolated from male Kunming mice that had been exposed to formaldehyde (10–50 μmol/L) *in vitro*, and observed both DNA breaks and DPCs in cells exposed to a higher formaldehyde concentration (75 μmol/L) [69]. Using the same assay to analyze the liver cells of the newborns of formaldehyde-treated pregnant female mice, DNA strand breaks and DPCs were detected at formaldehyde concentrations over 1.0 and 2.0 mg/kg, respectively, while most fetal liver DNA formed DPCs at the highest exposure level of 20.0 mg/kg [79]. However, both DNA breaks and DPCs reported in the last two studies [69, 79] were measured by the same comet assay, which could technically affect the accuracy of DPCs.

5.2 Oxidative Stress

Although formaldehyde is known to cause genotoxicity (DNA and chromosomal damage) and cytotoxicity (cell death or apoptosis), the mechanism is unclear. Limited evidence shows that oxidative DNA damage by reactive oxygen species (ROS) could play an important role. It is well known that excessive ROS production can cause developmental toxicity through oxidative damage to key cellular components such as DNA, proteins and lipids.

Formaldehyde was shown to synergize with a water-soluble radical initiator, 2,2′-azobis-[2-(2-imidazolin-2-yl)propane] dihydrochloride to increase cellular ROS and cell death via necrosis in Jurkat cells [98]. ROS-mediated oxidative damage resulting from formaldehyde exposure has been detected in distal cells and tissues, including reproductive tissues. Rodents exposed to formaldehyde by inhalation exhibited lipid peroxidation in liver [99] and brain [100]. Malondialehyde (MDA), a lipid peroxidation product commonly used as a biomarker of oxidative damage [101], was significantly increased in the testicular tissues of male mice treated with formaldehyde at 20 mg/kg [95]. Formaldehyde may exert these oxidative stress effects in reproductive tissues indirectly, mediated by an inflammatory response to lung damage upon inhalation. Male Wistar rats exposed to formaldehyde by

inhalation for 30 – 90 minutes per day for 4 days exhibited local and systemic inflammatory responses (increased leukocytes) [102]. The authors proposed that formaldehyde exposure may affect lung resident cells, including macrophages and mast cells that could mediate the lung inflammatory response and the systemic release of inflammatory mediators. The inflammatory mediators may trigger systemic immune responses.

Both the induction and suppression of antioxidant enzymes by formaldehyde has been demonstrated in male reproductive tissues. These enzymes, including glutathione peroxidase (GSH-Px), SOD, CAT, and GSH, protect cells against oxidative damage and a change in their activity levels may indicate the level of oxidative damage in target tissues and/or cells. Zeng *et al.* found that GSH-Px levels and GSH levels were lowered in formaldehyde exposed testicular tissue in mice, while SOD and CAT levels were significantly elevated [59]. A more recent study found significantly reduced levels of SOD and GSH-Px and higher amounts of MDA in the testicular tissue of male Wistar rats [101]. These studies show that formaldehyde induces the antioxidant defense mechanism in rodent testicular tissue and may impair its effects. Reduced amounts of the trace metals, copper and zinc, cofactors of SOD, in the testicles of male mice [95], could contribute to the reduced SOD activity. Activity of testicular G-6PD, an enzyme that protects red blood cells and tissues against oxidative damage, was decreased in male dosed with formaldehyde (21, 42, and 84 mg/m^3) by static inhalation for 5 days [62].

5.3 Other Possible MOAs

5.3.1 Dehydrogenases—Additional enzyme activities and protein levels and or functions have been shown to be impacted by formaldehyde exposure in reproductive tissues/cells and may contribute to reproductive toxicity. Lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) are involved in the maturation of spermatogenic cells, testis and spermatozoa and with the energy metabolism of spermatozoa. In a Chinese study of Kunming mice, SDH activity was measured by UV spectrophometry in testicular tissue after exposure to formaldehyde [65]. After 6 days, SDH activity decreased with increasing formaldehyde levels (0.2, 2, 20 mg/kg injected into the stomach, once a day, for 5 days) and was positively correlated with sperm cell counts, and negatively correlated with the abnormal rate of sperm heads. Thus, SDH activity is a potential biomarker of damage to testicular damage. Decreased SDH activity was also observed at all formaldehyde concentrations in a study with a similar exposure regimen in male mice, and was proposed to be a biomarker of effect, appearing after other observed toxic effects on germ cells [95]. Both SDH and LDH activities were decreased in male mice dosed with formaldehyde (21, 42, and 84 mg/m^3) by static inhalation for 5 days, compared with controls [62]. Additionally, one human study showed that a single nucleotide polymorphism (SNP) in the gene encoding aldehyde dehydrogenase 2 (ALDH2), 504 glu/glu, was associated with enhanced formaldehyde metabolism as evident by increased levels of formic acid in urine [103].

5.3.2 Heat shock proteins—Increased synthesis of heat shock protein 70 (Hsp70), a molecular chaperone involved in protein folding and repair that is rapidly induced in response to damage resulting from physical or chemical stress [104], was detected immunohistochemically in spermatogenic cells from the seminiferous tubules of male Wistar rats after subchronic periods of exposure to formaldehyde (13 weeks) at cytotoxic doses [51]. While spermatogenic cells of the testicular tissue normally synthesize Hsp70 during prophase of meiosis I [105] that can be detected by immunoreaction [106], its increased synthesis suggests that formaldehyde induces chemical stress and subsequent protein damage in these cells. Heat shock proteins regulate apoptosis [107], a possible fate

of stressed cells and another potential mechanism underlying formaldehyde-induced adult male reproductive toxicity.

5.3.3 Apoptosis—Apoptosis rate (measured by TUNEL assay) and expression of the *Fas* gene (measured by histochemistry) were increased and were significantly correlated ($r = 0.8832$, $p < 0.05$), in the testicular tissue of rats exposed (by daily i.p. injection continuously for 14 days) to 1.0 and 10.0 mg/kg/day formaldehyde. Morphological abnormalities of the testes and an increased number of abnormal sperm were also observed in the exposed rats [55]. The mechanisms determining the stress response of testicular cells and tissues and the balance between repair and apoptosis/necrosis requires further clarification.

5.3.4 Epigenetic alterations—Formaldehyde-induced male reproductive toxicity could also be mediated through aberrant DNA methylation. Abnormal DNA methylation of a key spermatogenesis gene has been associated with male gametogenic defects [108] and chemical exposures, e.g. acrylamide, may disrupt genomic imprinting in mitotic spermatogonia and primary spermatocytes [109]. As a reactive methyl donor known to enter the one-carbon metabolism (methyl) pool and interact with enzymes in the associated pathway [110–111], formaldehyde could potentially alter DNA methylation. Additionally, oxidative stress-related damage to sperm DNA impedes the process of methylation [112], representing an indirect mechanism by which formaldehyde could influence DNA methylation in sperm DNA though more epigenetic studies are warranted.

5.3.5 Sex hormones—A few studies showed that serum testosterone levels were decreased in male mice [62] and rats [51], subjected to formaldehyde exposure by inhalation and in male rats exposed by injection [54–55], representing another possible mechanism through which formaldehyde could disrupt male reproductive function.

5.3.6 Hypothalamus–pituitary–adrenal gland axis—It is possible that formaldehyde may exert adverse effects on the reproductive system without reaching it, through a stress-induced mechanism. The multiple adverse health effects associated with chronic formaldehyde exposure in humans [2] potentially indicates systemic stress and such environmentally mediated systemic stress can negatively impact the reproductive system, as previously reviewed [113]. Experimental data in animals and humans suggests that chronic or severe stress leads to anovulation and amenorrhea in women [114] and to decrease in sperm count, motility, and morphology in men [115]. Stress-induced reproductive toxicity could be mediated by effects on the endocrine or other regulatory systems.

The hypothalamus–pituitary–adrenal (HPA) gland axis responds to stress such as chemical exposures by increasing the secretion of corticotropin releasing hormone (CRH) in the hypothalamus, adrenocorticotropin hormone (ACTH) in the anterior pituitary gland, and adrenal corticosteroids in the adrenal gland. Altered hypothalamic-pituitary-adrenal (HPA) axis functioning was shown to occur after repeated low-level formol exposure in a rat model of multiple chemical sensitivity [116]. Similarly, prolonged exposure to low levels of formaldehyde in female C3H/He mice a led to a dose-dependent increase in the number of corticotropin releasing hormone (CRH)-immunoreactive (ir) neurons in the hypothalamus and in the adrenocorticotropin hormone (ACTH)-ir cells and ACTH mRNA in the pituitary [117]. Mice with allergies responded to lower levels of formaldehyde.

In the formalin test, injection of formalin into the rat paw induces a characteristic bi-phasal finching response to the induced persistent and inflammatory pain. Sex differences in response to the formalin test were noted and were initially attributed to estradiol effects [118]. Later, estrogen replacement in ovariectomized female rats was found to exert an antihyperalgesic effect on the inflammatory pain response to formalin injection, at least in

part, by restoring the maximum serum corticotrophin response to the stress [119]. In male rats, both male gonadal hormones and estrogen were shown to play a role in formalin-pain responses [120]. These experiments demonstrate that the corcitotrophin response to formalin injection, is modulated by reproductive hormones, and provide additional evidence that formaldehyde perturbs the closely related endocrine and reproductive systems.

5.4 Comparison with Other Reproductive Toxicants

The potential mechanisms by which formaldehyde causes reproductive and developmental toxicity may share similarities with those proposed for other suspected/known reproductive toxicants recognized by California's Proposition 65 (ethanol, benzene, primary and environmental tobacco smoke) [121] or by the National Toxicology Program's (NTP) Center for the Evaluation of Risks to Human Reproduction (Butyl Benzyl Phthalate (BBP) [122], Di-n-Butyl Phthalate (DBP) [123], Di-n-Hexyl Phthalate (DnHP) [124], and Di-Isodecyl Phthalate (DIDP) [125]).

The ingestion of **ethanol**, of which the aldehyde, acetaldehyde, is a major metabolite produced in the liver, can result in abnormal fetal development, including teratogenic defects, and fetal alcohol syndrome, in humans and experimental animal models. These effects are mediated in part by the induction of oxidative stress [126]. There is little data on reproductive or developmental toxicity associated with other aldehydes.

