

Review Article

Atrazine and Pregnancy Outcomes: A Systematic Review of Epidemiologic Evidence

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Atrazine (ATR) is a commonly used agricultural herbicide that has been the subject of epidemiologic studies assessing its relation to reproductive health problems. This review evaluates both the consistency and the quality of epidemiologic evidence testing the hypothesis that ATR exposure, at usually encountered levels, is a risk factor for birth defects, small for gestational age birth weight, prematurity, miscarriages, and problems of fetal growth and development. We followed the current methodological guidelines for systematic reviews by using two independent researchers to identify, retrieve, and evaluate the relevant epidemiologic literature on the relation of ATR to various adverse outcomes of birth and pregnancy. Each eligible paper was summarized with respect to its methods and results with particular attention to study design and exposure assessment, which have been cited as the main areas of weakness in ATR research. As a quantitative meta-analysis was not feasible, the study results were categorized qualitatively as positive, null, or mixed. The literature on ATR and pregnancy-related health outcomes is growing rapidly, but the quality of the data is poor with most papers using aggregate rather than individual-level information. Without good quality data, the results are difficult to assess; however, it is worth noting that none of the outcome categories demonstrated consistent positive associations across studies. Considering the poor quality of the data and the lack of robust findings across studies, conclusions about a causal link between ATR and adverse pregnancy outcomes are not warranted. *Birth Defects Res (Part B)* 101:215–236, 2014. © 2014 Wiley Periodicals, Inc.

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INTRODUCTION

Atrazine (ATR) is a triazine agricultural herbicide commonly used to control a wide spectrum of broadleaf weeds and grasses primarily around corn, but also in areas that cultivate sugar cane, and sorghum (Bridges, 2008). After application to soil, ATR degrades with an estimated half-life ranging from a few weeks to several months, but it may also be detected in surface waters and, to a lesser extent, in groundwater (Solomon et al., 2008; Sullivan et al., 2009b).

A substantial proportion of the U.S. population resides outside of major metropolitan centers in areas that are close to agricultural activity (U.S. Census Bureau, 2011). People who work in agriculture or reside near agricultural fields may have higher levels of exposure to (ATR) through spray drift than the general population (Clayton et al., 2003; Curwin et al., 2007a, 2007b). Occupational exposure to ATR may occur during manufacturing and formulation operations and during application. Nonoccupa-

tional exposure might arise from drinking water or, to a lesser extent, through dietary exposure. As the use of ATR is widespread, concerns have been raised about its effect on the environment and human health (Gammon et al., 2005). In response to these concerns, epidemiology of ATR exposure has become a rapidly expanding field with multiple studies evaluating various endpoints published over the last two decades.

This increased concern regarding human health is not supported by experimental animal studies. ATR and its metabolites have been evaluated for reproductive and developmental toxicity in regulation-compliant experiment

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studies in rats and rabbits (Scialli et al., 2014, companion paper). Administration of ATR to pregnant rats at dose levels up to 100 mg/kg body weight per day did not produce any apparent change in malformations in spite of maternal toxicity in the high-dose group. There were no adverse effects on embryo/fetal viability or growth. Administration of ATR to pregnant rabbits at up to 75 mg/kg bw/day did not increase the incidence of malformations (Infurna et al., 1988). Body weight gain was impaired in the high-dose group over the course of the pregnancy with significant loss of maternal body weight during the treatment period. There was a decrease in live fetuses and in fetal body weight in the high-dose group, which is consistent with the treatment-related effects on maternal weight gain. At the next lowest ATR dose there were no marked alterations in fetal viability or growth in spite of a transient decrease in maternal weight gain during a portion of the treatment period.

In animals, ATR is metabolized to desethylatrazine or deisopropylatrazine and ultimately to diaminochlorotriazine. Plants metabolize ATR to hydroxyatrazine. These four metabolites were tested in pregnant rats at high dose levels and no change in number of malformations occurred at any dose level with any metabolite (Scialli et al., 2014, companion paper). Two-generation testing of ATR in rats using dietary administration failed to identify adverse effects of treatment on fertility, growth, or viability with dose levels up to the equivalent of 38.7 mg/kg bw/day (DeSesso et al., 2014, companion paper). The maximum permissible ATR concentration in drinking water in the United States is 3 µg/l. A 60-kg woman drinking 2 l/day of water would ingest ATR at 6 µg/day or 0.1 µg/kg bw/day. The lack of developmental effects of ATR with respect to malformations, viability, or growth in experimental animal studies was demonstrated at exposure levels that are four to five orders of magnitude higher than maximum anticipated human exposure from drinking water. These negative findings notwithstanding, the conclusions about the effects of environmental exposures on pregnancy outcomes in humans should take into consideration human data that come primarily from epidemiologic studies.

The previously summarized epidemiologic evidence pertaining to the association between ATR and human health outcomes focused primarily on cancer risks (International Agency for Research on Cancer, 1999; MacLennan et al., 2002; Sathiakumar et al., 2011; Boffetta et al., 2013). In recent years, however, more attention has focused on the postulated endocrine effects of ATR (Roy et al., 2009; Hayes et al., 2011; Vandenberg et al., 2012), leading to hypotheses that ATR, and more broadly triazines, may affect reproductive health and may produce birth defects and other adverse outcomes of pregnancy (Hayes, 2005; Lubinsky, 2012).

With respect to epidemiologic data, this issue was evaluated in only one previous review, which concluded that the evidence pertaining to the association between triazines, taken as a group, and reproductive endpoints was "inadequate" (Weselak et al., 2007). This conclusion was based on only seven reports available at that time. Since then, more than a dozen additional relevant studies have been published, and for this reason, a new examination of epidemiologic evidence is warranted.

The current review, systematically evaluates both the consistency and the quality of the epidemiologic studies testing the hypothesis that exposure to ATR (alone or considered together with other triazines), at commonly encountered levels, is a risk factor for adverse pregnancy outcomes, including congenital malformations, premature delivery, miscarriages, and problems of fetal growth and development.

APPROACH

The guidelines of the current methodological literature on systematic reviews were followed (Sutton et al., 1998; Shea et al., 2007; Moher et al., 2009). In particular, study methods were cross-checked against "assessment tool of multiple systematic reviews" (AMSTAR) guidelines (Shea et al., 2007), which represent an extension of a previously published instrument (Moher et al., 1999). The AMSTAR tool includes the following 11 items: (1) an a priori statement of research questions and inclusion/exclusion criteria; (2) duplicate literature searches by two or more coauthors; (3) the use of at least two electronic search engines followed by a supplemental search of reviews, textbooks, and secondary references with key words and medical subject headings statements reported in the methods section; (4) a statement of whether or not studies were excluded based on publication status and language; (5) a list of studies included and excluded from the review; (6) a summary of study characteristics that met the inclusion criteria; (7) a formal assessment of the individual study quality; (8) consideration of study quality in drawing conclusions; (9) whenever possible, pooling of study results in a quantitative meta-analysis accompanied by a test for heterogeneity; (10) assessment of publication bias; and (11) a statement of sources of support (Shea et al., 2007). With the exception of items 9 and 10, which are contingent on the feasibility of a formal meta-analysis, the AMSTAR checklist is applicable to any systematic review.

Identification and Selection of Studies

The initial literature search was conducted using PubMed, Ovid, EMBASE, and Google Scholar electronic databases. Using various combinations of keywords "ATR," "triazines," "herbicides," "exposure," "humans," "health," "outcomes," "epidemiology," "pregnancy," "birth," "congenital," "defects," "malformations," "prematurity," "gestational age (GA)," "delivery," and "neonatal," we selected relevant articles that investigated pregnancy outcomes associated with ATR and/or all triazine exposures in humans. The electronic database search strategy with corresponding medical subject headings and general keyword terms, and numbers of articles retrieved and examined at each stage of this process are presented in Appendix A.

Secondary references of retrieved articles were reviewed to identify publications not captured by the electronic search. Additional literature searches were conducted to identify relevant reports that were not published in the peer-reviewed literature. A list of studies retrieved, evaluated but excluded from the review, and the reasons for exclusions are provided in Appendix B.

The search and selection of relevant studies was conducted independently by two study authors (JSM and MG) with all disagreements resolved by consensus. The criteria for inclusion into the present review were as follows:

- (1) Studies of human populations assessing exposures to:
 - (a) ATR;
 - (b) Triazine herbicides considered as a group; or
 - (c) Corn growing areas used as a surrogate for ATR exposure in the United States (National Agricultural Statistics Service, 2006).
- (2) Endpoints of interest that fall into three broad categories:
 - (a) Congenital malformations (birth defects);
 - (b) Pregnancy loss: miscarriages and stillbirths;
 - (c) Other pregnancy outcomes:
 - i. Continuous variables—birth weight (BW), head circumference, and GA;
 - ii. Categorical variables—premature delivery, low birth weight, small for gestational age (SGA) newborn weight, and small head circumference
- (3) The association between exposure(s) of interest and any of the above outcomes either was assessed by the authors or could be assessed based on the information provided in the publication.
- (4) Publication appeared in English before May 1, 2013 (end of literature search).

Literature Review

Each study that met the inclusion criteria was examined independently by the same two coauthors (JSM and MG) who conducted the literature search. Data from each study were tabulated, and the resulting summary tables were again cross-checked with disagreements resolved by consensus. Information extracted from each study for the purposes of this review included

- (1) Description of the study: for example, design, year of publication, and location;
- (2) Exposure assessment: for example, individual-level or aggregate, measured or estimated, biomarker- or questionnaire-based;
- (3) Exposure categorization for example, binary, ordinal, or continuous;
- (4) Outcomes ascertainment and definitions: for example, SGA below 10th or 5th percentile, individual-level or aggregate, abstracted from medical records or self-reported; and
- (5) Measure of association and a measure of precision: for example, an odds ratio (OR) or a linear regression coefficient accompanied by a 95% confidence interval (CI) or a *p*-value.

Two studies (Limousi et al., 2013; Migeot et al., 2013) did not report the results in terms of ORs, but did provide information sufficient to reconstruct the two-by-two tables. For those studies the ORs and the 95% CIs were calculated by one of the authors (MG) using OpenEpi statistical software (Sullivan et al., 2009a).

Although strength of evidence provided by any epidemiologic study depends on multiple factors, including overall design, selection of participants, ability to minimize exposure, and outcome misclassification and control for confounders (Vandenbroucke et al., 2007), some of those factors are particularly relevant for studies of ATR. A previous review of the literature indicated that the main limitations of epidemiologic studies relating pregnancy outcomes to ATR include suboptimal exposure assessment and reliance on ecologic rather than individual-level measures (Weselak et al., 2007). To allow a more formal assessment of design strength, each study was assigned to one of three tiers. Tier 1 included studies that relied on individual-level measures for both exposure and outcome. Studies that used aggregate measures of exposure, but individual-level outcome assessment were assigned to Tier 2. Tier 3 included studies that used ecologic measures for both exposure and outcome, and were considered methodologically the weakest.