Benzene has been shown to cause human and animal reproductive toxicity [127–128]. Chromosome abnormalities, specifically aneuploidies (numerical chromosomal changes), were detected in the sperm of male workers occupationally exposed to benzene levels above [129–132] and below [133] 1 ppm, the current U.S. Permissible Exposure Limit for benzene (8 hr time-weighted average) set by OSHA. As with formaldehyde, benzene exerts some of its adverse reproductive effects through the generation of ROS and oxidative stress. In one study, benzene metabolites were shown to induce DNA double strand breaks as well as increased homologous recombination via ROS in Chinese hamster ovary cells [134]. Using a CD-1 mouse model, Badham and colleagues showed increased oxidative stress in fetal tissue from embryos exposed to benzene *in utero* by measuring the ratios of reduced to oxidized glutathione, and increased levels of ROS in male fetuses using flow cytometry and a ROS-sensitive fluorescent probe [135].

Both benzene and formaldehyde are constituents of **cigarette smoke**, exposure to which is associated with increased risk of infertility [136] and delayed conception [137] in women, lowered semen quality in men [138] and a number of adverse obstetrical outcomes including SAB [139], preterm birth [140] and low birth weight [141]. At a recent Environmental Mutagen Society annual conference in Fort Worth, TX (2010), scientists presented new evidence supporting smoking as a male and female germ cell mutagen in humans [142–143].

The oxidative metabolism of **trichloroethylene** has been associated with epididymal damage and aberrant sperm production in mice following systemic toxicity [144]. **Phthalate** esters induce male fetal endocrine toxicity and postnatal reproductive malformations in several animal models, by disrupting androgen production and testosterone synthesis during the sexual differentiation period of development *in utero* [145–146], with different potencies reported among different phthalate compounds [147]. Data in humans is limited but possible associations between phthalate exposure and disturbance of normal sperm function, such as fewer motile sperm, low sperm concentration and motility, sperm malformations, and increased DNA damage have been reported, as reviewed [146]. Lower plasma testosterone levels were also observed in workers occupationally exposed to phthalates [148].

While several potential mechanisms of formaldehyde reproductive toxicity exist and overlap with mechanisms proposed for other known or suspected reproductive toxicants, the lack of an accepted mechanism should not detract from the strength of any empirical evidence supporting a link between formaldehyde exposure and reproductive and developmental toxicity.

6. Current Research Gaps and Future Directions

Gaps in the data on formaldehyde exposure and reproductive/developmental toxicity require further research. As described above, only 18 studies have evaluated these effects in humans, and the majority has focused on developmental outcomes in females (Table 1). These predominantly retrospective epidemiological studies were potentially limited by recall and selection biases and inadequate exposure assessment. None of the studies offer a plausible biological mechanism by which reproductive and developmental toxicity occurs. Additionally, molecular epidemiological studies investigating male reproductive (sperm study) toxicity are lacking.

Therefore, new molecular epidemiological studies are required that are designed to investigate developmental and reproductive toxicity in both males and females. These studies should be designed to minimize recall and selection bias, incorporate exposure assessment including biomarkers of internal dose and exclusion of confounding exposures, and include the collection of biospecimens (serum, sperm, etc.) to allow for the simultaneous investigation of multiple mechanisms. For example, formaldehyde can bind covalently to protein to form crosslinks, or with human serum albumin [149] or the N-terminal valine of hemoglobin [150] to form molecular adducts, potential biomarkers of formaldehyde exposure. Sperm aneuploidy could be analyzed by fluorescent *in situ* hybridization and assessed as a biomarker of male reproductive toxicity. Well-designed molecular epidemiological studies could also be leveraged to identify biomarkers of susceptibility such as SNPs and DNA repeat sequences associated with developmental and reproductive outcomes. The identification of such alleles could also inform mechanism.

There is a need for both animal and human studies examining the effect of formaldehyde exposure on reproductive and developmental toxicity in the current generation as well as in subsequent generations (transgenerational effects). Most of the current mechanistic data comes from animal studies and it is not clear how relevant these findings are to human outcomes. Further, the animal studies vary in study design, route and duration of exposure, number of animals studied and length of follow-up time, all of which could influence outcome.

Another key issue requiring further study is how formaldehyde reaches the reproductive or closely related endocrine systems. As formaldehyde is reactive and is usually rapidly metabolized by reduction, oxidation and reduced glutathione-dependent pathways, determination of how it reaches distal sites is important. A single study showed that formaldehyde and its metabolites, in ^{14}C-labeled form, could cross the placenta and become concentrated in fetal brain and liver of mouse from where they are eliminated more slowly than from maternal tissue [74]. This study requires replication in the mouse as well as in other species.

Alternatively, formaldehyde could adversely impact the reproductive system without reaching it through stress-induced effects on the HPA gland axis, endocrine or other regulatory systems. This hypothesis requires further investigation. As discussed above, effects on the HPA gland axis which were demonstrated in mice following exposure to formaldehyde should be examined in exposed people. Similarly, systemic effects of formaldehyde on the endocrine system should be examined.

Finally, if the association between formaldehyde exposure and reproductive/developmental toxicity is strengthened, regulation in the workplace and the environment should be adjusted accordingly in the interest of public health.

7. Conclusion

In this review, we comprehensively summarize human and animal studies of reproductive and developmental toxicity associated with formaldehyde, from the literature. From our meta-analysis, which includes data from recent epidemiological studies, our calculated relative risk remains similar to that of Collins *et al.* [10]; however, the more precise confidence intervals presented here indicate a consistently increased risk for both SAB and all combined pregnancy outcomes. Empirical evidence from animal studies also shows a strong association between both reproductive and developmental toxicity and formaldehyde exposure, at multiple doses and routes of exposures, in various species. While gaps in our understanding of the reproductive toxicity of formaldehyde need to be addressed by further epidemiological studies, animal studies and mechanistic studies, we conclude that human reproductive and developmental toxicities resulting from formaldehyde exposure could potentially be a threat to human health, particularly given its widespread exposure in the general population including its most susceptible members, women of child-bearing age and young children.

Acknowledgments

We truly appreciate the comments and suggestions provided by Prof. **Martyn Smith** of UC Berkeley and Prof. **David Eastmond** of UC Riverside. We would also like to thank our collaborators in China, Dr. **Xiaojiang Tang** and Prof. **Xu Yang**, for identifying and collecting most of the Chinese studies. We are very grateful to our student assistant, Ms. **Bin Tu**, for translating some of the Chinese papers. **Anh Duong**, a previous Berkeley undergrad student, was supported in part by the Council for Education and Research on Toxics (CERT). This study was supported in part by the National Institute of Environmental Health Sciences grants R01ES017452 (L. Zhang). Additional support was provided by the Northern California Center for Occupational and Environmental Health.

Abbreviations

ACTH	Adrenocorticotropin hormone
CI	Confidence Interval
CRH	Corticotropin releasing hormone
DPC	DNA Protein Crosslinking
EPA	Environmental Protection Agency
FDR	Fecundability Density Ratio
GSH	Glutathione
GSH-PX	Glutathione Peroxidase
HPA	Hypothalamus–pituitary–adrenal
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
i.g.	intragastric injection
i.m.	intramuscular injection
i.p.	intraperitoneal injection
i.v.	intravenous injection

ir	immunoreactive
LDH	Lactate Dehydrogenase
MDA	Malondialehyde
MN	Micronuclei
MOA	Mechanism of Action
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
ppm	parts per million
RoC	Report on Cancer
ROS	Reactive Oxygen Species
RR	Relative Risk
SAB	Spontaneous Abortion
SCE	Sister Chromatid Exchange
SDH	Succinate Dehydrogenase
SE	Standard Error
SOD	Superoxide dismutase

References

1. IARC. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol. IARC Monographs on the Evaluation of Carcinogenic Risks to Human. 2006; 88
2. Tang X, Bai Y, Duong A, Smith MT, Li L, Zhang L. Formaldehyde in China: production, consumption, exposure levels, and health effects. Environ Int. 2009; 35:1210–1224. [PubMed: 19589601]
3. Baan R, Grosse Y, Straif K, Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V. A review of human carcinogens--Part F: chemical agents and related occupations. Lancet Oncol. 2009; 10:1143–1144. [PubMed: 19998521]
4. NTP. Report on Carcinogens. 12. 2011.
5. EPA. Toxicological Review of Formaldehyde Inhalation Toxicity. 2010. EPA/635/R-10/002C
6. USEPA. Guidelines for Reproductive Toxicity Risk Assessment. 1996.
7. Staples, RE. Teratogenicity of Formaldehyde. In: Gibson, J., editor. Formaldehyde Toxicity. Hemisphere Publishing Corporation; Washington: 1983.
8. Feinman, SE. Formaldehyde genotoxicity and teratogenicity. In: Feinman, SE., editor. Formaldehyde Sensitivity and Toxicity. CRC Press, Inc; Boca Raton, FL: 1988. p. 167-178.
9. WHO. Formaldehyde, Environmental Health Criteria, International Program on Chemical Safety. World Health Organization; 1989. p. 219
10. Collins JJ, Ness R, Tyl RW, Krivanek N, Esmen NA, Hall TA. A Review of Adverse Pregnancy Outcomes and Formaldehyde Exposure in Human and Animal Studies. Regulatory Toxicology and Pharmacology. 2001; 34:17–34. [PubMed: 11502153]
11. Ward JB Jr, Hokanson JA, Smith ER. Sperm count, morphology and fluorescent body frequency in autopsy service workers exposed to formaldehyde. Mutation Research. 1984; 130:417–424. [PubMed: 6513969]

12. Lindbohm ML, Hemminki K, Bonhomme MG, Anttila A, Rantala K, Heikkila P, Rosenberg MJ. Effects of paternal occupational exposure on spontaneous abortions. American Journal of Public Health. 1991; 81:1029–1033. [PubMed: 1853994]
13. Shumilina AV. Menstrual and child bearing functions of female workers occupationally exposed to the effects of formaldehyde (Russian). Meditsina Truda I Promyshlennaya Ekologiya. 1975; 19:18–21.
14. Olsen JH, Dossing M. Formaldehyde induced symptoms in day care centers. Am Ind Hyg Assoc J. 1982; 43:366–370. [PubMed: 7090990]
15. Taskinen HK, Kyyrönen P, Sallmén M, Virtanen SV, Liukkonen TA, Huida O, Lindbohm ML, Anttila A. Reduced fertility among female wood workers exposed to formaldehyde. American Journal of Industrial Medicine. 1999; 36:206–212. [PubMed: 10361608]
16. Vorvick, L.; Storck, S. Miscarriage, Medline Plus Medical Encyclopedia. US National Library of Medicine & National Institutes of Health; 2009.
17. Hemminki K, Mutanen P, Saloniemi I. Spontaneous abortions in hospital staff engaged in sterilising instruments with chemical agents. British Medical Journal. 1982; 285:1461–1463. [PubMed: 6814601]
18. Hemminki K, Kyyronen P, Lindbohm ML. Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs, and other potential hazards in hospitals, based on registered information of outcome. Journal of Epidemiology and Community Health. 1985; 39:141–147. [PubMed: 3925060]
19. Axelsson G, Lutz C, Rylander R. Exposure to solvents and outcome of pregnancy in university laboratory employees. British Journal of Industrial Medicine. 1984; 41:305–312. [PubMed: 6743577]
20. Seitz, T.; Baron, S. Health-hazard-evaluation report HETA 87–349–2022. Rockcastle Manufacturing; Mount Vernon, Kentucky: 1990. p. 36Medium: X; Size
21. Stucker I, Caillard JF, Collin R, Gout M, Poyen D, Hemon D. Risk of spontaneous abortion among nurses handling antineoplastic drugs, Scandinavian Journal of Work. Environment and Health. 1990; 16:102–107.
22. John EM, Savitz DA, Shy CM. Spontaneous abortions among cosmetologists. Epidemiology. 1994; 5:147–155. [PubMed: 8172989]
23. Saurel-Cubizolles MJ, Hays M, Estryn-Behar M. Work in operating rooms and pregnancy outcome among nurses. Int Arch Occup Environ Health. 1994; 66:235–241. [PubMed: 7843833]
24. Taskinen H, Kyyronen P, Hemminki K, Hoikkala M, Lajunen K, Lindbohm ML. Laboratory work and pregnancy outcome. Journal of Occupational Medicine. 1994; 36:311–319. [PubMed: 8195901]
25. CDCP. Birth Defects. Centers for Disease Control and Prevention; Atlanta, GA: 2009.
26. Ericson A, Kallen B, Zetterstrom R. Delivery outcome of women working in laboratories during pregnancy. Archives of Environmental Health. 1984; 39:5–10. [PubMed: 6712285]
27. Dulskiene V, Grazuleviciene R. [Environmental risk factors and outdoor formaldehyde and risk of congenital heart malformations]. Medicina (Kaunas). 2005; 41:787–795. [PubMed: 16227712]
28. Zhu JL, Knudsen LE, Andersen AM, Hjollund NH, Olsen J. Laboratory work and pregnancy outcomes: a study within the National Birth Cohort in Denmark. Occup Environ Med. 2006; 63:53–58. [PubMed: 16361406]
29. Grazuleviciene R, Dulskiene V, Vencloviene J. Formaldehyde exposure and low birth weight incidence. J Occup Health. 1998; 40:61–67.
30. Maroziene L, Grazuleviciene R. Maternal exposure to low-level air pollution and pregnancy outcomes: a population-based study. Environ Health. 2002; 1:6. [PubMed: 12495448]
31. OSHA, Occupational Safety and Health Administration. Occupational Exposure to Formaldehyde Fact Sheet. Department of Labor; 1995.
32. Greenland, S. Causation and causal inference. Lippincott Raven; Philadelphia: 1998.
33. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7:177–188. [PubMed: 3802833]
34. Greenland, S. Meta-analysis. Lippincott Raven; Philadelphia: 1988.

35. Petitti, D. Statistical Methods in Meta-analysis. Meta-Analysis, Decision Analysis, and Cost-Effectiveness Analysis. Oxford University Press; New York: 1994.
36. Poole C, Greenland S. Random-effects meta-analyses are not always conservative. Am J Epidemiol. 1999; 150:469–475. [PubMed: 10472946]
37. Shore RE, Gardner MJ, Pannett B. Ethylene oxide: an assessment of the epidemiological evidence on carcinogenicity. Br J Ind Med. 1993; 50:971–997. [PubMed: 8280635]
38. Bhatia R, Lopipero P, Smith AH. Diesel exhaust exposure and lung cancer. Epidemiology. 1998; 9:84–91. [PubMed: 9430274]
39. Setia MS, Steinmaus C, Ho CS, Rutherford GW. The role of BCG in prevention of leprosy: a meta-analysis. Lancet Infect Dis. 2006; 6:162–170. [PubMed: 16500597]
40. Steinmaus C, Smith AH, Jones RM, Smith MT. Meta-analysis of benzene exposure and non-Hodgkin lymphoma: biases could mask an important association. Occup Environ Med. 2008; 65:371–378. [PubMed: 18417556]
41. Steinmaus CM, Nunez S, Smith AH. Diet and bladder cancer: a meta-analysis of six dietary variables. Am J Epidemiol. 2000; 151:693–702. [PubMed: 10752797]
42. Zhuo H, Smith AH, Steinmaus C. Selenium and lung cancer: a quantitative analysis of heterogeneity in the current epidemiological literature. Cancer Epidemiol Biomarkers Prev. 2004; 13:771–778. [PubMed: 15159309]
43. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994; 50:1088–1101. [PubMed: 7786990]
44. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997; 315:629–634. [PubMed: 9310563]
45. Breslow, N.; Day, N. Statistical Methods in Cancer Research Vol II: The Design and Analysis of Cohort Studies. International Agency for Research on Cancer; Lyon: 1987.
46. Trocho C, Pardo R, Rafecas I, Virgili J, Remesar X, Fernandez-Lopez JA, Alemany M. Formaldehyde derived from dietary aspartame binds to tissue components in vivo. Life Sci. 1998; 63:337–349. [PubMed: 9714421]
47. FDA. Common Ingredients in US Licensed Vaccines, Vaccines, Blood, & Biologics. US Food and Drug Admininstration; 2009.
48. Evison BJ, Chiu F, Pezzoni G, Phillips DR, Cutts SM. Formaldehyde-activated Pixantrone is a monofunctional DNA alkylator that binds selectively to CpG and CpA doublets. Mol Pharmacol. 2008; 74:184–194. [PubMed: 18413664]
49. Levovich I, Nudelman A, Berkovitch G, Swift LP, Cutts SM, Phillips DR, Rephaeli A. Formaldehyde-releasing prodrugs specifically affect cancer cells by depletion of intracellular glutathione and augmentation of reactive oxygen species. Cancer Chemother Pharmacol. 2008; 62:471–482. [PubMed: 18030472]
50. Golalipour MJ, Azarhoush R, Ghafari S, Gharravi AM, Fazeli SA, Davarian A. Formaldehyde exposure induces histopathological and morphometric changes in the rat testis. Folia Morphol (Warsz). 2007; 66:167–171. [PubMed: 17985313]
51. Ozen OA, Akpolat N, Songur A, Kus I, Zararsiz I, Ozacmak VH, Sarsilmaz M. Effect of formaldehyde inhalation on Hsp70 in seminiferous tubules of rat testes: an immunohistochemical study. Toxicol Ind Health. 2005; 21:249–254. [PubMed: 16463957]
52. Zhou DX, Qiu SD, Zhang J, Tian H, Wang HX. The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. Asian J Androl. 2006b; 8:584–588. [PubMed: 16751993]
53. Ozen OA, Yaman M, Sarsilmaz M, Songur A, Kus I. Testicular zinc, copper and iron concentrations in male rats exposed to subacute and subchronic formaldehyde gas inhalation. J Trace Elem Med Biol. 2002; 16:119–122. [PubMed: 12195726]
54. Chowdhury AR, Gautam AK, Patel KG, Trivedi HS. Steroidogenic inhibition in testicular tissue of formaldehyde exposed rats. Indian J Physiol Pharmacol. 1992; 36:162–168. [PubMed: 1473844]
55. Zhou DX, Qiu SD, Zhang J, Wang ZY. Reproductive toxicity of formaldehyde to adult male rats and the functional mechanism concerned. Sichuan Da Xue Xue Bao Yi Xue Ban. 2006a; 37:566–569. [PubMed: 16909603]

56. Majumder PK, Kumar VL. Inhibitory effects of formaldehyde on the reproductive system of male rats. Indian J Physiol Pharmacol. 1995; 39:80–82. [PubMed: 7705878]
57. Odeigah PG. Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutat Res. 1997; 389:141–148. [PubMed: 9093377]
58. Cassidy SL, Dix KM, Jenkins T. Evaluation of a testicular sperm head counting technique using rats exposed to dimethoxyethyl phthalate (DMEP), glycerol alpha-monochlorohydrin (GMCH), epichlorohydrin (ECH), formaldehyde (FA), or methyl methanesulphonate (MMS). Arch Toxicol. 1983; 53:71–78. [PubMed: 6882214]
59. Zeng, Ce; Peng, X.; Qiao, Y. [Reproductive toxcity of formaldehyde to male mice]. The Chinese Journal of Human Sexuality. 2003; 12:1–4.
60. Wang X, Duan L, Yan Y, Yang X. Study on formaldehyde's effect on germ cells in male mice. Journal of Central China Normal University. 2005a; 39:373–378.
61. Xing, S-y; Ye, L.; Wang, N-n. [Toxic effect of formaldehyde on reproduction and heredity in male mice]. Journal of Jilin University. 2007; 33:716–718.
62. Wang N, Yang Y, Zhang L, Ye L. Reproductive toxicity of formaldehyde on male mice. Community Medical Journal. 2006c; 4:13–15.
63. Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR, Moss OR. Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. Toxicology. 1986; 41:253–266. [PubMed: 3775776]
64. Stott WT, Watanabe PG. Kinetic interaction of chemical mutagens with mouse sperm in vivo as it relates to animal mutagenic effects. Toxicol Appl Pharmacol. 1980; 55:411–416. [PubMed: 7434354]
65. Xie Y, Yi Y, Tang M. [Toxicity of formaldehyde on germ cells of male mice]. J Environ Health. 2003; 20:84–85.
66. Yi, J-h; Zhang, J-h; Gao, Y-x. [Experiment on the effects of formaldehyde on the sperm toxicity of mice]. Ind Hlth and Occup Dis. 2000; 26:263–264.
67. Huang Y, Yan W, Tang L. [Study of Formaldehyde's Effects on Mice Sperm]. China Journal of Medicine. 2002; 12:77–78.
68. Peng G, Yang X, Zhao W. [Study of DPC Induced by Liquid Formaldehyde in the Liver and Testicles of Mice]. J Med Res. 2006; 35:9–12.
69. Wang X, Duan L, Li C. Study on formaldehyde-induced DNA damage in mice testicular cells. J Environ Health. 2006a; 23:128–131.
70. Wang W, Tang M, Yi Y. [The effects of formaldehyde on estrous cycle and ovary of female mice]. Practical Preventitive Medicine. 2002; 9:641–643.
71. Meng D, Hui Z, Yang J, Yuan J, Ling Y, He C. Reduced egg production in hens associated with avian influenza vaccines and formalin levels. Avian Dis. 2009; 53:16–20. [PubMed: 19431998]
72. Anwar MI, Khan MZ, Muhammad G, Bachaya A, Babar AM. Effects of dietary formalin on the health and testicular pathology of male Japanese quails (Coturnix coturnix Japonica). Vet Hum Toxicol. 2001; 43:330–333. [PubMed: 11757989]
73. Khan MZ, Ali Z, Muhammad G, Khan A, Mahmood F. Pathological effects of formalin (37% formaldehyde) mixed in feed or administered into the crops of White Leghorn cockerels. J Vet Med A Physiol Pathol Clin Med. 2003; 50:354–358. [PubMed: 14633228]
74. Thrasher JD, Kilburn KH. Embryo toxicity and teratogenicity of formaldehyde. Arch Environ Health. 2001; 56:300–311. [PubMed: 11572272]
75. Tang, Q-n; Hao, J-g; Xia, Q.; Ru, J-g; Zhang, C.; Huang, Y-f. [Toxic Effect of Formaldehyde on Reproduction and Development of Rats]. Endemic Diseases Bulletin. 2006; 21:15–18.
76. Martin WJ. A teratology study of inhaled formaldehyde in the rat. Reprod Toxicol. 1990; 4:237–239. [PubMed: 2136043]
77. Saillenfait AM, Bonnet P, de Ceaurriz J. The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Food Chem Toxicol. 1989; 27:545–548. [PubMed: 2792978]