Once relevant data were compiled, the entire body of literature was organized according to the outcomes of interest. This grouping allowed an evaluation of consistency of findings across studies that addressed the same or similar hypotheses. Qualitatively, all study results were categorized as “positive” if the measures of association were statistically significantly different from the null when comparing the highest to the lowest exposure category or there was evidence of a significant trend. The result was considered “null” if the associations were not statistically significant or if the results were opposite to the hypothesized direction. In those instances when the methods section of an article indicated evaluation of multiple outcomes but the results section reported only selected findings, it was assumed that the nonreported results were null. The results were categorized as “mixed” if a study included several analyses (e.g., using alternative exposure categorization cutoffs) with no consistency across findings.

RESULTS

Overview of the Available Literature

Our search identified 22 epidemiologic studies reporting on the association between ATR or related exposures and various pregnancy outcomes in humans. Of those, 12 studies assessed congenital malformations (Munger et al., 1992; Garry et al., 1996; Meyer et al., 2006; Rull et al., 2006a; Mattix et al., 2007; Weselak et al., 2008; Ochoa-Acuña and Carbajo, 2009; Winchester et al., 2009; Waller et al., 2010; Agopian et al., 2013a, 2013b, 2013c), nine studies examined pregnancy outcomes other than malformations (Munger et al., 1997; Savitz et al., 1997; Arbuckle et al., 2001; Dąbrowski et al., 2003; Villanueva et al., 2005; Ochoa-Acuña et al., 2009; Rinsky et al., 2012; Limousi et al., 2013; Migeot et al., 2013), and one study (Chevrier et al., 2011) included both types of endpoints. Three studies included in the review were conducted in Canada (Savitz et al., 1997; Arbuckle et al., 2001; Weselak et al., 2008), four in France (Villanueva et al., 2005; Chevrier et al., 2011; Limousi et al., 2013; Migeot et al., 2013), one in Poland (Dąbrowski et al., 2003), and the remaining 14 in the United States. Not all studies were entirely independent. The three Canadian studies used data from the

Ontario Farm Family Health Study (Savitz et al., 1997; Arbuckle et al., 2001; Weselak et al., 2008), two French studies (Limousi et al., 2013; Migeot et al., 2013) were based on the same data from the district of Deux-Sèvres in the Poitou-Charentes region, and three case-control studies of birth defects in Texas (Agopian et al., 2013a, 2013b, 2013c) performed analyses of the state birth defects registry. The publication dates ranged from 1992 through 2013 with the most recent paper (Limousi et al., 2013) still in press at the time of this review.

Studies of Congenital Malformations (Birth Defects)

Methods and results of studies assessing the association between ATR and related exposures and congenital malformations (birth defects) are summarized in Table 1. Of the 13 studies in this category, most examined malformations grouped according to organ systems or evaluated all birth defects combined. Only four reports focused on a specific malformation. Of those that did, two studies (Waller et al., 2010; Agopian et al., 2013b) limited their analyses to abdominal wall defects, including gastroschisis and omphalocele, one examined the relation of ATR to hypospadias (Meyer et al., 2006), one (Agopian et al., 2013b) addressed a similar hypothesis for choanal atresia or stenosis, and one (Rull et al., 2006a) focused on neural tube defects (NTDs) and specifically anencephaly and spina bifida.

Studies of birth defects that examined both exposure and outcome at individual levels (Tier 1). Among four studies in this category, only one (Chevrier et al., 2011) used biomarkers of ATR exposure. This case-cohort study was nested within the prospective PELAGIE (Perturbateurs endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance) cohort from the Brittany region of France. Maternal urine samples were examined for pesticide exposure biomarkers before the 19th week of gestation. Exposure in this study population was mainly environmental (i.e., via drinking water) since ATR use in France was limited to 1 kg/ha/year in 1997 and was banned completely in 2003, 2 years following its ban in the EU. The PELAGIE cohort samples were collected between 2002 and 2006. The main analyses compared persons with no ATR or any of its derivatives in the urine (reference category) to those who had detectable levels of ATR or ATR mercapturate (a glutathione-derived metabolite), which are thought to reflect direct exposure. Another, more inclusive, exposure category was comprised of all persons with at least one ATR metabolite, some of which are the products of ATR degradation in the environment and may not result from direct contact (Barr et al., 2007). Birth defects were categorized, as described previously (Garlantezec et al., 2009), as major or minor malformations using the classification scheme from the European Surveillance of Congenital Anomalies (EUROCAT). Only major malformations (combined) were used as the endpoint of interest and the OR (95% CI) estimates were 1.0 (0.3, 3.1) for the directly exposed group (positive for ATR or ATR mercapturate) and 1.2 (0.7, 2.1) for persons with at least one ATR metabolite detected in the urine.

Another study assessing all congenital malformations combined was conducted in Canada (Weselak et al., 2008). The exposure assessment and outcome ascertainment were based on self-reports using questionnaires administered to husbands and wives. Pre- and postconception exposures to triazines, but not specifically ATR, were assessed separately. Exposures were categorized as "any" versus "direct" with the second category defined as applying chemicals to crops or mixing or otherwise handling them around the farm or yard. Using generalized estimating equations models to control for correlated observations, the OR reflecting the association of birth defect affected pregnancies with any pre- or postconception triazine exposures were 0.64 (95% CI, 0.31–1.31) and 1.03 (95% CI, 0.53–2), respectively. The corresponding ORs (95% CIs) for direct exposures were 0.49 (0.21, 1.1) and 0.78 (0.3, 2.01).

Three studies assessed exposure in terms of the types and intensity of agricultural activity around the participants' homes (Meyer et al., 2006; Rull et al., 2006a; Ochoa-Acuña and Carbajo, 2009). In a case-control study conducted in Arkansas, Meyer et al. (2006) compared newborns with hypospadias, identified through a population-based birth defect registry, to two controls matched on maternal race and exhibiting no congenital malformation based on birth certificates. Information on proximity of the maternal residence to specific crops (e.g., corn) was combined with the pesticide use data. The exposure variable was defined as an estimated amount of pesticide (in pounds) applied or persisting within 500 m of each maternal residence during the period from 6 to 16 weeks of gestation. When exposure to ATR was expressed as a continuous variable (per 0.5 lb. applied), the OR was 1.00 (95% CI, 0.98–1.01). In the analyses comparing the lowest ATR exposure level to the moderate and high categories, the ORs (95% CIs) were 0.68 (0.45, 1.04) and 1.02 (0.58, 1.79), respectively.

A similarly designed study conducted in Indiana examined the association between various birth defects and the number of hectares used for corn cultivation within a 500-m radius from the maternal street address (Ochoa-Acuña and Carbajo 2009). There were no specific data on exposure to ATR or any pesticide, either individually or geographically. Exposure to corn, the proxy exposure measure, was used as a continuous variable (per 10-ha increase) or dichotomized using a cutoff of 3.4 ha. The birth defects were categorized according to affected organ systems as abdominal cavity, craniofacial, cardiac, limb, neural tube, other nervous system, respiratory, or urogenital. The only category that demonstrated a statistically significant association with corn crop acreage in proximity to maternal residence was limb defects with an adjusted OR of 1.76 (95% CI, 1.12–2.78) in the analysis that expressed exposure as a binary variable, and 1.22 (95% CI, 1.01–1.47) in the corresponding analyses per 10-ha increase.

Rull et al., (2006a) pooled data from two case-control studies of NTDs (Shaw et al., 1995, 1999) and linked information on maternal residence with reports on pesticide use from the California Department of Pesticide Regulation. For each pesticide, a mother was considered exposed if she resided during the periconceptional period within 1000 m of a crop treated with the agent in question. The

Table 1
Summary of Studies Evaluating the Association between ATR/Triazine Herbicide (or Surrogate) Exposure and Birth Defects

| Study reference location, tier | Outcome definition (N); total sample size | Exposure assessment | Statistical analysis | Control variables | Measure of association (95% CI or <i>p</i> -value) |
|---------------------------------------|---|---|---|--|--|
| Agopian et al. (2013a), Texas Tier 2 | Nonsyndromic choanal atresia or stenosis (N = 280); total N (cases + controls) = 4000 | County-level estimates based on crop type and acreage of ATR use; low: <25th, medium: 25–75th, high: >90th percentile | Logistic regression | Season of conception; infant sex; birth year; and maternal race/ethnicity, education, age, and smoking | Using ATR as continuous variable: OR = 1.49 (1.09–2.04) By category: low: OR = 1.0 (ref) Medium-low: OR = 0.93 (0.68, 1.29) Medium-high: 1.35 (0.90, 2.01) High: OR = 1.79 (1.17, 2.74) |
| Agopian et al. (2013b), Texas Tier 2 | Gastrochisis (N = 1161); total N (cases + controls) = 9551 | County-level estimates based on crop type and acreage of ATR use; low: <25th, medium: 25–75th, high: >90th percentile | Logistic regression | Month of conception, infant sex, birth year, and maternal race/ethnicity, education, age, birthplace, history of previous live births, and smoking | Low: OR = 1.0 (ref) Medium-low: OR = 1.11 (0.94, 1.32) Medium-high: 0.82 (0.64, 1.04) High: OR = 1.22 (0.96, 1.56) |
| Agopian et al. (2013c), Texas Tier 2 | Male genital malformations (N = 16,433); hypospadias (N = 8909), severe (third and fourth degree) hypospadias (N = 738), small penis (N = 670), cryptorchidism (N = 4,324); total N (cases + controls) = 32,866 | County-level acreage of crops for which ATR is used; low: <25th, medium-low: 25–75th, medium: 75–90, high: >90th percentile | Logistic regression | Season of conception, birth year, and maternal race/ethnicity, education, age, history of previous live births, birthplace, and smoking | All male genital malformations Low: OR = 1.0 (ref) Medium-low: OR = 1.19 (1.12, 1.26) Medium: OR = 1.20 (1.11, 1.29) High: OR = 0.96 (0.87, 1.05) Hypospadias Medium-low: OR = 1.07 (1.00, 1.15) Medium: OR = 1.09 (1.00, 1.20) High: OR = 1.00 (0.89, 1.11) Severe hypospadias Medium-low: OR = 1.44 (1.17, 1.77) Medium: OR = 1.18 (0.90, 1.55) High: OR = 1.00 (0.71, 1.41) Small penis: Medium-low: 1.05 (0.86, 1.28) Medium: OR = 1.38 (1.07, 1.77) High: OR = 0.74 (0.52, 1.04) Cryptorchidism Medium-low: 1.17 (1.08, 1.28) Medium: OR = 1.14 (1.01, 1.28) High: OR = 0.93 (0.80, 1.07) |
| Chevrier et al. (2011), France Tier 1 | Major congenital malformations (N = 88); total analytic subcohort, N = 579 | Prenatal urine levels of ATR or metabolites (desethyl ATR, hydroxy ATR, hydroxydesethyl ATR, ATR mercapturate); detectable versus nondetectable | Logistic regression with backward elimination | Year of enrollment, season at conception, mother's occupational exposure to solvents, GA at birth, and simazine exposure | No ATR or metabolites: OR = 1.0 (ref) At least one analyte: OR = 1.2 (0.7, 2.1) ATR or ATR mercapturate (direct exposure): OR = 1.0 (0.3, 3.1) |