78. Katakura Y, Kishi R, Okui T, Ikeda T, Miyake H. Distribution of radioactivity from 14C-formaldehyde in pregnant mice and their fetuses. Br J Ind Med. 1993; 50:176–182. [PubMed: 8435351]

79. Wang B, Liu DD. Detection of formaldehyde induced developmental toxicity assessed with single cell gel electrophoresis. Fen Zi Xi Bao Sheng Wu Xue Bao. 2006b; 39:462–466. [PubMed: 17117557]

80. Fontignie-Houbrechts N. Genetic effects of formaldehyde in the mouse. Mutat Res. 1981; 88:109–114. [PubMed: 7207492]

81. Fontignie-Houbrechts N, Moutschen-Dahmen M, Degraeve N, Gloor H. Genetic effects in the mouse of formaldehyde in combination with adenosine and hydrogen peroxide. Mutat Res. 1982; 104:371–376. [PubMed: 7110172]

82. Marks TA, Worthy WC, Staples RE. Influence of formaldehyde and Sonacide (potentiated acid glutaraldehyde) on embryo and fetal development in mice. Teratology. 1980; 22:51–58. [PubMed: 6777891]

83. Al-Saraj AA. Teratogenic effect of formaldehyde in rabbits. Iraqi Journal of Veterinary Sciences. 2009; 23:1–4.

84. Magras IN. Formaldehyde vapour effects in chicken embryo. Anat Histol Embryol. 1996; 25:197–200. [PubMed: 8976614]

85. Hayretdag S, Kolankaya D. Investigation of the Effects of Pre-Incubation Formaldehyde Fumigation on the Tracheal Epithelium of Chicken Embryos and Chicks. Turk J Vet Anim Sci. 2008; 32:263–267.

86. Overman DO. Absence of embryotoxic effects of formaldehyde after percutaneous exposure in hamsters. Toxicol Lett. 1985; 24:107–110. [PubMed: 3975924]

87. Songur A, Kus I, Sahin S, Sogut S, Ozen O, Yaman M, Sarsilmaz M. The changes of zinc, copper, and iron levels in the lung tissue after formaldehyde inhalation during the early postnatal period of rats. Eur J Gen Med. 2005; 2:62–68.

88. Songur A, Akpolat N, Kus I, Ozen OA, Zararsiz I, Sarsilmaz M. The effects of the inhaled formaldehyde during the early postnatal period in the hippocampus of rats: A morphological and immunohistochemical study. Neuroscience Research Communications. 2003; 33:168–178.

89. Guy ER, Abbott FV. The behavioral response to formalin in preweanling rats. Pain. 1992; 51:81–90. [PubMed: 1454409]

90. Harris C, Dixon M, Hansen JM. Glutathione depletion modulates methanol, formaldehyde and formate toxicity in cultured rat conceptuses. Cell Biol Toxicol. 2004; 20:133–145. [PubMed: 15250539]

91. Hansen JM, Contreras KM, Harris C. Methanol, formaldehyde, and sodium formate exposure in rat and mouse conceptuses: a potential role of the visceral yolk sac in embryotoxicity. Birth Defects Res A Clin Mol Teratol. 2005; 73:72–82. [PubMed: 15578646]

92. Laschinski G, Vogel R, Spielmann H. Cytotoxicity test using blastocyst-derived euploid embryonal stem cells: a new approach to in vitro teratogenesis screening. Reprod Toxicol. 1991; 5:57–64. [PubMed: 1807538]

93. Osinowo OA, Bale JO, Oyedipe EO, Eduvie LO. Motility and eosin uptake of formaldehyde-treated ram spermatozoa. J Reprod Fertil. 1982; 65:389–394. [PubMed: 6178824]

94. Zhang L, Freeman LE, Nakamura J, Hecht SS, Vandenberg JJ, Smith MT, Sonawane BR. Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. Environ Mol Mutagen. 2010; 51:181–191. [PubMed: 19790261]

95. Tang M, Xie Y, Yi Y, Wang W. [Effects of formaldehyde on germ cells of male mice]. Wei Sheng Yan Jiu. 2003; 32:544–548. [PubMed: 14963899]

96. Natarajan AT, Darroudi F, Bussman CJ, van Kesteren-van Leeuwen AC. Evaluation of the mutagenicity of formaldehyde in mammalian cytogenetic assays in vivo and vitro. Mutat Res. 1983; 122:355–360. [PubMed: 6656818]

97. de Graaf B, Clore A, McCullough AK. Cellular pathways for DNA repair and damage tolerance of formaldehyde-induced DNA-protein crosslinks. DNA Repair (Amst). 2009; 8:1207–1214. [PubMed: 19625222]

98. Saito Y, Nishio K, Yoshida Y, Niki E. Cytotoxic effect of formaldehyde with free radicals via increment of cellular reactive oxygen species. Toxicology. 2005; 210:235–245. [PubMed: 15840437]

99. Im H, Oh E, Mun J, Khim JY, Lee E, Kang HS, Kim E, Kim H, Won NH, Kim YH, Jung WW, Sul D. Evaluation of toxicological monitoring markers using proteomic analysis in rats exposed to formaldehyde. J Proteome Res. 2006; 5:1354–1366. [PubMed: 16739987]

100. Lu Z, Li CM, Qiao Y, Yan Y, Yang X. Effect of inhaled formaldehyde on learning and memory of mice. Indoor Air. 2008; 18:77–83. [PubMed: 18333987]

101. Ozen OA, Kus MA, Kus I, Alkoc OA, Songur A. Protective effects of melatonin against formaldehyde-induced oxidative damage and apoptosis in rat testes: an immunohistochemical and biochemical study. Syst Biol Reprod Med. 2008; 54:169–176. [PubMed: 18942024]

102. Lino dos Santos Franco A, Damazo AS, Beraldo de Souza HR, Domingos HV, Oliveira-Filho RM, Oliani SM, Costa SK, Tavares de Lima W. Pulmonary neutrophil recruitment and bronchial reactivity in formaldehyde-exposed rats are modulated by mast cells and differentially by neuropeptides and nitric oxide. Toxicol Appl Pharmacol. 2006; 214:35–42. [PubMed: 16427670]

103. Cheng XM, Zhao J, Feng B, Wen PE, Shao H. [Relationship between susceptibility of formaldehyde metabolism and genetic polymorphisms of ALDH2 and cytochrome P4502E1]. Zhonghua Yu Fang Yi Xue Za Zhi. 2008; 42:582–587. [PubMed: 19115622]

104. Craig EA. The heat shock response. CRC Crit Rev Biochem. 1985; 18:239–280. [PubMed: 2412760]

105. Dix DJ. Hsp70 expression and function during gametogenesis. Cell Stress Chaperones. 1997; 2:73–77. [PubMed: 9250397]

106. Dix DJ, Allen JW, Collins BW, Poorman-Allen P, Mori C, Blizard DR, Brown PR, Goulding EH, Strong BD, Eddy EM. HSP70–2 is required for desynapsis of synaptonemal complexes during meiotic prophase in juvenile and adult mouse spermatocytes. Development. 1997; 124:4595–4603. [PubMed: 9409676]

107. Takayama S, Reed JC, Homma S. Heat-shock proteins as regulators of apoptosis. Oncogene. 2003; 22:9041–9047. [PubMed: 14663482]

108. Navarro-Costa P, Nogueira P, Carvalho M, Leal F, Cordeiro I, Calhaz-Jorge C, Goncalves J, Plancha CE. Incorrect DNA methylation of the DAZL promoter CpG island associates with defective human sperm. Hum Reprod. 2010; 25:2647–2654. [PubMed: 20685756]

109. Wang Z, Lu S, Liu C, Yao N, Zhang C, Ma X. Acrylamide disturbs genomic imprinting during spermatogenesis. Front Biosci (Elite Ed). 2010; 2:165–170. [PubMed: 20036867]

110. ATSDR. Agency for Toxic Substances and Disease Registry: Toxicological Profile for Formaldehyde. Atlanta, GA: 1999.

111. Kalasz H. Biological role of formaldehyde, and cycles related to methylation, demethylation, and formaldehyde production. Mini Rev Med Chem. 2003; 3:175–192. [PubMed: 12570834]

112. Tunc O, Tremellen K. Oxidative DNA damage impairs global sperm DNA methylation in infertile men. J Assist Reprod Genet. 2009; 26:537–544. [PubMed: 19876730]

113. Negro-Vilar A. Stress and other environmental factors affecting fertility in men and women: overview. Environ Health Perspect. 1993; 101 Suppl 2:59–64. [PubMed: 8243408]

114. Barnea ER, Tal J. Stress-related reproductive failure. J In Vitro Fert Embryo Transf. 1991; 8:15–23.

115. McGrady AV. Effects of psychological stress on male reproduction: a review. Arch Androl. 1984; 13:1–7. [PubMed: 6152527]

116. Sorg BA, Bailie TM, Tschirgi ML, Li N, Wu WR. Exposure to repeated low-level formaldehyde in rats increases basal corticosterone levels and enhances the corticosterone response to subsequent formaldehyde. Brain Res. 2001; 898:314–320. [PubMed: 11306018]

117. Sari DK, Kuwahara S, Tsukamoto Y, Hori H, Kunugita N, Arashidani K, Fujimaki H, Sasaki F. Effect of prolonged exposure to low concentrations of formaldehyde on the corticotropin releasing hormone neurons in the hypothalamus and adrenocorticotropic hormone cells in the pituitary gland in female mice. Brain Res. 2004; 1013:107–116. [PubMed: 15196973]

118. Aloisi AM, Albonetti ME, Carli G. Sex differences in the behavioural response to persistent pain in rats. Neurosci Lett. 1994; 179:79–82. [PubMed: 7845629]

119. Mannino CA, South SM, Quinones-Jenab V, Inturrisi CE. Estradiol replacement in ovariectomized rats is antihyperalgesic in the formalin test. J Pain. 2007; 8:334–342. [PubMed: 17140856]
120. Aloisi AM, Ceccarelli I. Role of gonadal hormones in formalin-induced pain responses of male rats: modulation by estradiol and naloxone administration. Neuroscience. 2000; 95:559–566. [PubMed: 10658636]
121. OEHHA. O.o.E.H.H. Assessment. Chemicals Listed Effective May 20, 2011 As Known To The State Of California To Cause Cancer: Proposition 65. California: 2011.
122. NTP-CERHR. D.o.H.a.H. Services. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Butyl Benzyl Phthalate (BBP). Research Triangle Park; NC: 2003.
123. NTP-CERHR. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-n-Butyl Phthalate (DBP). Research Triangle Park; NC: 2003.
124. NTP-CERHR. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-n-Hexyl Phthalate (DnHP). Research Triangle Park; NC: 2003.
125. NTP-CERHR. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-Isodecyl Phthalate (DIDP). Research Triangle Park; NC: 2003.
126. Brocardo PS, Gil-Mohapel J, Christie BR. The Role of Oxidative Stress in Fetal Alcohol Spectrum Disorders. Brain Res Rev. 2011; 9:9.10.1016/j.brainresrev.2011.1002.1001
127. Wilbur S, Wohlers D, Paikoff S, Keith LS, Faroon O. ATSDR evaluation of health effects of benzene and relevance to public health. Toxicol Ind Health. 2008; 24:263–398. [PubMed: 19022880]
128. Lupo PJ, Symanski E, Waller DK, Chan W, Langlois PH, Canfield MA, Mitchell LE. Maternal Exposure to Ambient Levels of Benzene and Neural Tube Defects among Offspring: Texas, 1999–2004. Environ Health Perspect. 2011; 119:397–402. [PubMed: 20923742]
129. Li X, Zheng LK, Deng LX, Zhang Q. [Detection of numerical chromosome aberrations in sperm of workers exposed to benzene series by two-color fluorescence in situ hybridization]. Yi Chuan Xue Bao. 2001; 28:589–594. [PubMed: 11480168]
130. Liu S, Zheng L, Deng L, Tang G, Zhang Q. [Detection of numerical chromosome aberrations in sperm of workers exposed to benzene series by two-color fluorescence in situ hybridization]. Zhonghua Yu Fang Yi Xue Za Zhi. 2000; 34:17–19. [PubMed: 11860889]
131. Liu XX, Tang GH, Yuan YX, Deng LX, Zhang Q, Zheng LK. [Detection of the frequencies of numerical and structural chromosome aberrations in sperm of benzene series-exposed workers by multi-color fluorescence in situ hybridization]. Yi Chuan Xue Bao. 2003; 30:1177–1182. [PubMed: 14986438]
132. Zhao T, Liu XX, He Y, Deng LX, Zheng LK. [Detection of numerical aberrations of chromosomes 7 and 8 in sperms of workers exposed to benzene series by two-color fluorescence in situ hybridization]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2004; 21:360–364. [PubMed: 15300634]
133. Xing C, Marchetti F, Li G, Weldon RH, Kurtovich E, Young S, Schmid TE, Zhang L, Rappaport S, Waidyanatha S, Wyrobek AJ, Eskenazi B. Benzene exposure near the U.S. permissible limit is associated with sperm aneuploidy. Environ Health Perspect. 2010; 118:833–839. [PubMed: 20418200]
134. Winn LM. Homologous recombination initiated by benzene metabolites: a potential role of oxidative stress. Toxicol Sci. 2003; 72:143–149. [PubMed: 12604843]
135. Badham HJ, Renaud SJ, Wan J, Winn LM. Benzene-initiated oxidative stress: Effects on embryonic signaling pathways. Chem Biol Interact. 2010; 184:218–221. [PubMed: 19913523]
136. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: a systematic review and meta-analysis. Hum Reprod. 1998; 13:1532–1539. [PubMed: 9688387]
137. Zenzes MT, Wang P, Casper RF. Cigarette smoking may affect meiotic maturation of human oocytes. Hum Reprod. 1995; 10:3213–3217. [PubMed: 8822447]
138. Ramlau-Hansen CH, Thulstrup AM, Storgaard L, Toft G, Olsen J, Bonde JP. Is prenatal exposure to tobacco smoking a cause of poor semen quality? A follow-up study. Am J Epidemiol. 2007; 165:1372–1379. [PubMed: 17369608]

139. George L, Granath F, Johansson AL, Anneren G, Cnattingius S. Environmental tobacco smoke and risk of spontaneous abortion. Epidemiology. 2006; 17:500–505. [PubMed: 16837826]
140. Fantuzzi G, Aggazzotti G, Righi E, Facchinetti F, Bertucci E, Kanitz S, Barbone F, Sansebastiano G, Battaglia MA, Leoni V, Fabiani L, Triassi M, Sciacca S. Preterm delivery and exposure to active and passive smoking during pregnancy: a case-control study from Italy. Paediatr Perinat Epidemiol. 2007; 21:194–200. [PubMed: 17439527]
141. Jaddoe VW, Troe EJ, Hofman A, Mackenbach JP, Moll HA, Steegers EA, Witteman JC. Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study. Paediatr Perinat Epidemiol. 2008; 22:162–171. [PubMed: 18298691]
142. Yauk C. Mutagenic effects of cigarette smoke on sperm in mice and humans. Environmental and Molecular Mutagenesis. 2010; 51:687–687.
143. DeMarini DM. Is tobacco smoke a germ-cell mutagen. Environmental and Molecular Mutagenesis. 2010; 51:687–687.
144. Lamb JC, Hentz KL. Toxicological review of male reproductive effects and trichloroethylene exposure: assessing the relevance to human male reproductive health. Reprod Toxicol. 2006; 22:557–563. [PubMed: 16938429]
145. Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, Gray LE Jr. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci. 2000; 58:339–349. [PubMed: 11099646]
146. Lyche JL, Gutleb AC, Bergman A, Eriksen GS, Murk AJ, Ropstad E, Saunders M, Skaare JU. Reproductive and developmental toxicity of phthalates. J Toxicol Environ Health B Crit Rev. 2009; 12:225–249. [PubMed: 20183522]
147. Hannas BR, Furr J, Lambright CS, Wilson VS, Foster PM, Gray LE Jr. Dipentyl Phthalate Dosing during Sexual Differentiation Disrupts Fetal Testis Function and Postnatal Development of the Male Sprague-Dawley Rat with Greater Relative Potency than Other Phthalates. Toxicol Sci. 2011; 120:184–193. [PubMed: 21177253]
148. Pan G, Hanaoka T, Yoshimura M, Zhang S, Wang P, Tsukino H, Inoue K, Nakazawa H, Tsugane S, Takahashi K. Decreased serum free testosterone in workers exposed to high levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): a cross-sectional study in China. Environ Health Perspect. 2006; 114:1643–1648. [PubMed: 17107847]
149. Pala M, Ugolini D, Ceppi M, Rizzo F, Maiorana L, Bolognesi C, Schiliro T, Gilli G, Bigatti P, Bono R, Vecchio D. Occupational exposure to formaldehyde and biological monitoring of Research Institute workers. Cancer Detect Prev. 2008; 32:121–126. [PubMed: 18639989]
150. Bono R, Vincenti M, Schiliro T, Scursatone E, Pignata C, Gilli G. N-Methylenvaline in a group of subjects occupationally exposed to formaldehyde. Toxicol Lett. 2006; 161:10–17. [PubMed: 16153790]

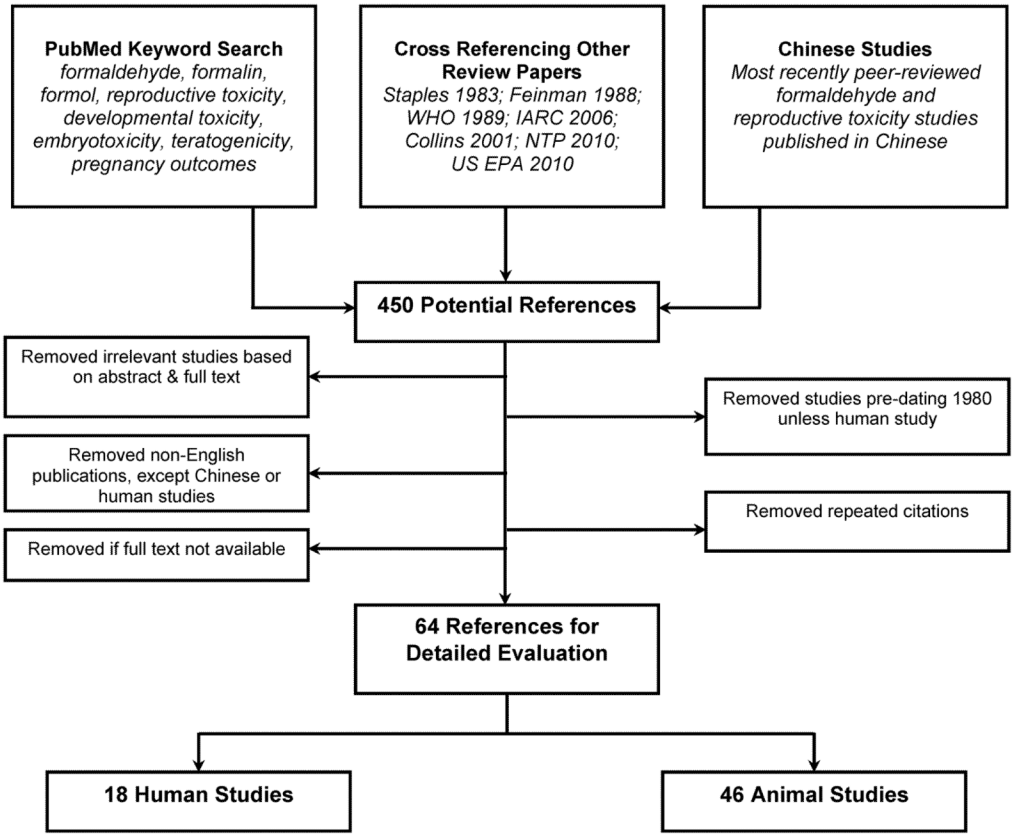

Figure 1. Flow diagram of study selection process

This figure depicts the logic of the study selection process, the results of which are included for review in this paper.