Table 1
Continued

| Study reference location, tier | Outcome definition (N); total sample size | Exposure assessment | Statistical analysis | Control variables | Measure of association (95% CI or <i>p</i> -value) |
|--|--|--|-----------------------------|---|--|
| Garry et al. (1996), Minnesota Tier 2 | Birth defects: CNS (N = 248), circulatory / respiratory (N = 439), gastrointestinal (N = 181), urogenital (N = 519), musculoskeletal/integumental (N = 842), chromosomal (N = 374), all anomalies (N = 3,791), combined anomalies (NS); total study, N = 187,512 | County cluster level ATR use (lbs); using different cutoffs for high versus low | Logistic regression | Quote: "including, but not exclusively" maternal age, education, smoking, alcohol consumption, previous miscarriage, and earlier births with anomalies | "Atrazine use showed a significant increase for all birth anomalies (OR = 1.13; CI, 1.04–1.24), and for combined birth anomalies (OR = 1.33; 95% CI, 1.19–1.49) at only one herbicide use level (>100,000 vs. <100,000). At herbicide use levels defined by MDA as high-use areas (>25,000), atrazine did not show a significant increase in birth anomalies compared to low-use areas" *Results for other birth defects examined in this study are not reported, but based on the text were not statistically significant. |
| Mattix et al. (2007), Indiana Tier 3 | 1990–2001 monthly rates of abdominal wall defects: gastroschisis and omphalocele (ecologic study, sample sizes NS) | Monthly surface water ATR levels from US Geological Survey | Correlation analyses | None | Using CDC data Correlation coefficient = 0.6 (<i>p</i> = 0.0392) Using Indiana state registry data Correlation coefficient = 0.69 (<i>p</i> = 0.0125) |
| Meyer et al. (2006), Arkansas Tier 1 | Hypospadias (N = 354); total N (cases + controls) = 1081 | Amount of pesticides within 500 m of each maternal residence during 70-day window 6–16 gestational weeks | Logistic regression | Maternal age and race, paternal education level, weight gain during pregnancy, GA at birth, timing of first prenatal care visit, parity, and number of cigarettes smoked per day during pregnancy | Per 0.5 lb ATR applied OR = 1.00 (0.98, 1.01) Ordinal: Low (0 lb): OR = 1.0 (ref) 0 to <3.6 lb: OR = 0.68 (0.45, 1.04) ≥3.6 lb: OR = 1.02 (0.58, 1.79) |
| Munger et al. (1992), Iowa Tier 3 | 1983–1989 rates of birth defects:* All defects, cardiac defects, urogenital defects, limb reduction defects (ecologic study, sample size NS) | Residence in 18 communities with ATR contamination: yes versus no | Rate ratio (RR) calculation | None | All defects: RR = 2.6 (2.0–3.4) Cardiac defects: RR = 3.1 (2.1–4.6) Urogenital defects: RR = 3.5 (2.2–5.3) Limb reduction defects: RR = 6.9 (4.2–11.0) |
| Ochoa-Acuña and Carbajo (2009), Indiana Tier 1 | *List not available Defects of abdominal cavity (N = 39), craniofacial (N = 110), heart (N = 114), limb (N = 105), neural tube (N = 9), other nervous system | Number of hectares (ha) of cornfields within 500 meters of home | Logistic regression | Not specified (after backward elimination) | Abdominal cavity <3.4 versus ≥3.4 ha: OR = 1.50 (0.72, 3.10) Per 10 ha corn: OR = 0.84 (0.57, 1.25) Craniofacial <3.4 versus ≥3.4 ha: OR = 1.36 (0.88, 2.10) Per 10 ha corn: OR = 1.10 (0.89, 1.33) |

Table 1
Continued

| Study reference location, tier | Outcome definition (N); total sample size | Exposure assessment | Statistical analysis | Control variables | Measure of association (95% CI or <i>p</i> -value) |
|---|--|--|---|--|---|
| | (<i>N</i> = 31), respiratory (<i>N</i> = 107), urogenital (<i>N</i> = 146); total study (for corn exposure), <i>N</i> = 24,108 | | | | Heart <3.4 versus ≥3.4 ha: OR = 1.26 (0.82, 1.93) Per 10 ha corn: OR = 1.11 (0.91, 1.35) Limb <3.4 versus ≥3.4 ha: OR = 1.76 (1.12, 2.78) Per 10 ha corn: OR = 1.22 (1.01, 1.47) Neural tube <3.4 versus ≥3.4 ha: OR = 3.57 (0.62, 20.5) Per 10 ha corn: OR = 1.02 (0.51, 2.03) Other nervous system <3.4 versus ≥3.4 ha: OR = 0.91 (0.40, 2.08) Per 10 ha corn: OR = 1.03 (0.69, 1.54) Respiratory <3.4 versus ≥3.4 ha: OR = 1.40 (0.88, 2.23) Per 10 ha corn: OR = 1.06 (0.87, 1.30) Urogenital <3.4 versus ≥3.4 ha: OR = 0.98 (0.67, 1.44) Per 10 ha corn: OR = 0.83 (0.67, 1.03) Any NTD: OR = 0.9 (0.4, 1.8) Anencephaly: OR = 1.3 (0.5, 3.2) Spina bifida: OR = 0.6 (0.2, 1.6) |
| Rull et al. (2006a), California Tier 1 | NTDs: any NTD (<i>N</i> = 731), anencephaly (<i>N</i> = 307), and spina bifida (<i>N</i> = 390); Total <i>N</i> (cases + controls) = 1671 | Triazine/triazole use within 1000 m of maternal residence during periconceptional period | Logistic regression; binary for any NTDs, polytomous for anencephaly and spina bifida | Study population, time of delivery, maternal education, ethnicity, periconceptional smoking and vitamin use, and the uncategorized (other) pesticides and physiochemical categories listed | |
| Waller et al. (2010), Washington State Tier 2 | Gastrochisis (<i>N</i> = 805); total, <i>N</i> (cases + controls) = 4421 | Residential proximity to the closest geographic site with surface water level of >3 µg/l | Logistic regression | For continuous log-distance: maternal age, parity, smoking status, and season of conception for ordinal categories: same but without season of conception | Using log-transformed distance as continuous variable: OR = 0.80 (0.63–1.01) For ordinal categories: >50 km: OR = 1.0 (ref) 25–50 km: OR = 1.41 (1.19–1.66) <25 km: OR = 1.60 (1.10–2.34) All offspring |
| Weselak et al. (2008), Canada Tier 1 | All birth defects (<i>N</i> = 108), total study, <i>N</i> = 3412 | Self-reported exposure to triazines 3 months pre-conception and 3 months postconception: yes versus no | Generalized estimated equations | None for any farm use; income and father's education level for direct pre-conception activity; maternal weight gain during pregnancy and number of years mother lived on the farm before the pregnancy for direct postconception | Any preconception use: OR = 0.64 (0.31, 1.31) Any postconception farm use: OR = 1.03 (0.53, 2.00) Direct pre-conception activity: OR = 0.49 (0.21, 1.10) Direct postconception activity: OR = 0.78 (0.30, 2.01) |

Table 1
Continued

| Study reference location, tier | Outcome definition (N); total sample size | Exposure assessment | Statistical analysis | Control variables | Measure of association (95% CI or <i>p</i> -value) |
|--|--|--|--|--|--|
| Winchester et al. (2009), US nationwide Tier 3 | All birth defects, spina bifida/meningocele; hydro-/microcephalus; other CNS; heart and other circulatory and respiratory; rectal atresia/stenosis; omphalocele/gastroschisis; other GI anomalies; malformed genitalia and other urogenital anomalies; cleft lip/palate; adactyly polydactyly, syndactyly; diaphragmatic hernia; Down syndrome and other chromosomal anomalies; musculoskeletal; other defects; TEF; ecologic study, sample sizes NS | Monthly surface water ATR between 1996 and 2002 "modeled with the seasonal factor using multivariate regression models" **No other explanation provided | Linear regression of log-odds of birth defects* versus ATR exposure *No explanation of log-odds calculations provided Nonspecified logistic regression | For linear regression—none For logistic regression—"maternal risk factors, maternal demographics and years" plus levels of nitrate and other pesticides *No other explanation provided | Linear regression All defects combined: $\beta = 0.0115, p < 0.001$ Logistic regression Spina bifida: OR = 1.018 (0.988, 1.050) Circulatory: OR = 1.006 (0.994, 1.018) TEF: OR = 1.016 (0.978, 1.056) GI: OR = 1.024 (0.999, 1.051) Uro/Gen: OR = 1.007 (0.994, 1.021) Cleft lip: OR = 1.024 (1.009, 1.040) Poly/syn/adactyly: OR = 1.023 (1.007, 1.039) Club foot: OR = 1.014 (0.995, 1.033) Musc/Skel: OR = 1.008 (0.999, 1.018) Down syndrome: OR = 1.027 (1.005, 1.049) Other NS: OR = 1.011 (1.002, 1.025) *Results for other birth defects examined in this study are not reported, but based on the text were not statistically significant |

OR, odds ratio; CI, confidence interval; CNS, central nervous system; Uro/Gen, urogenital; Musc/Skel, muscular and skeletal; Chrom, chromosomal; GI, gastrointestinal; TEF, tracheoesophageal fistula; NS, not specified.

time interval of interest included the calendar month of conception and the month after conception. In the analyses evaluating exposures to triazines/triazoles, all results were null with adjusted OR of 0.9 (95% CI, 0.4–1.8) for any NTD, 1.3 (95% CI, 0.5–3.2) for anencephaly, and 0.6 (95% CI, 0.2–1.6) for spina bifida (Rull et al., 2006a). No results were available specifically for ATR.