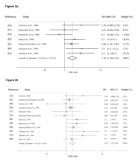

Figure 2. Forest plot for studies of spontaneous abortion (SAB) and all reproductive outcomes combined

These Forest plots show that ORs equal to or above 1.01 were found in (A) 5 of the 7 (71%) studies in the SAB analysis, and (B) 9 of the 12 (75%) studies in the all outcomes analysis. Most of the confidence intervals in the all outcomes analysis were well above 1, indicating higher significance. *ORs in (A) were calculated from SAB dat a reported in Axelsson et al. (1984), Hemminki et al. (1982), and Saurel-Cubizzoles et al. (1994), and recalculated from the data provided in Hemminki et al. (1985) as described in the footnote to Table 1. ORs in (B) were calculated from data on congenital malformations reported in Ericson et al. (1984), and from data on SAB and congenital malformations combined in Hemminki et al. (1985) as these outcomes were based on separate controls.

Figure 3a

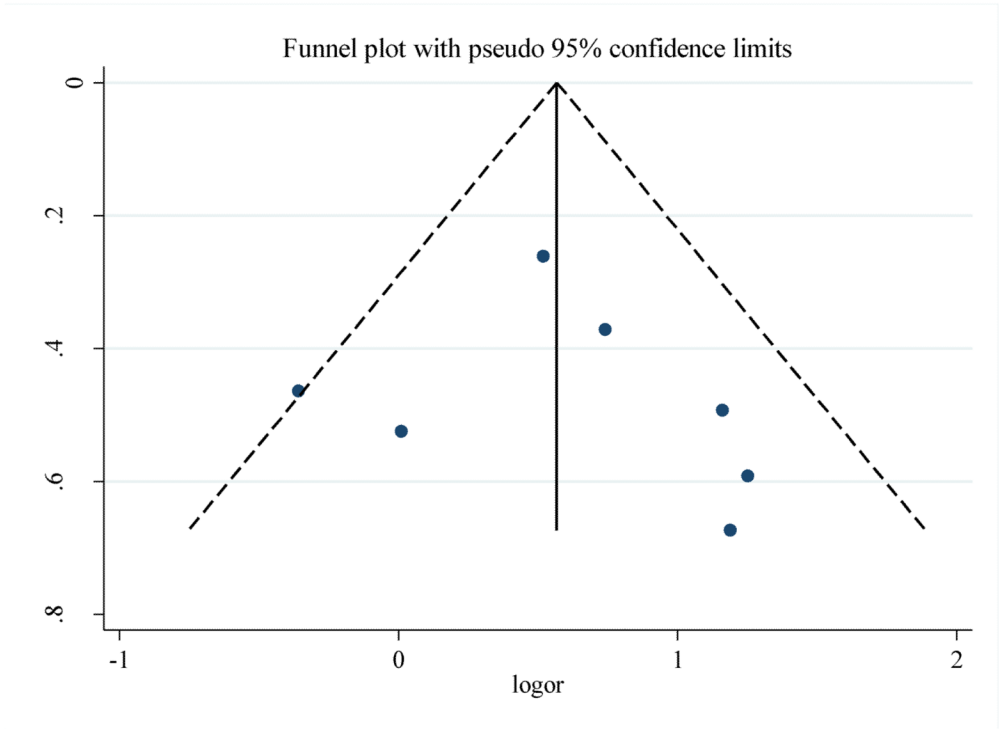

Figure 3b

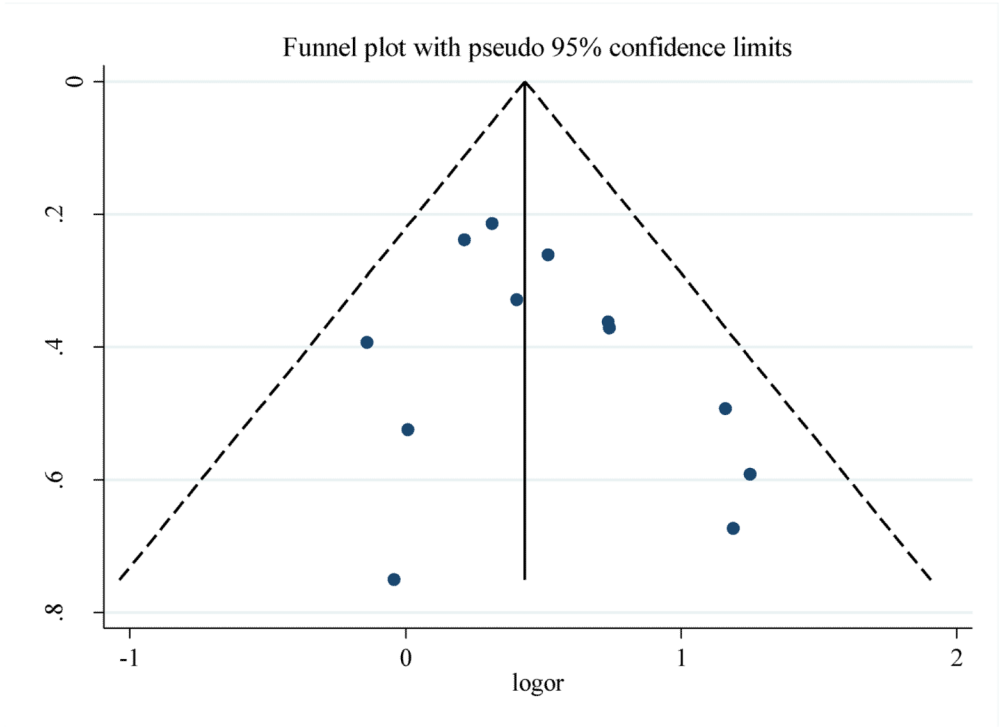

Figure 3. Funnel plot of studies of spontaneous abortion (SAB) and all reproductive outcomes combined

Publication bias is not apparent in the analysis of (A) SAB and (B) all outcomes.

Table 1

Summary of all human toxicity studies.

Outcome & Study*	Study Type	Study Population	Exposure Source	Location	Formaldehyde Concentration**	Outcome (95% CI)
Fertility						
Shumilina (1975) [13]	Cross-sectional	130 high exposed, 316 low exposed, 200 unexposed	Female factory workers	Russia	1.5 – 4.5 mg/m^3	Menstrual disorders in FA exposed 2.5x higher than in the unexposed
Olsen & Dossing (1982) [14]	Cross-sectional	66 exposed, 26 unexposed	Female daycare workers	Denmark	0.43 mg/m^3	Menstrual irregularities in about 30–40% of FA exposed and 0% of unexposed; higher rate of vaginal irritation & pain during micturition
Taskinen *et al.* (1999) [15]	Cohort	119 low exposed, 77 moderate, 39 high, 367 unexposed	Female wood workers	Finland	0.01 – 1.00 ppm	Reduced fertility, FDR = 0.64 (95% CI 0.43–0.92), *p* 0.02 at high FA exposure (mean 0.33 ppm)
Ward *et al.* (1984) [11]	Cross-sectional	11 exposed, 11 unexposed	Male autopsy workers	Texas	0.1 – 5.8 ppm	No statistically significant difference in sperm morphology or count between FA exposed and unexposed
Spontaneous Abortion						
Shumilina (1975) [13]	Cross-sectional	130 high exposed, 316 low exposed, 200 unexposed	Female factory workers	Russia	0.05 – 4.5 mg/m^3	No difference between FA exposed and unexposed in “premature births or abortions”
Hemminki *et al.* (1982) [17]	Cohort	1100 unexposed pregnancies, 50 formaldehyde exposed pregnancies.	Female hospital workers	Finland	N/A	Adjusted rates of 8.4% SAB in FA exposed and 8.3% in unexposed
Axelsson *et al.* (1984) [19]	Cohort	1160 total pregnancies among 745 females, 10 exposed	Female lab workers	Sweden	N/A	30% SAB rate in FA exposed, 11.5% in unexposed
Hemminki *et al.* (1985) [18]	Case-control	164 cases, 464 controls	Female hospital nurses	Finland	N/A	COR: 0.7 (0.28–1.73)& of SAB for FA exposure; 6 exposed in cases and 24 exposed in controls
Seitz & Baron (1990) [20]	Cohort	365 pregnancies	Female fabric workers (Rockcastle facility)	Kentucky	0.14 – 0.79 ppm	Miscarriage rate: fabric workers at Rockcastle, 14%; working elsewhere, 13%; not working outside home, 5%.
Stucker et al. (1990) [21]	Cohort	139 exposed (cytostatic drug) pregnancies, 357 unexposed; 113 FA-	Female hospital nurses	France	N/A	FA did not modify the risk of SAB associated with exposure to cytostatic drugs

Outcome & Study*	Study Type	Study Population	Exposure Source	Location	Formaldehyde Concentration**	Outcome (95% CI)
		exposed or exposure unknown				
John *et al.* (1994) [22]	Cohort	244 formaldehyde exposed and 132 unexposed	Cosmetologists	US	N/A	AOR: 2.1 (1.0–4.3) of SAB for FA exposure
Saurel-Cubizolles *et al.* (1994) [23]	Cohort	724 pregnancies: 316 formol exposed, 408 unexposed	Female hospital nurses	France	N/A	11.1% SAB in formaldehyde exposed, 6.9% in unexposed
Taskinen *et al.* (1994) [24]	Case-control	206 cases, 329 controls	Female lab workers	Finland	0.01 – 7 ppm	OR: 3.5 (1.1–11.2) of SAB for FA exposure
Taskinen *et al.* (1999) [15]	Cohort	52 cases with same work place from preconception through year of SAB, in high, medium and low exposure categories	Female wood workers	Finland	0.01 – 1.00 ppm	OR 3.2 (1.2–8.3) of SAB for high FA exposure
Lindbohm *et al.* (1991) [12]	Cohort	596 formaldehyde exposed pregnancies, 54 SAB cases	Paternal occupational exposure	Finland	“Moderate/High”	OR: 1.0 (0.8–1.4) of SAB for FA exposure
Lindbohm *et al.* (1991) [12]	Cohort	1212 formaldehyde exposed pregnancies, 110 SAB cases	Paternal occupational exposure	Finland	“Low”	OR 1.1 (0.9–1.4) of SAB for FA exposure
Congenital Malformations						
Ericson (1984) [26]	Nested case-control	26 cases, 50 controls	Female lab workers	Sweden	N/A	OR = 0.96 (0.22–4.18) of SAB for FA exposure; 3 exposed in cases and 6 in controls
Hemminki *et al.* (1985) [18]	Case-control	34 cases, 95 controls	Female hospital nurses	Finland	N/A	COR: 1.74 (0.40–7.60)# of CM in FA exposed; 8.8% cases FA exposed, 5.5 % controls
Saurel-Cubizolles *et al.* (1994) [23]	Cohort	641 pregnancies: 271 exposed, 370 unexposed	Female hospital nurses	France	N/A	5.2% CM in FA exposed, 2.2% in unexposed
Dulskiene & Grazuleviciene (2005) [27]	Case-control controls	184 cases, 479	Ambient exposure	Lithuania	> 2.4 μg/m^3	OR= 1.24 (0.81–2.07) of heart CM for FA exposure
Zhu *et al.* (2006) [28]	Cohort	983 pregnancies: 218 high exposure, 364 medium exposure, 401 low exposure	Female lab workers	Denmark	N/A	AOR: 1.5 (0.8–2.9) of CM for high FA exposure
Low Birth Weight						

Outcome & Study*	Study Type	Study Population	Exposure Source	Location	Formaldehyde Concentration**	Outcome (95% CI)
Shumilina (1975) [13]	Cross-sectional	130 high exposed, 316 low exposed, 200 unexposed	Female factory workers	Russia	0.05 – 4.5 mg/m^3	No cases in high-exposed group, 2 cases in low-exposed group, 2 cases in control group
Grazuleviciene *et al*. (1998) [29]	Cross-sectional	4,343 births, 244 cases	Ambient exposure	Lithuania	<1.94 to >4.67 μg/m^3	Adjusted RR 1.37 (0.90–2.09) of low birth weight for FA exposures> 3.5 ug/m^3 compared to <3.5 ug/m^3
Maroziene & Grazuleviciene (2002) [30]	Cross-sectional	3,988 births, 140 cases	Ambient exposure	Lithuania	Mean 3.14 μg/m^3 and tertiles <2.00, 2.01–3.9 and >3.9 μg/m^3§	OR adjusted for gestational age 2.09 (1.03–4.26) of low birth weight for >3.9 ug/m^3 FA
Zhu *et al*. (2006) [28]	Cohort	983 pregnancies: 218 high exposure, 364 medium exposure, 401 low exposure	Female lab workers	Denmark	N/A	AOR: 1.2 (0.6–2.2) of low birth weight at high FA exposure index
Preterm Birth						
Shumilina *et al*. (1975) [13]	Cross-sectional	130 high exposure, 316 low, 200 unexposed	Female factory workers	Russia	1.5 – 4.5 mg/m^3	No difference between exposed and unexposed in "premature births or abortions"
Maroziene & Grazuleviciene (2002) [30]	Cross-sectional	3,988 births, 203 cases	Ambient exposure	Lithuania	Mean 3.14 μg/m^3 and tertiles <2.00, 2.01–3.9 and >3.9 μg/m^3§	AOR: 1.37 (0.91–2.05) of preterm birth for FA exposure
Zhu *et al*. (2006) [28]	Cohort	983 pregnancies: 218 high exposure, 364 medium exposure, 401 low exposure	Female lab workers	Denmark	N/A	AOR: 0.7 (0.3–1.7) of preterm birth at high FA exposure index
Other Developmental Toxicity						
Seitz & Baron (1990) [20]	Cohort	365 pregnancies	Female fabric workers	Kentucky	0.14 – 0.79 ppm	RR: 6.9 (3.6–13.1) of stillbirth, premature birth, birth defect for FA exposure

*Categorized by pregnancy outcome, then listed chronologically by publication year

**Values are as reported, not converted, 1 ppm = 1.23 mg/m^3

&The reported crude OR is 0.6, but a crude OR of 0.70 is calculated based the data in their Table 2

#The reported crude OR is 1.8, but a crude OR of 1.74 is calculated based on the data in their Table 6

§Obtained from additional Table 1 in [30]

Abbreviations: AOR adjusted odds ratio | CM congenital malformation | COR crude odds ratio | CRR crude relative risk | FA formaldehyde | FDR fecundability density ratio | OR odds ratio | N/A not available | ppm parts per million | RR relative risk | SAB spontaneous abortion | TWA time weighted average

Table 2

Studies not included in the meta-analysis and respective exclusion criteria.

Study	Outcome	Result (RR and CI or other)	Reason not used
Shumilina (1975) [13]	Low birth weight (< 2500 g)	No cases in the high exposed group, 2 cases in the unexposed group.	No RR, poorly described methods
Shumilina (1975) [13]	Menstrual irregularities	47.5% vs. 18.6 %	Poorly described methods
Shumilina (1975) [13]	Premature births and SAB	“no differences…”	No RR, poorly described methods
Olsen & Dossing (1982) [14]	Menstrual irregularities	Menstrual irregularities in about 30–40% of exposed and 0% of unexposed	No RR
Ward *et al*. (1984) [11]	Sperm count and morphology	“no statistically significant differences”	No RRs
Seitz & Baron (1990) [20]	SAB	Rate: exposed = 14%; other work = 13%; home = 5%	Subjects used as own unexposed controls, methods not well described.
Stucker *et al.* (1990) [21]	SAB	No RR for formaldehyde	Large numbers with unknown exposure

Table 3

Results of the meta-analysis of formaldehyde and adverse pregnancy outcomes.

		Fixed effects			Shore		Random effects			Heterogeneity	
	N	RR	CI_L	CI_U	CI_L	CI_U	RR	CI_L	CI_U	X^2	p
Maternal exposure only											
Spontaneous abortion	7	1.76	1.29	2.41	1.20	2.59	1.80	1.19	2.70	8.99	0.17
Self-reported exposure data	4	2.04	1.40	2.97	--**	--	--	--	--	1.89	0.60
Not self-reported	3	1.29	0.74	2.25	0.52	3.21	1.31	0.52	3.25	5.34	0.07
All outcomes	12	1.54	1.27	1.88	1.27	1.88	1.55	1.27	1.89	11.18	0.43
Self-reported exposure data	5	1.95	1.35	2.81	--	--	--	--	--	2.83	0.59
Not self-reported	7	1.40	1.11	1.78	1.11	1.78	1.41	1.11	1.79	6.19	0.40
Maternal and paternal exposure*											
Spontaneous abortion	8	1.29	1.04	1.59	0.94	1.76	1.58	1.06	2.35	16.03	0.02
All outcomes	13	1.34	1.14	1.57	1.10	1.62	1.45	1.17	1.80	17.36	0.14

* Includes all the maternal exposure studies plus the Lindbohm *et al.*, 1991 study on paternal formaldehyde exposure

** Shore and random effects models were only done when heterogeneity was present.

Table 4

Differences between current meta-analysis and Collins *et al*. for spontaneous abortion

	Current Meta-Analysis			Collins *et al*, 2001				
Study	**RR**	**CI_{lower}**	**CI_{upper}**	**RR**	**CI_{lower}**	**CI_{upper}**	**Difference**	**Reason for Difference**
Hemminki *et al*. (1982) [17]	1.01	0.36	2.82	1.0	0.7	1.3	Minor	Different method for CI calculation
Axelsson *et al*. (1984) [19]	3.29	0.88	12.34	3.3	1.2	9.2	Minor	Different method for CI calculation
Hemminki *et al*. (1985) [18]	0.70	0.28	1.73	0.7	0.3	1.8	Minor	Different method for CI calculation
Stucker *et al.* (1990) [21]	Not used			1.0	0.5	2.0	Yes	Rate of unknown exposures is high
Lindbohm *et al*. (1991) [12]	Not used			1.0	0.8	1.4	Yes	Paternal exposure
John *et al.* (1994) [22]	2.1	1.0	4.3	2.1	1.0	4.3	No	
Saurel-Cubizolles *et al*. (1994) [23]	1.68	1.01	2.82	Not used			Yes	Unknown
Taskinen *et al*. (1994) [24]	3.5	1.1	11.2	3.5	1.1	11.2	No	
Taskinen *et al*. (1999) [15]	3.2	1.2	8.3	2.3	1.4	3.6	Yes	High exposed (Current) vs. all exposed (Collins)

RR: relative risk; CI: confidence interval

Table 5

Summary of all animal reproductive & developmental toxicity studies

Reference	Study Animal	Total Study Size	Animal Age	Exposure Type	Dosage	Duration	Toxic Effects
Reproductive toxicity studies							
Rats							
Ozen (2002) [53]	Male albino Wistar rats	42	Adult	Inhalation	0–25 mg/L	8h/d, 5d/wk for 4 or 13 wks	Growth retardation, altered levels of trace elements; damage in testicular tissues
Ozen (2005) [51]	Male albino Wistar rats	18	Adult	Inhalation	0–10 ppm	91d	↓ seminiferous tubules and testosterone levels
Zhou (2006b) [52]	Sprague-Dawley rats	30	Adult	Inhalation	0, 10, 30 mg/m^3	12 h/d for 2 wks	↓ testicular weight; seminiferous tubule atrophy; ↓ sperm cells
Golalipour (2007) [50]	Male albino Wistar rats	28	6–7 wks	Inhalation	1.5 ppm	18wks	↓ germ cells, thickening of seminiferous tubules; displacement of Sertoli and germinal cells; smaller seminiferous tubules
Chowdhury (1992) [54]	Male Charles rats	40	Unknown	Injection (i.p.)	5, 10, or 15 mg/kg	30d	Leydig cell impairment; ↓ testes weight and serum testosterone; steroidogenic inhibition
Majumder (1995) [56]	Male albino Wister rats	Unknown	Unknown	Injection (i.p.)	10 mg/kg	30d	↓ DNA & tissue protein content in testis, prostate, epididymis; ↓ sperm motility & viability
Odeigah (1997) [57]	Male albino rats	1224	12–14 wks	Injection (i.p.)	0.125–.500 mg/kg	5x daily	More lethal mutations; reduced fertile matings; ↑ sperm head abnormalities