Studies of birth defects that examined aggregate data (Tiers 2 and 3). Several studies were based on individual-level outcome ascertainment, but relied on ecologic (usually area-based) exposure assessment. Three case-control studies conducted in Texas used essentially the same design in evaluating the relation of residential ATR exposure to different birth defects (Agopian et al., 2013a, 2013b, 2013c). All three studies assessed ATR exposure by linking county-level data for pesticide use and crop acreages to maternal county of residence at delivery and delivery year. The congenital malformations of interest in these three studies were, choanal atresia or choanal stenosis (Agopian et al., 2013a), abdominal wall defects (Agopian et al., 2013b) and various male genital defects (Agopian et al., 2013c). All cases were ascertained from the Texas Birth Defects Registry and controls were selected from live-born infants without congenital malformations. ATR exposures in all analyses were categorized as low (<25th percentile), medium (25–75th percentile), medium-high (75–90th percentile), and high (>90th percentile). In the study of gastroschisis (Agopian et al., 2013b), there was no statistically significant association with medium-low, medium, and high category-specific adjusted ORs of 1.11 (95% CI, 0.94–1.32), 0.82 (95% CI, 0.64–1.04), and 1.22 (95% CI, 0.96–1.56), respectively. After stratification on maternal age (<25 vs. ≥25 years), the association was statistically significant (OR = 1.97; 95% CI, 1.19–3.26) among older women in the highest ATR exposure category. The authors justified their maternal age analysis based on the higher incidence of gastroschisis in infants born to younger women and the possibility that an ATR-associated risk would be more difficult to detect against a higher age-related background. In the another study conducted by the same group and using the same methods (Agopian et al., 2013a), there was a statistically significant positive relationship between choanal atresia and ATR exposure category when the highest level of exposure (>90th percentile) was compared with the first quartile (OR = 1.79; 95% CI, 1.17–2.74) with evidence of a linear trend ($p = 0.002$). The third case-control study in this series (Agopian et al., 2013c) focused on male genital abnormalities. There was no evidence of a dose response relation for all defects combined or separately for hypospadias, small penis, and cryptorchidism. The OR in the highest relative to the lowest ATR category ranged between 0.74 and 1 across all analyses. The authors posited that their findings could be explained by an inverted U-shaped dose-response relationship. The studies using the Texas Birth Defects Registry did not indicate whether the reported results were chosen from a larger set of investigated associations involving other specific birth defects.

Waller et al. (2010) used Washington state birth certificates to conduct a case-control study that examined the association between gastroschisis and proximity of the participants' zip code of residence to a point source

known to have an elevated (>3 $\mu\text{g}/\text{l}$) concentration of ATR in surface water. When the persons residing >50 km away from a high ATR concentration site were compared with those living within the 25 to 50 km and <25 km radius, the OR (95% CIs) were 1.41 (1.19, 1.66) and 1.60 (1.10, 2.34), respectively. In the analyses that used log-transformed distance to a high ATR site as a continuous variable, the inverse association with gastroschisis (OR = 0.8) did not reach statistical significance. The rationale for using 25- and 50-km cut points was not provided.

In a study of birth defects conducted in rural Minnesota, Garry et al. (1996) linked data on children of licensed, male private pesticide applicators to the state birth defects registry. The authors reported that females constituted less than 1% of pesticide applicators and were not considered in the study. Each birth was characterized with respect to paternal exposure to various pesticides, including ATR. Pesticide exposure was ascertained from a database containing county cluster-level information on crop acreage and amount of pesticide applied, and each exposure variable was dichotomized using different outcomes. The categories of birth defects included those involving central nervous, circulatory/respiratory, gastrointestinal, urogenital, and musculoskeletal/integumental systems. Separate analyses evaluated chromosomal abnormalities, all congenital malformations taken together, and combinations of birth defects. The full set of results was not reported. ATR use was associated with significant increase in all birth anomalies (OR = 1.13; CI, 1.04–1.24) and in combined birth anomalies (OR = 1.33; 95% CI, 1.19–1.49), but only at one cutoff level (>100,000 vs. <100,000 lb). Based on the Minnesota Department of Agriculture definition (>25,000 lb), high ATR use areas did not show a significant change in birth anomalies compared with low-use areas.

Three studies used aggregate-level data for both exposure and outcome (Tier 3). The earliest of available reports in this category (Munger et al., 1992) presented an analysis comparing rates of birth defects in 18 communities with elevated levels of ATR in surface water to all other, presumably less-exposed (2.2 vs. 0.6 $\mu\text{g}/\text{liter}$) communities in Iowa. The rate ratio (the authors used the term "relative risk") was 2.6 (95% CI, 2.0–3.4) for all congenital malformations, 3.1 (95% CI, 2.1–4.6) for cardiac abnormalities, 3.5 (95% CI, 2.2–5.3) for urogenital defects, and 6.9 (95% CI, 4.2–11.0) for limb reduction. This study was published as an abstract, and did not appear to be followed up, at least with respect to birth defects, with a peer-reviewed publication. The details of methods and results remain unclear.

In another study that used aggregate data, Mattix et al. (2007) performed an ecologic analysis evaluating monthly rates of abdominal wall defects in Indiana using data from the Centers for Disease Control and Prevention and from the Indiana State Department of Health. The rates of abdominal wall defects were then compared with the average monthly concentrations of ATR in surface water in Indiana; and the resulting correlation coefficients using the Centers for Disease Control and Prevention and the Department of Health data were 0.6 ($p = 0.04$) and 0.69 ($p = 0.01$), respectively (Mattix et al., 2007).

Study characteristics:

Tier 1: Individual-level exposure (E) and outcome (O)

- E: prenatal urine levels ; O: medical records (Chevrier et al. 2011)
- E: nearby pesticide application; O: registry (Meyer et al. 2006)
- E: nearby crop use; O: registry (Ochoa-Acuna & Carbajo 2009)
- E: nearby pesticide application; O: medical records (Rull et al. 2006)
- E: questionnaire; O: questionnaire (Weselak et al. 2008)

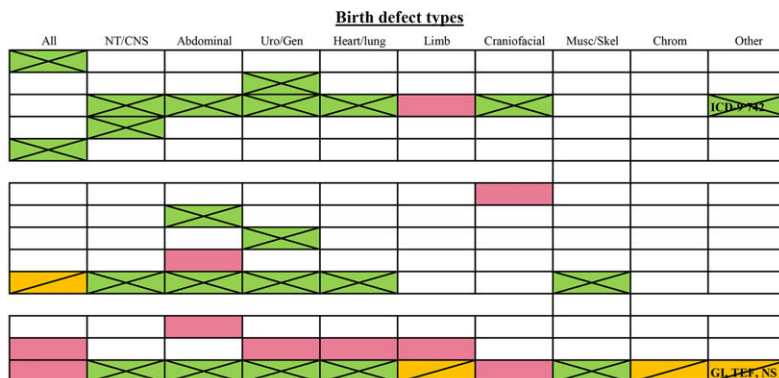
Tier 2: Ecologic exposure and individual-level outcome

- E: county-level use; O: registry (Agopian et al. 2013a)
- E: county-level use; O: registry (Agopian et al. 2013b)
- E: county-level use; O: registry (Agopian et al. 2013c)
- E: zip code proximity to source; O: registry (Waller et al. 2010)
- E: county cluster-level use; O: registry (Garry et al. 1996)

Tier 3: Ecologic exposure and outcome

- E: monthly water levels; O: monthly rates (Mattix et al. 2007)
- E: community type; O: community rates (Munger et al. 1992)
- E: monthly water levels; O: monthly rates (Winchester et al. 2009)

* Three analyses based on the same data (Texas Birth Defects Registry linked with United States Geological Survey)



Acronyms and abbreviations:

NT/CNS= neural tube and central nervous system, Uro/Gen = urogenital; Musc/Skel=muscular and skeletal; Chrom=chromosomal; ICD-9-CM 742 = "other" congenital abnormalities of the nervous system; GI=gastrointestinal; TEF=tracheoesophageal fistula, NS= other not specified

Legend:

- Positive result
- X null result
- mixed results
- No data

Fig. 1. Overview of study findings on the associations between ATR/triazine herbicide (or surrogate) exposures and birth defects.

Winchester et al. (2009) extended the Mattix et al. (2007) analyses by conducting a national ecologic study of the association between various categories of birth defects and surface water concentrations of agrichemicals. The study used 22 birth defect categories (listed in Table 1), but results were presented for only a subset of outcomes. Elevated OR per log increase in ATR concentration were observed for cleft lip, adactyly, Down syndrome, and "other" defects. For all other categories, the results were either not significantly different from the null or not mentioned (Winchester et al., 2009).

Evaluation of consistency of studies of birth defects. As shown in Figure 1, few studies examined the same or similar outcomes. The most commonly addressed endpoints were all congenital malformations combined (five studies), abdominal abnormalities (six studies), and urogenital defects (six studies). None of these three outcome categories demonstrated consistent positive associations across studies. For other types of birth defects an assessment of consistency is difficult due to the few observations. Figure 1 also shows that most positive results (7 of 10) were reported in studies that used aggregate data to characterize both the exposure and outcome (Tier 3). In contrast, most associations in studies of individual-level data (Tier 1) were consistent with the null hypothesis.

Studies of Pregnancy Outcomes Other Than Birth Defects

Table 2 summarizes studies that examined the association of ATR and related exposures with various pregnancy outcomes other than birth defects. Most of the 10 studies in this category focused on outcomes of live births, although two studies (Savitz et al., 1997; Arbuckle et al., 2001) evaluated the relation of ATR to pregnancy loss defined as spontaneous abortion or miscarriage. In two

studies, Munger et al. (1997) and Chevrier et al. (2011) examined "fetal growth restriction" or "intrauterine growth retardation" as the outcomes of interest, but defined these conditions using diagnostic criteria for SGA in a newborn, rather than the prenatal diagnosis of a fetus, as currently recommended (American College of Obstetricians and Gynecologists, 2001; Zhang et al., 2010). For those studies the term "SGA" is used for consistency.

Studies of pregnancy outcomes that examined only individual-level data (Tier 1). The case-cohort study by Chevrier et al. (2011) assessed the relation of ATR exposure biomarkers to various pregnancy outcomes, which, in addition to birth defects, included SGA (called "fetal growth restriction" by the authors) and small head circumference. Both SGA and small head circumference were defined as values that were below the 5th percentile for French population reference data (Chevrier et al., 2011). As in the birth defect analyses, the reference categories included subjects with no ATR or any of its derivatives in the urine and the two comparison groups were defined as direct exposure (detectable levels of ATR or ATR mercapturate) or any exposure (at least one of several ATR metabolites found in the urine). In the SGA analyses, the OR (95% CI) estimates were 1.6 (0.8–3.1) for the directly exposed group and 1.5 (1.0, 2.2) for individuals with any exposure. The corresponding OR (95% CI) in the small head circumference analyses were 1.3 (0.5, 3.3) and 1.7 (1.0, 2.7). When BW, birth length, and head circumference were used as continuous variables, only BW was statistically significantly associated with direct ATR exposure (β coefficient = -151; 95% CI, -293 to -9).

Two studies evaluated the relationship between triazine exposures and pregnancy outcomes in Canada (Savitz et al., 1997; Arbuckle et al., 2001). Both studies used data from the Ontario Farm Family Health Study. Data collection methods were the same as in the previously cited study of congenital malformations (Weselak

Table 2
Summary of Studies Evaluating the Association between ATR/Triazine Herbicide (or Surrogate) Exposure and Pregnancy Outcomes Other Than Birth Defects

| Study reference, location, tier | (N); total sample size | Exposure assessment | Statistical analysis | Control variables | Measure of association (95% CI or p-value) |
|---|---|--|--|---|---|
| Arbuckle et al. (2001), Canada Tier 1 | Spontaneous abortion (N = 395); total study N = 3936 | Self-reported exposure to ATR 3 months preconception and 3 months postconception: binary | Logistic regression | None | Preconception exposure: All GAs: OR = 1.2 (0.9, 1.7) <12 weeks gestation: OR = 1.3 (0.8, 2.0) 12-19 weeks gestation: OR = 1.1 (0.7, 1.9) Postconception exposure: All GAs: OR = 0.8 (0.5, 1.2) <12 weeks gestation: OR = 0.7 (0.3, 1.5) 12-19 weeks gestation: OR = 0.8 (0.4, 1.6) SGA: No ATR or metabolites: OR = 1.0 (ref) At least one analyte: OR = 1.5 (1.0, 2.2) ATR or ATR mercapturate (direct exposure): OR = 1.6 (0.8, 3.1) SHC: At least one analyte: OR = 1.7 (1.0, 2.7) Direct exposure: OR = 1.3 (0.5, 3.3) BW: At least one analyte: $\beta = -57$ (-130, 17) Direct exposure: $\beta = -151$ (-293, -9) BL: At least one analyte: $\beta = -0.22$ (-0.5, 0.1) Direct exposure: $\beta = -0.44$ (-1.1, 0.2) HC: At least one analyte: $\beta = -0.18$ (-0.4, 0.1) Direct exposure: $\beta = -0.39$ (-0.9, 0.1) |
| Chevrier et al. (2011), France Tier 1 | SGA newborn weight* (N = 178), and small head circumference (SHC, N = 103), each defined as less than percentile, BW, birth lengths (BL) and head circumference (HC)—continuous variables; total analytic subcohort, N = 579 *The authors used term "fetal growth restriction" | Prenatal urine levels of ATR or metabolites (desethylatrazine, hydroxyatrazine, hydroxydesethylatrazine, ATR mercapturate) | Logistic regression with backward elimination for FGR and SHC, linear regression for BW, BL, and HC | For SGA: smoking status at enrollment, high blood pressure before/during pregnancy, thawing and refreezing process, and acetochlor exposure For SHC: residence district, alcohol consumption at enrollment (except for the models on dealkylated and hydroxylated metabolites), thawing and refreezing process, cesarean delivery, and parity For BW: year of enrollment, education level, smoking status at enrollment, high blood pressure before/during pregnancy, thawing and refreezing process, prepregnancy BMI, child's sex, shellfish intake (except for the models on dealkylated and hydroxylated metabolites), GA at birth, squared GA, and alachlor exposure For BL: year of enrollment, smoking status at enrollment, high blood pressure before/during pregnancy, season at conception, prepregnancy BMI, child's sex, shellfish intake, GA at birth, squared GA, and alachlor exposure For HC: residence district, smoking status at enrollment, prepregnancy BMI, child's sex, cesarean delivery, parity, shellfish intake, GA at birth, squared GA, and simazine exposure | |
| Dabrowski et al. (2003), Poland Tier 1 | BW, and PD—continuous variables; total study, N = 494 | Self-reported exposure to triazines | Not specified comparison of observed and expected values (expected apparently based on data in the nonexposed) | None | BW: Obs-Exp = -259 gm (p = 0.433) PD Obs-Exp = -2 weeks (p = 0.177) |

Table 2
Continued

| Study reference, location, tier | (N); total sample size | Exposure assessment | Statistical analysis | Control variables | Measure of association (95% CI or p-value) |
|--|---|---|---|--|--|
| Limousi et al. (2013), France Tier 2 | SGA newborn weight (N = 914); total study, N = 10,784 | Presence of ATR in community water supply | Nitrate-specific crude association | None | Result for ATR stratified by nitrite levels: Nitrite* tertile 1: OR = 1.05 (0.83, 1.31) Nitrite tertile 2: OR = 0.72 (0.50, 1.03) Nitrite tertile 3: OR = 0.80 (0.60, 1.08) *Calculated by MG |
| Migeot et al. (2013), France Tier 2* | SGA newborn weight (N = 827); total study (adjusted analysis), N = 3346 | Presence of ATR in community water supply | Nitrate-specific crude association | None | Result for ATR stratified by nitrite levels: Nitrite* tertile 1: OR = 1.14 (0.89, 1.47) Nitrite tertile 2: OR = 0.80 (0.55, 1.17) Nitrite tertile 3: OR = 0.83 (0.71, 0.98) *Calculated by MG |
| *Used the same data as Limousi et al. (2013) Munger et al. (1997), Iowa Tier 3 | Rates of SGA (ecologic study, sample size NS) newborn weight* by community *Authors used term "intrauterine growth retardation" defined as <10th percentile BW; total sample size NS | Community-level concentrations of ATR in drinking water | Linear regression | Community-level percent of women that smoked and had no date of last menstrual period recorded | β -coefficient = 1.80 Standardized β = 0.32 (p = 0.001) |
| Ochoa-Acuña et al. (2009), Indiana Tier 2 | Preterm delivery (N = 1777) and SGA (N = 3172) newborn weight; total study, N = 24,154 | Drinking water ATR concentrations calculated by linear interpolation between sampling dates | Log-binomial models; results expressed as PRs | Mother's ethnicity, level of education, month prenatal care began, smoking status, and quarter of the year in which baby was conceived | Preterm delivery first month: <25th percentile: PR = 1.0 (ref) 25-75th percentile: PR = 0.98 (0.87, 1.11) >75th percentile: PR = 1.07 (0.93, 1.22) Preterm delivery last month: <25th percentile: PR = 1.0 (ref) 25-75th percentile: PR = 1.04 (0.93, 1.18) >75th percentile: PR = 0.87 (0.72, 1.04) SGA third trimester <25th percentile: PR = 1.0 (ref) 25-75th percentile: PR = 1.19 (1.08-1.32) >75th percentile: PR = 1.17 (1.03-1.34) SGA entire pregnancy <25th percentile: PR = 1.0 (ref) 25-75th percentile: PR = 1.06 (0.98-1.15) >75th percentile: PR = 1.14 (1.03-1.24) |
| Rinsky et al. (2012), Kentucky Tier 2 | Preterm delivery (N = 8915); total study N = 71,768 | County-level drinking water ATR concentrations | Logistic regression | Maternal age, race/ethnicity, education, smoking status, and prenatal care | Method 1 (\leq LOD = 0 μ g/l) Low: OR = 1.0 (ref) Moderate: OR = 0.94 (0.89, 1.00) High: OR = 1.22 (1.16, 1.29) Method 2 (\leq LOD = 1/2 LOD of test) Low: OR = 1.0 (ref) Moderate: OR = 0.90 (0.85, 0.95) High: OR = 1.20 (1.14, 1.27) Method 3 (\leq LOD = 1/2 lowest LOD) Low: OR = 1.0 (ref) Moderate: OR = 1.02 (0.96, 1.09) High: OR = 1.26 (1.19, 1.32) |

Table 2
Continued

| Study reference, location, tier | (N); total sample size | Exposure assessment | Statistical analysis | Control variables | Measure of association (95% CI or p-value) |
|---|---|---|----------------------|---|--|
| Savitz et al. (1997), Canada Tier 1 | Miscarriage (N = 395), preterm delivery (N = 128), and SGA (N = 276) newborn weight; total study, N = 3427 | Self-reported paternal farm exposure to triazines and ATR | Logistic regression | <p>For miscarriage: mother's age, parity, mother's and father's education, income and off-farm work, mother's smoking and alcohol use, and conception to interview interval</p> <p>For preterm delivery: mother's age and ethnicity, mother's and father's education, income, mother's off-farm job, mother's smoking and caffeine use, primary language, and month of conception</p> <p>For SGA same as above plus father's age, parity, father's off-farm job, father's cigarette smoking and alcohol use during pregnancy, mother's alcohol use, child's sex, and conception to interview interval</p> | <p>For miscarriage: Crop triazines: OR = 1.4 (0.9, 2.2) Crop ATR: OR = 1.5 (0.9, 2.4) Yard triazines: OR = 1.4 (0.8, 2.3) Yard ATR: OR = 1.2 (0.6–2.3)</p> <p>For preterm delivery: Crop triazines: OR = 1.5 (0.6, 3.8) Crop ATR: OR = 2.4 (0.8, 7.0) Yard triazines: OR = 3.2 (1.2, 8.9) Yard ATR: OR = 4.9 (1.6–15.0)</p> <p>For SGA Crop triazines: OR = 0.8 (0.4, 1.5) Crop ATR: OR = 0.5 (0.2, 1.3) Yard triazines: OR = 0.7 (0.3, 1.5) Yard ATR: OR = 0.5 (0.2–1.5)</p> |
| Villanueva et al. (2005), France Tier 2 | LBW—less than 2500 gm (N = 163); Preterm delivery—GA < 37 weeks (N = 137); SGA newborn weight—<10th percentile for GA (N = 241); total study N = 3510 | Community water levels of ATR | Logistic regression | <p>Maternal age, sex of the newborn, and percentage of samples below the detection limit from May to September</p> | <p>For preterm delivery: Raw water T1: OR = 1.0 (ref) Raw water T2: OR = 1.34 (0.84 to 2.14) Raw water T3: OR = 1.15 (0.62 to 2.13) Treated water T2: OR = 1.22 (0.73 to 2.06) Treated water T3: OR = 1.93 (0.85 to 4.35)</p> <p>For LBW: Raw water T2: OR = 0.70 (0.44 to 1.12) Raw water T3: OR = 0.78 (0.45 to 1.36) Treated water T2: OR = 1.29 (0.77 to 2.15) Treated water T3: OR = 0.92 (0.45 to 1.86)</p> <p>For SGA raw water T2: OR = 0.81 (0.56 to 1.18) Raw water T3: OR = 0.65 (0.41 to 1.03) Treated water T2: OR = 0.96 (0.62 to 1.48) Treated water T3: OR = 0.97 (0.53 to 1.79)</p> |

OR, odds ratio; CI, confidence interval; LOD, limit of detection; SHC, small head circumference; LBW, low birth weight; SGA, small for gestational age; BW, birth weight (continuous); PD, pregnancy duration (continuous).

et al., 2008) and based on self-reports using questionnaires administered to husbands and wives. Savitz et al. (1997) examined exposures to ATR and triazines in relation to three outcomes: miscarriage, preterm delivery, and SGA BW. All paternal exposures were categorized as encountered while working with crops or in the yard. Of the 12 reported measures of association (three outcomes \times two categories of chemicals \times two circumstances of exposure), only two demonstrated a statistically significant association. Yard (but not crop) ATR and triazine exposures were found to be related to premature delivery with OR of 3.2 (95% CI, 1.2–8.9) and 4.9 (95% CI, 1.6–15.0), respectively (Savitz et al., 1997). The second Canadian study (Arbuckle et al., 2001) focused on spontaneous abortions and assessed women's exposure to triazines, ATR, and various other pesticides, separately pre- and postconception. Compared with unexposed pregnancies, those with reported preconception exposures to ATR had an OR of 1.2 with a 95% CI from 0.9 to 1.7. The same analysis for postconception exposure produced an OR of 0.8 (95% CI, 0.5–1.2). The results for all triazines combined were essentially the same.