Reference	Study Animal	Total Study Size	Animal Age	Exposure Type	Dosage	Duration	Toxic Effects
Zhou (2006a) [55]	Male Rats	40	8–10 wks	Injection (i.p.)	0.10–10 mg/kg	14d x daily	↓ testicular weight, sperm counts, sperm motility, serum testosterone; ↑ apoptosis rate of sperm cells
Cassidy (1983) [58]	Male Wistar rats	80	10 wks	Oral	100 & 200 mg/kg	Once	↑ sperm head abnormalities
Mice							
Maronpot (1986) [63]	Male and female B6C3F1 mice	20	6 wks	Inhalation	0, 2, 4, 10, 20, or 40 ppm	5–6 h/d for 13 wks	Hypoplasia of uterus and ovaries in female mice
Zeng (2003) [59]	Male KM mice	15	4 wks	Inhalation	0, 1, 3 mg/m^3	6 h/d for 7d	Death of epithelial cells of seminiferous tubules; ST walls degrading; GSH-Px ↓
Wang (2005a) [60]	Male mice	25	7–10 wks old	Inhalation	0.5, 1.0, or 3.0 mg/m^3	72h	Higher micronuclei frequency in early spermatids; ↓ sperm
Wang (2006c) [62]	Male KM mice	60	Unknown	Inhalation	21, 42, 84 mg/m^3	2 h/d × 6 d/wk for 13 wks	LDH, G-6PD, SDH, serum testosterone & activity of germ cells ↓; deformity rate ↑
Xing (2007) [61]	Male KM mice	180	Unknown	Inhalation	21, 42, or 84 mg/m^3	2 h/d for 6/wk for 13 wks	Damaged testicular cells; ↑ spermatozoa aberration rate, ↓ sperm survival rate, sperm count, G-6PD & SDH activity
Stott (1980) [64]	Male CF-1 mice	Unknown	10–12 wks	Injection (i.p., i.v.)	1–150mg/kg	Once	Linear relationship between sperm head DNA alkylation and administered dosages of formaldehyde
Yi (2000) [66]	Male ICR mice	25	6–8 wks	Injection (i.p.)	4, 10, or 30 mg/kg	5d	↓ sperm count, ↑ abnormal sperm

Reference	Study Animal	Total Study Size	Animal Age	Exposure Type	Dosage	Duration	Toxic Effects
Huang (2002) [67]	Male KM mice	40	7 wks	Injection (i.g.)	0.2, 2.0, 20.0 mg/kg	7d	Sperm degeneration; ↓ sperm count; ↑ deformed sperm
Wang (2002) [70]	Female KM mice	48	7 wks	Injection (i.g.)	0, 1.25, 2.50, or 5 mg/kg	5d	Irregular estrous cycles, damaged & smaller ovaries; damaged oocytes; fibrosis in reproductive tissue; decreased number of mitochondrioa
Xie (2003) [65]	Male KM mice	30	Adult	Injection (i.g.)	0, 2.0, 20.0 mg/kg	5d	Germ cells denatured; ↓ sperm count, ↑ sperm cell deformity rate
Wang (2005) [60]	Male mice	25	7–10 wks	Injection (i.g.)	0.2, 2.0, 20.0 mg/kg	7d	Tissue coefficient decreases with higher concentrations; less active and fewer sperm; increased sperm deformity
Wang (2006a) [69]	Male KM mice	Unknown	7–10 wks	Injection (i.g.)	0–100 umol/L	Once	DPC present; DNA breakage in testicular cells
Peng (2006) [68]	Male Kunming	30	5 wks	Injection (i.p.)	20 mg/kg	5d	Causes DNA protein crosslinking
Ward (1984) [11]	Male B6C3F1 mice	10	4 months	Oral	0 or 100 mg/kg	5d	Small but non-significant increase in abnormal sperm cells
Other Animals							
Meng (2009) [71]	Laying hens	59,000 hens	16 weeks	Injection (i.m.)	Formalin of varying amounts	Once	Lower egg production, estradiol levels; degeneration of combs and ovarian follicles
Anwar (2001) [72]	Male Japanese quail	75	35 days	Oral	0–20 ml/mg	8 wks	↓ testes weight & smaller seminiferous tubule diameter; ↑

Reference	Study Animal	Total Study Size	Animal Age	Exposure Type	Dosage	Duration	Toxic Effects
							vacuolation in germinal epithelial of seminiferous tubules
Khan (2003) [73]	White leghorn cockerels	120	10 wks	Oral	2.5, 5.0, 10 ml/kg	2x daily for 8 wks	Smaller seminiferous tubule diameter; reduced testes weight & volume
Developmental toxicity studies prenatal exposure							
Rats							
Thrasher (2001) [74]	Wistar rats	Unknown	Pregnant rats	Inhalation	0.5 & 1.5 mg/m^3	4h/d for 4 months	Damage to blastomeres, increased rate of embryo degeneration; increased chromosome aberrations & aneuploidy
Thrasher (2001) [74]	Wistar rats	Unknown	Pregnant rats	Inhalation	0.012 & 1.0 mg/m^3	10–15d	↑ overall body weight; involution of lymphoid tissues; mild hypertrophy of Kupffer's cells in fetus
Sailenfait (1989) [77]	Sprague-Dawley rats	25	Pregnant rats	Inhalation	0–40 ppm (0–37%)	6h/d for 15d gestation	at 40ppm, maternal weight loss; slightly feototoxic
Martin (1990) [76]	Sprague-Dawley rats	125	Pregnant1 3–14 week-old rats	Inhalation	2–10 ppm	6h/d for 10d	↓ maternal food consumption & weight gain; ↓ bone ossification; corpora lutea, implantation sites, dead fetuses, fetal weights all unaffected
Tang (2006) [75]	Wistar rats	120	Unknown	Inhalation	0, 5, 25, 120 mg/m^3	1h/d for 7 wks	Corpus luteum, placental weight, total body weight & live birth rate ↓; ↑ fetus abnormality; pups had shorter limbs

Reference	Study Animal	Total Study Size	Animal Age	Exposure Type	Dosage	Duration	Toxic Effects
Thrasher (2001) [74]	Rats	Unknown	Unknown	Injection (i.g.)	8 mg/kg	Once/day throughout pregnancy	↑ pre & post-implantation deaths by 2x; altered fetal liver enzyme activity
Thrasher (2001) [74]	Mongrel rats	Unknown	Unknown	Oral	0.5 mg/kg	22d	Many abnormalities in pups, but non conclusive
Mice							
Thrasher (2001) [74]	ICR mice	Unknown	Unknown	Injection (tail vein)	0.05 ml of 1% formaldehyde solution	Once	Formaldehyde elimination slower in fetal tissue
Katakura (1993) [78]	ICR mice	Unknown	Fetuses	Injection (tail vein)	0.05 ml of 1% formaldehyde solution	Once	Formaldehyde elimination slower in fetal than maternal tissue
Fontignie-H (1981) [80]	Male Q strain mice	85	3 months old	Injection (i.p.)	50 mg/kg, 35% formaldehyde solution	Once	No observed effect despite ↑ embryo mortality, and pre- & post implantation deaths
Fontignie-H (1982) [81]	Male Q strain mice	18	3 months old	Injection (i.p.)	Unknown	Once	↑ pre& post-implantation deaths
Wang (2006b) [79]	Pregnant female mice	Unknown	10–13 wks	Injection (i.p.)	0, 0.2, 1.0, 2.0, 20.0 mg/kg	14d	DNA breakage at 1.0 mg/kg; DPC at 2.0 mg/kg; more severe in fetal liver cells than maternal
Marks (1980) [82]	CD-1 Albino mice	174	Pregnant mice	Oral	74, 148, or 185 mg/kg/day	18d	22/34 pregnant mice died early; no effect on malformation or fetus
Other Animals							
Al-Saraj (2009) [83]	Female rabbits	33	Pregnant rabbits	Inhalation	12 ppm formaldehyde	Constant throughout gestation	Meromelia, encephalocele, oligodactyly, umbilical hernia, short tail
Magras (1996) [84]	Chicken eggs	1011 eggs	Embryos	vapor in incubation chamber	40% formalin	1–6x per day for 3d	Intact eggs unaffected; embryotoxicity in

Reference	Study Animal	Total Study Size	Animal Age	Exposure Type	Dosage	Duration	Toxic Effects
							29:100 of perforated eggs; main effects were early and late prenatal death, congenital abnormalities and deformities
Hayretdag (2008) [85]	Chicken eggs	1464 eggs	18-day old embryos	Fumigation of intact & perforated eggs	42 ml or 56 ml of 40% formalin	Once for 20 or 40 min	Shortening and loss of cilia; vascuolisation and swelling of mitochondria, spoiling of cristae in tracheal epithelium; effects increased with duration
Overman (1985) [86]	Hamsters	Unknown	Pregnant hamsters	Topical	0.5 ml FA (37% solution)	Once for 2h	No significant effect on fetal weight, length or malformation
Developmental toxicity studies– post-natal exposure							
Rats							
Songur (2003) [88]	Albino Wistar Rats	113	Neonatal	Inhalation	0–12 ppm	30d	Causes increased Hsp70 synthesis and damaged neurons in hippocampus
Songur (2005) [87]	Albino Wistar rats	75	Neonatal	Inhalation	0–12 ppm	30d	Increased tissue SOD, copper and iron, decreased zinc in lung tissue, suggestive of oxidative damage
Guy (1992) [89]	Long-Evans rats	Unknown	1–20 days old	Injection (sub-cutaneous)	1 or 2.5% formalin	up to 20d	Specific and non-specific pain responses detected at 1 day old; type of response varied by age; intensity of response decreased with age
***Ex vivo & in vitro* animal studies**							
Ex vivo embryo studies							

Reference	Study Animal	Total Study Size	Animal Age	Exposure Type	Dosage	Duration	Toxic Effects
Harris (2004) [90]	Sprague-Dawley rats	26 controls, 114 treated	Embyros GD 10–11	In culture medium	3 & 6 μg/ml	Once	↓ embryo viability & rotation; blister formation; dysmorphogenesis; embryolethal
Hansen (2005) [91]	CD-1 mice & Sprague-Dawley rats	N/A	Embryos GD 10	In culture medium	1.0–8.0 μg/ml	Constant	Dose-dependent loss of viability; incomplete axial rotation and neural tube closure
Hansen (2005) [91]	CD-1 mice & Sprague-Dawley rats	Unknown	Embyros GD 10	Injection	0.003–2.0 μg	Once	Dose-dependent loss of viability; incomplete axial rotation and neural tube closure at lower doses than in culture medium
In vitro studies							
Osinowo (1982) [93]	Ram	N/A	Sperm	Direct washing	0.0025–0.04%	Once for 30 min	sperm motility reversibly inhibited at 0.05 – 0.01%, irreversibly at 0.04%
Laschinski (1991) [92]	Mouse	N/A	ESCs	In culture	ID_{50} (440μM) ID_{90} (690μM)	24h	Cytotoxicity increased with increasing dose

Abbreviations: h hours; d days; wks weeks; ESCs embryonic stem cells; G-6PD glucose-6-phosphate dehydrogenase; GSH-Px glutathione peroxidase; LDH lactate dehydrogenase; N/A Not applicable; SDH succinate dehydrogenase; DPC DNA-protein crosslink; SOD superoxide dismutase; ESC embryonic stem cell; i.p. intraperitoneal, i.v. intravenous; i.m. intramuscular; i.g intragastric injection.