Another study that relied on self-reports to assess exposure to triazines considered as a group was conducted in Poland (Dąbrowski et al., 2003). Women who gave birth at 1 of 25 maternity hospitals in the central region of the country were asked to respond to a questionnaire that was administered by a physician 1 to 2 days after delivery. The questionnaire included items on pesticide use. The outcomes of interest were BW and pregnancy duration, and the analyses compared observed values for each outcome to those expected based on the data for unexposed women (the details of these calculations were not provided). For triazines, the difference in BW was -259 gm, and the difference in pregnancy length was -2 weeks. These differences were not statistically significant.

Studies of pregnancy outcomes that examined aggregate data (Tiers 2 and 3). This category includes six studies that evaluated area-based measures of ATR in the water supply (Munger et al., 1997; Villanueva et al., 2005; Ochoa-Acuña et al., 2009; Rinsky et al., 2012; Limousi et al., 2013; Migeot et al., 2013). Three of those studies were conducted in France. Villanueva et al. (2005) assessed the association between ATR concentration in the municipal drinking water in the district of Finistère and three pregnancy outcomes: low birth weight (BW <2500 gm), preterm delivery (GA <37 weeks), and SGA status (<10 th percentile), all obtained from the infants' health records (Villanueva et al., 2005). ATR levels were assessed separately for raw and treated water and divided into tertiles. None of the analyses demonstrated a significant association between ATR and endpoints of interest. Using the first tertile as reference, the OR for the highest tertiles ranged from 0.7 to 1.9 with all 95% CIs including unity. Two more recent, but similarly designed, French studies conducted in the district of Deux-Sèvres in the Poitou-Charentes region examined the relationship between the concentrations of ATR in municipality water and SGA (Limousi et al., 2013; Migeot et al., 2013). The results in both papers are presented for the association between SGA and water nitrates stratified on ATR detection (yes vs. no). For the purposes of the present review, the

association between ATR and SGA was examined using data provided in the papers. Nitrate tertile-specific ORs (95% CI) for the first study (Migeot et al., 2013) were 1.14 (0.89, 1.47), 0.80 (0.55, 1.17), and 0.83 (0.71, 0.98). The corresponding ORs (95% CI) for the second study (Limousi et al., 2013) were 1.05 (0.83, 1.31), 0.72 (0.50, 1.03), and 0.8 (0.6, 1.08).

Two studies linking aggregate measures of ATR concentration in the water supply to individual-level pregnancy outcome data were conducted in the United States (Ochoa-Acuña et al., 2009; Rinsky et al., 2012). Ochoa-Acuña et al. (2009) obtained community water systems information on ATR concentrations from the Indiana Department of Environmental Management and ascertained cases of preterm delivery (<37 weeks GA) and SGA (<10 th percentile BW) from the state Birth Records Database. Exposure variable was based on the linear interpolation between sampling dates converted into an average ATR concentration for different gestational months and trimesters and for the entire pregnancy. The prevalence ratios (PRs) were calculated for preterm delivery in relation to ATR exposure in the first and the last months of pregnancy and for SGA in relation to average water ATR concentrations in the third trimester and throughout pregnancy (Ochoa-Acuña et al., 2009). None of the results for preterm delivery were appreciably different from the null. SGA analyses produced statistically significant measures of association comparing low (<25 th percentile) to high (>75 th percentile) categories of ATR exposure; for third trimester the PR was 1.17 (95% CI, 1.03–1.34) and for the entire pregnancy the PR was 1.14 (95% CI, 1.03–1.24). A similar study conducted in Kentucky linked county-level ATR concentration in public water supplies with preterm delivery data from birth certificates (Rinsky et al., 2012). Exposure was categorized as low, moderate, or high using three different methods of handling values below the limit of detection (LOD)—by substituting $<LOD$ with zero, by assigning the lowest laboratory-specific LOD value to all $<LOD$ samples, and by using one-half of the lowest laboratory-specific LOD. High exposure categories were significantly associated with premature delivery regardless of LOD coding with adjusted OR ranging from 1.2 (95% CI, 1.14–1.27) to 1.26 (95% CI, 1.19–1.32).

An ecologic study in Iowa (Munger et al., 1997) examined the association between ATR concentration in different communities served by the Rathbun water system and the corresponding rates of SGA (called "intrauterine growth retardation" by the authors), defined as <10 th percentile BW using California standards. Overall, the Rathbun communities had a greater proportion of live births with SGA compared with communities with other sources of water (relative risk 1.8; 95% CI, 1.2–2.6). In the linear regression models using community as the unit of analysis, the β coefficient for ATR was significant at 1.8.

Evaluation of consistency of studies of pregnancy outcomes other than birth defects. As shown in Figure 2, the most commonly addressed endpoints in this group of studies were SGA (six reports) and premature delivery (four reports). As with data for birth defects, none of the outcome categories demonstrated

Study characteristics:

Tier 1: Individual-level exposure (E) and outcome (O)

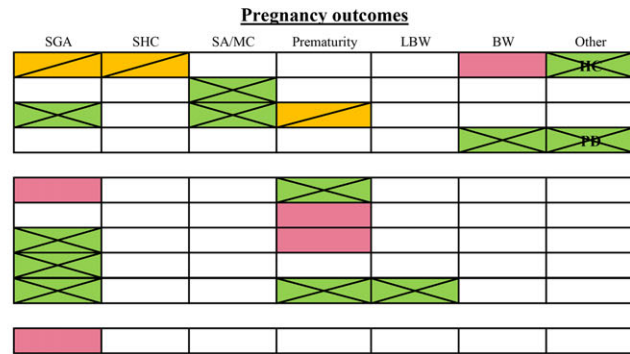
- E: prenatal urine levels ; O: medical records (Chevrier et al. 2011)
- E: questionnaire; O: questionnaire (Arbuckle et al. 2001)*
- E: questionnaire; O: questionnaire (Savitz et al. 1997)*
- E: questionnaire; O: questionnaire (Dabrowski et al. 2003)

Tier 2: Ecologic exposure and individual-level outcome

- E: community water levels; O: registry (Ochoa-Acuna et al. 2009)
- E: county water levels; O: registry (Rinsky et al. 2012)
- E: community water levels; O: health records (Limousi et al. 2013)**
- E: community water levels; O: health records (Migeot et al. 2013)**
- E: community water levels; O: health records (Villanueva et al. 2005)

Tier 3: Ecologic exposure and outcome

- E: community water levels; O: community rates (Munger et al. 1997)



* Two analyses based on the same data (Ontario Farm Family Health Study)

** Two analyses based on the data (French study conducted in the district of Deux-Sèvres)

Acronyms and abbreviations: SGA= small for gestational age; SHC=small head circumference; SA=spontaneous abortion; MC=miscarriage; LBW=low birth weight; BW=birth weight (continuous); HC= head circumference (continuous); PD=pregnancy duration (continuous)

Legend: Positive result (pink), null result (green with X), mixed results (yellow with diagonal lines), No data (white)

Fig. 2. Overview of study findings on the associations between ATR/triazine herbicide (or surrogate) and pregnancy outcomes other than birth defects.

consistent positive associations across studies. For most outcomes, a weight of evidence assessment was not possible because they were examined in only one or two studies.

DISCUSSION

The epidemiologic literature on the association between ATR and pregnancy outcomes has increased over time. The present review incorporated 21 studies published between January 1992 and May 2013. Of those, five papers were published in the first 10 years (1992–2001), 11 in the second decade (2002–2011), and 5 in just 16 months since January 2012. Despite reasonably many studies, a formal meta-analysis was not feasible because the methodology and the presentation of findings were too heterogeneous to allow meaningful pooling of results. Some of the studies used individual-level data and some were ecologic, some reported results for ATR and some of all triazines combined, some calculated measures of association and some mentioned their findings in the text without providing numeric values. More importantly, even if study results could be pooled, there was a good reason not to conduct a meta-analysis. As noted in the current edition of The Cochrane Collaboration handbook “meta-analyses of studies that are at risk of bias may be seriously misleading. If bias is present in each (or some) of the individual studies, meta-analysis will simply compound the errors, and produce a wrong result that may be interpreted as having more credibility” (Higgins and Green, 2011). As discussed below, this consideration certainly applies to the current ATR literature.

Despite the growing body of literature, data remain of limited quality with most (13 of 21) studies using aggregate rather than individual-level information. There appears to be an agreement that exposure assessment is perhaps the most challenging aspect of epidemiologic

studies evaluating health effects of pesticides (McCauley et al., 2006; Shirangi et al., 2011). In the present review, even those studies that used individual-level data relied on suboptimal methods, including recall or use of surrogate measure, such as proximity to crops rather than direct measures of exposure.

The use of geocoded information has become particularly popular in recent years, and it was used in several studies included in this review. This approach has its advantages and disadvantages. On the one hand, geographic information systems (GISs) based methods of assessing proximity to agricultural crops may be superior to questionnaire-derived measures that have been shown to produce marked exposure misclassification (Rull et al., 2006b). On the other hand, GIS measures may be subjected to their own types of error that stem from inadequate or arbitrarily drawn boundaries, failure to consider seasonality, and assumption that all similar crops are treated the same way (Gunier et al., 2001; Rull and Ritz, 2003).

The use of area-based measures linked to place of residence is further complicated by the need to consider “windows of susceptibility,” which are particularly important for studies of birth defects. Use of residence at the time of birth, as was done in several studies reviewed here, may not be relevant because 20 to 30% of women in the United States are reported to change address during pregnancy (Khoury et al., 1988; Canfield et al., 2006).

Importantly, residential mobility during pregnancy was shown to be related to risk factors of adverse pregnancy outcomes, including age, smoking, and socioeconomic status (Miller et al., 2010), all of which may act as confounders (Bell and Belanger, 2012). Other potential confounders in studies of reproductive outcomes include place of birth, medical and obstetric history (e.g., infections and medication use), lifestyle characteristics other than smoking (e.g., nutrition and alcohol use), as well as

numerous pregnancy-related factors, such as initiation of prenatal care, weight gain during gestation, and prenatal complications (Shi and Chia, 2001; Chao et al., 2010). Among studies included in the current review, there appeared to be no consistency in terms of control for confounders. For example, while most (but not all) studies of birth defects considered maternal age, smoking, and education as possible confounders, only 1 of the 13 analyses (Meyer et al., 2006) controlled for initiation of prenatal care, one (Weselak et al., 2008) controlled for maternal weight gain during pregnancy, and none of the studies adjusted for family income.

Exposures to environmental risk factors other than ATR may also act as confounders. According to the United States Department of Agriculture data for 2005 (National Agricultural Statistics Service, 2006), while ATR is the most common corn herbicide (applied to 97% of corn acreage), other often used compounds include glyphosate (31%), metolachlor, and acetochlor (23% each) as well as several insecticides (23% combined). In addition to other pesticide exposures, proximity to agricultural activity is associated with coexposures to a variety of fertilizers that are applied in particularly large quantities in corn-growing areas (Ochoa-Acuña and Carbajo, 2009). Further, residence in agricultural areas has been linked to adverse birth outcomes with a wide range of postulated causes, which (in addition to fertilizers and pesticides) include microbial toxins (Missmer et al., 2006; Gelineau-van Waes et al., 2009), diet and lifestyle (Giordano et al., 2008; Elliott et al., 2009), and socioeconomic problems (Auger et al., 2009), although in many studies the specific risk factors remained unknown (Batra et al., 2007; Gonzalez et al., 2008; Messer et al., 2010).

The need to consider multiple exposures simultaneously presents a difficult methodological problem (Boobis et al., 2011). On the one hand, it is possible that certain exposures act together, or perhaps interact, in ways that may influence health outcomes (Johns et al., 2012; Sexton, 2012). On the other hand, without an a priori hypothesis, it cannot be assumed that a study of mixed exposures will be more informative than a study focusing on any one specific exposure. Even with relatively simple mixtures, results from observational epidemiology literature are often difficult to interpret due to lack of consistency in methods and reporting across studies (Goodman et al., 2010; Kuo et al., 2013). More complex mixtures of chemical, physical, biological, and social exposures, such as those associated with agricultural activities, present a far greater challenge.

In the occupational setting, exposure is often ascertained based on self-assessment with an expectation that recall of activities, such as pesticide application, should be sufficiently accurate and reliable (Gartner et al., 2005). Specifically for ATR, however, Perry et al. (2006) showed that agreement between biomonitoring- and questionnaire-derived measures is generally poor.

A promising alternative to questionnaire- or GIS-based exposure assessment is the measurement of ATR metabolites in urine samples (Barr et al., 2007). To date, only one study (Chevrier et al., 2011) measured urinary biomarkers of ATR; however, it is not clear if a one-time spot

urine sample collected for that study was sufficient to accurately and reliably characterize exposure. Moreover, exposures observed by Chevrier et al. (2011) may not be relevant to the U.S. populations because most of the samples for that study were collected after ATR was banned in France.

The direction of bias attributable to exposure misclassification is difficult to assess. It is often assumed that exposure misclassification is nondifferential (i.e., independent of the outcome) and, if so, bias is further assumed to result in the erroneous attenuation of the association leading to false-negative results (Copeland et al., 1977; Cantor et al., 1992). It has been shown, however, that neither of these assumptions may hold in practice (Sorahan and Gilthorpe, 1994; Wacholder et al., 1995; Dosemeci et al., 1990).

Another possible source of false-negative results is insufficient statistical power of individual studies. Low study power could have influenced the current review if there was a predominance of positive, but statistically nonsignificant results. This predominance of nonsignificant positive associations does not characterize the literature on ATR, as the null results were almost equally likely to be positive or inverse. The ranges of statistically nonsignificant RR and OR estimates comparing the highest and the lowest exposure categories across most commonly evaluated birth defects were 0.6 to 3.6 for NTD/central nervous system abnormalities, 0.8 to 1.5 for abdominal defects, and 0.7 to 1.0 for defects of the genitourinary tract. For SGA (the most commonly examined nonbirth defect outcome), the corresponding range was between 0.5 and 1.6.

Many studies were limited to live births and ascertained congenital anomalies and other pregnancy outcomes from birth certificates. Limiting outcomes to those observed among live births present a challenge because many birth defects may result in either spontaneous or induced abortions (Game and Bergman, 1999; ESHRE Capri Workshop Group, 2008). It has been reported that inclusion of elective terminations would produce a more than 50% increase in the estimated proportions of affected pregnancies for some birth defects, including gastroschisis and omphalocele, NTDs, and several chromosomal abnormalities (Forrester et al., 1998). When the loss of cases with pregnancy termination is independent of exposure, the resulting measures of association would suffer from a loss of precision. If, however, the exposure is associated with the probability of exclusion due to loss of pregnancy, the observed results will be biased toward or away from the null (Cragan and Khoury, 2000).

Several studies were limited by nonspecific outcome definitions such as "major congenital anomalies" or "any birth defect." This practice is usually dictated by sample size constraints, but nevertheless limits meaningful conclusions (Olsen and Skov, 1993; Kogevinas and Sala, 1998). Even those studies that subdivided birth defects according to affected organ (e.g., abdominal wall) or organ system (e.g., central nervous system) combined disparate conditions into a single heterogeneous category (Sarnat and Flores-Sarnat, 2004; Barkovich et al., 2009; Garne et al., 2012). Conversely, it has been shown that

certain birth defects affecting different organs may have a common cause (Lammer et al., 1985; Stevenson et al., 2004).

It was proposed nearly 25 years ago that a proper study of birth defects should use disease classification that is based on principles of embryology and genetics rather than anatomic site (Olsen, 1988). Such study is only possible with large comprehensive population-based databases, allowing detailed outcome ascertainment. To date, studies that analyzed population-based registry data to examine the association between ATR and specific birth defects used inadequate exposure assessment, while studies with better exposure information tended to be underpowered to assess specific malformations.

Gastroschisis (a defect of the abdominal wall lateral to the midline) is an example of a congenital defect that was investigated in relation to ATR exposure. An association between residence in geographic areas where ATR was used and risk of gastroschisis was found in one ecologic (Mattix et al., 2007) and one case-control study (Waller et al., 2010). Another case-control study (Agopian et al., 2013b) reported an association in the subanalysis limited to older mothers, but not in the total study population, while two earlier studies (Ochoa-Acuña and Carbajo, 2009; Winchester et al., 2009) indicated no evidence that ATR or surrogate exposures were linked to gastroschisis or other abdominal wall defects. Regardless of the reported results, all of these studies suffered from inadequate exposure characterization. It is important that the results of guideline compliant developmental toxicity safety tests in animals do not support a role for ATR in the induction of gastroschisis. No case of gastroschisis was observed among 1116 rat fetuses or 349 rabbit fetuses in the two embryo-fetal developmental studies of ATR, and only a single case of gastroschisis was observed among a total of 3925 rat fetuses of dams treated with ATR metabolites in four embryo-fetal development studies (Scialli et al., 2014, companion paper).

Several studies in this review illustrated how availability of data on several exposure metrics and several endpoints presented an opportunity to simultaneously test multiple hypotheses. The appropriate methodology for dealing with multiple comparisons remains the focus of long-standing debate (Rothman, 1990; Sabatti, 2007; Attia et al., 2009). It can be argued that changing type I error cutoff for statistical significance (e.g., a Bonferroni adjustment) may not be necessary as long as studies are designed and carried out based on an a priori formulated hypothesis that takes into consideration both epidemiologic and mechanistic evidence. Recent advances in genomic research led to the development of novel approaches toward reducing the probability of false-positive findings without increasing the risk of false-negative results (Datta and Datta, 2005; Chatterjee and Wacholder, 2009; Dai et al., 2012; Mukherjee et al., 2012). These approaches are applicable in other areas of epidemiology that involve multiple hypothesis testing.

In conducting a review of studies that included multiple comparisons, one also needs to keep in mind that

not all tested associations are equally likely to be reported (Dickersin and Min, 1993; Dwan et al., 2008), or if reported, highlighted in the conclusions (Kyzas et al., 2007). An uncritical review of main reported findings may leave an impression that studies are in agreement; however, inconsistencies may become evident when results of different analyses are organized systematically.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

Our review indicates that the number of studies assessing the relation of ATR exposure to adverse pregnancy outcomes has been increasing. However, no study has provided data of sufficient quality to draw definitive conclusions. The previously cited review by Weselak et al. (2007) summarizing the literature on the relation of triazine herbicides to congenital anomalies and other adverse pregnancy outcomes assessed evidence as "inadequate" because studies available at that time were based on ecologic exposure measures or self-reports. Although our review identified more than a dozen additional studies, there is no reason to disagree with this assessment.

There appears to be a particular need for studies with individual-level assessment of exposure. Before drawing conclusions, it may be important to evaluate exposure measurement error by validating the methodology used in a particular study against an alternative, more accurate approach (Jurek et al., 2006). The correction for measurement error can be formally carried out through quantitative sensitivity analyses that examine "the extent to which results are affected by changes in methods, values of variables, or assumptions" (Last, 2001). The methodology of sensitivity analyses is now well developed both in terms of basic theory (Greenland, 1996) and with respect to practical applications (Lash and Fink, 2003; Maldonado et al., 2003; Goodman et al., 2007).

Without good quality studies, evaluation of consistency across findings is of limited value. Nevertheless, a systematic examination of the available data indicates no consistency of results for any of the outcomes. In view of the poor quality epidemiologic data and the lack of robust findings, claims about a causal link between ATR and adverse pregnancy outcomes are not warranted. The largely negative epidemiologic evidence is consistent with the experimental animal studies.

CONFLICT OF INTEREST

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APPENDIX A

Summary of Electronic PubMed Search Strategies to Identify Studies Evaluating the Association between ATR (or Surrogate) Exposure and Birth Defects or Other Pregnancy Outcomes

| Search set | Search terms | Number of titles | Number of new abstracts | New full text articles reviewed | New articles added to the review |
|------------|---|------------------|-------------------------|---------------------------------|----------------------------------|
| 1 | ("Triazines"[MeSH terms] or "triazines"[all fields] or "triazine"[all fields]) and exposure[all fields] and "humans"[MeSH terms] | 425 | 83 | 22 | 11 |
| 2 | ("ATR"[MeSH terms] or "ATR"[all fields]) and exposure[all fields] and "humans"[MeSH terms] | 152 | 22 | 11 | 1 |
| 3 | ("ATR"[MeSH terms] or "ATR"[all fields]) and ("epidemiology"[Subheading] or "epidemiology"[all fields] or "epidemiology"[MeSH terms]) and "humans"[MeSH terms] | 48 | 2 | 2 | 1 |
| 4 | ("ATR"[MeSH terms] or "ATR"[all fields]) and ("pregnancy"[MeSH terms] or "pregnancy"[all fields]) and "humans"[MeSH terms] | 18 | 2 | 1 | 1 |
| 5 | ("Triazines"[MeSH terms] or "triazines"[all fields] or "triazine"[all fields]) and ("congenital abnormalities"[MeSH terms] or ("congenital"[all fields] and "abnormalities"[all fields]) or "congenital abnormalities"[all fields] or ("congenital"[all fields] and "malformations"[all fields]) or "congenital malformations"[all fields]) and "humans"[MeSH terms] | 89 | 0 | 0 | 0 |
| 6 | ("Triazines"[MeSH terms] or "triazines"[all fields]) and ("herbicides"[MeSH terms] or "herbicides"[all fields] or "herbicides"[pharmacologic action]) and ("epidemiology"[subheading] or "epidemiology"[all fields] or "epidemiology"[MeSH terms]) and "humans"[MeSH terms] | 43 | 0 | 0 | 0 |
| 7 | ("Triazines"[MeSH terms] or "triazines"[all fields] or "triazine"[all fields]) and ("herbicides"[MeSH terms] or "herbicides"[all fields] or "herbicides"[pharmacologic action]) and ("delivery, obstetric"[MeSH terms] or ("delivery"[all fields] and "obstetric"[all fields]) or "obstetric delivery"[all fields] or "delivery"[all fields])) and "humans"[MeSH terms] | 7 | 1 | 1 | 0 |
| 8 | ("Triazines"[MeSH terms] or "triazines"[all fields] or "triazine"[all fields]) and ("infant, premature"[MeSH terms] or ("infant"[all fields] and "premature"[all fields]) or "premature infant"[all fields] or "prematurity"[all fields]) and "humans"[MeSH terms] | 11 | 0 | 0 | 0 |
| 9 | ("Triazines"[MeSH terms] or "triazines"[all fields] or "triazine"[all fields]) and ("GA"[MeSH terms] or ("gestational"[all fields] and "age"[all fields]) or "GA"[all fields])) and "humans"[MeSH terms] | 14 | 0 | 0 | 0 |
| 10 | ("Triazines"[MeSH terms] or "triazines"[all fields] or "triazine"[all fields]) and neonatal[all fields] and "humans"[MeSH terms] | 26 | 0 | 0 | 0 |
| 11 | ("ATR"[MeSH terms] or "ATR"[all fields]) and neonatal[ALL FIELDS] and "humans"[MeSH terms] | 4 | 0 | 0 | 0 |
| 12 | ("Triazines"[MeSH terms] or "triazines"[all fields] or "triazine"[all fields]) and ("herbicides"[MeSH terms] or "herbicides"[all fields] or "herbicide"[all fields] or "herbicides"[pharmacologic action]) and ("toxicity"[subheading] or "toxicity"[all fields]) and "humans"[MeSH terms] | 108 | 1 | 1 | 0 |

APPENDIX B

STUDIES FOR WHICH FULL-TEXT ARTICLES RETRIEVED DURING ELECTRONIC OR MANUAL SEARCHES WERE EXAMINED, BUT NOT INCLUDED IN THE REVIEW

Barr DB, Panuwet P, Nguyen JV, Udunka S, Needham LL. 2007. Assessing exposure to atrazine and its metabolites using biomonitoring. *Environ Health Perspect* 115(10):1474–1478.

Reason for exclusion: Exposure assessment study.

Birnbaum LS, Fenton SE. 2003. Cancer and developmental exposure to endocrine disruptors. *Environ Health Perspect* 111(4):389–394.

Reason for exclusion: Review (references examined).

Boffetta P, Adami HO, Berry SC, Mandel JS. 2013. Atrazine and cancer: a review of the epidemiologic evidence. *Eur J Cancer Prev* 22:169–180.

Reason for exclusion: Review (references examined).

Catenacci G, Maroni M, Cottica D, Pozzoli L. 1990. Assessment of human exposure to atrazine through the determination of free atrazine in urine. *Bull Environ Contam Toxicol* 44(1):1–7.

Reason for exclusion: Exposure assessment study.

Clayton AC, Pellizzari ED, Whitmore RW, Quackenboss JJ, Adgate J, Sefton K. 2003. Distributions, associations, and partial aggregate exposure of pesticides and polynuclear aromatic hydrocarbons in the Minnesota Children's Pesticide Exposure Study (MNCPPES). *J Expo Anal Environ Epidemiol* 13(2):100–111.

Reason for exclusion: Exposure assessment study.

Cooper RL, Laws SC, Das PC, Narotsky MG, Goldman JM, Lee Tyrey E, Stoker TE. 2007. Atrazine and reproductive function: mode and mechanism of action studies. *Birth Defects Res B Dev Reprod Toxicol* 80:98–112.

Reason for exclusion: Review (references examined).

- Cragin LA, Kesner JS, Bachand AM, Barr DB, Meadows JW, Krieg EF, Reif JS. 2011. Menstrual cycle characteristics and reproductive hormone levels in women exposed to atrazine in drinking water. *Environ Res* 111(8):1293–1301.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H, Reynolds SJ, Alavanja MC. 2007a. Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg* 51(1):53–65.
- Reason for exclusion:* Exposure assessment study.
- Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H, Reynolds SJ, Alavanja MC. 2007b. Pesticide dose estimates for children of Iowa farmers and non-farmers. *Environ Res* 105:307–315.
- Reason for exclusion:* Exposure assessment study.
- Farr SL, Cooper GS, Cai J, Savitz DA, Sandler DP. 2004. Pesticide use and menstrual cycle characteristics among premenopausal women in the Agricultural Health Study. *Am J Epidemiol* 160:1194–1204.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Farr SL, Cai J, Savitz DA, Sandler DP, Hoppin JA, Cooper JS. 2006. Pesticide exposure and timing of menopause: the Agricultural Health Study. *Am J Epidemiol* 163:731–742.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Freeman NC, Shalat SL, Black K, Jimenez M, Donnelly KC, Calvin A, Ramirez J. 2004. Seasonal pesticide use in a rural community on the US/Mexico border. *J Expo Anal Environ Epidemiol* 14(6):473–478.
- Reason for exclusion:* Exposure assessment study.
- Gammon DW, Aldous CN, Carr WC, Jr., Sanborn JR, Pfeifer KF. 2005. A risk assessment of atrazine use in California: human health and ecological aspects. *Pest Manag Sci* 61(4):331–355.
- Reason for exclusion:* Review (references examined).
- Garaj-Vrhovac V, Zeljezic D. 2001. Cytogenetic monitoring of Croatian population occupationally exposed to a complex mixture of pesticides. *Toxicology* 165(2–3):153–162.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Garaj-Vrhovac V, Zeljezic D. 2002. Assessment of genome damage in a population of Croatian workers employed in pesticide production by chromosomal aberration analysis, micronucleus assay and Comet assay. *J Appl Toxicol* 22(4):249–255.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Garcia AM. 1998. Occupational exposure to pesticides and congenital malformations: a review of mechanisms, methods, and results. *Am J Ind Med* 33:232–240.
- Reason for exclusion:* Review (references examined).
- Hayes TB. 2005. Welcome to the revolution: integrative biology and assessing the impact of endocrine disruptors on environmental and public health. *Integr Comp Biol* 45:321–329.
- Reason for exclusion:* Review (references examined).
- Hines CJ, Deddens JA, Striley CA, Biagini RE, Shoemaker DA, Brown KK, Mackenzie BA, Hull RD. 2003. Biological monitoring for selected herbicide biomarkers in the urine of exposed custom applicators: application of mixed-effect models. *Ann Occup Hyg* 47(6):503–517.
- Reason for exclusion:* Exposure assessment study.
- International Agency for Research on Cancer. 1999. Atrazine. IARC Monogr Eval Carcinog Risks Hum 73:59–113.
- Reason for exclusion:* Review (references examined).
- Lubinsky M. 2012. Hypothesis: estrogen related thrombosis explains the pathogenesis and epidemiology of gastroschisis. *Am J Med Genet A* 158A(4):808–811.
- Reason for exclusion:* Review (references examined).
- Lucas AD, Jones AD, Goodrow MH, Saiz SG, Blewett C, Seiber JN, Hammock BD. 1993. Determination of atrazine metabolites in human urine: development of a biomarker of exposure. *Chem Res Toxicol* 6(1):107–116.
- Reason for exclusion:* Exposure assessment study.
- MacLennan PA, Delzell E, Sathiakumar N, Myers SL, Cheng H, Grizzle W, Chen VW, Wu XC. 2002. Cancer incidence among triazine herbicide manufacturing workers. *J Occup Environ Med* 44:1048–1058.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Payán-Rentería R, Garibay-Chávez G, Rangel-Ascencio R, Preciado-Martínez V, Muñoz-Islas L, Beltrán-Miranda C, Mena-Munguía S, Jave-Suárez L, Feria-Velasco A, De Celis R. 2012. Effect of chronic pesticide exposure in farm workers of a Mexico community. *Arch Environ Occup Health* 67(1):22–30.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Rocheleau CM, Romitti PA, Dennis LK. 2009. Pesticides and hypospadias: a meta-analysis. *J Pediatr Urol* 5:17–24.
- Reason for exclusion:* Review (references examined).
- Roy JR, Chakraborty S, Chakraborty TR. 2009. Estrogen-like endocrine disrupting chemicals affecting puberty in humans—a review. *Med Sci Monit* 15:RA137–RA145.
- Reason for exclusion:* Review (references examined).
- Saldana TM, Basso O, Hoppin JA, Baird DD, Knott C, Blair A, Alavanja MC, Sandler DP. 2007. Pesticide exposure and self-reported gestational diabetes mellitus in the Agricultural Health Study. *Diabetes Care* 30(3):529–534.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Sathiakumar N, MacLennan PA, Mandel J, Delzell E. 2011. A review of epidemiologic studies of triazine herbicides and cancer. *Crit Rev Toxicol* 41(Suppl 1):1–34.
- Reason for exclusion:* Review (references examined).
- Shirangi A, Nieuwenhuijsen M, Vienneau D, Holman CD. 2011. Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: a review of the literature. *Paediatr Perinat Epidemiol* 25:172–191.
- Reason for exclusion:* Review (references examined).
- Short P, Colborn T. 1999. Pesticide use in the U.S. and policy implications: a focus on herbicides. *Toxicol Ind Health* 15(1–2):240–275.
- Reason for exclusion:* Review (references examined).
- Suárez S, Rubio A, Sueiro RA, Garrido J. 2003. Sister chromatid exchanges and micronuclei analysis in lymphocytes of men exposed to simazine through drinking water. *Mutat Res* 537(2):141–149.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Swan SH. 2006. Semen quality in fertile US men in relation to geographical area and pesticide exposure. *Int J Androl* 29(1):62–68.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Swan SH, Kruse RL, Liu F, Barr DB, Drobnis EZ, Redmon JB, Wang C, Brazil C, Overstreet JW; Study for Future Families Research Group. 2003. Semen quality in relation to biomarkers of pesticide exposure. *Environ Health Perspect* 111(12):1478–1484.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Thorpe N, Shirmohammadi A. 2005. Herbicides and nitrates in ground-water of Maryland and childhood cancers: a geographic information systems approach. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 23(2):261–278.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review.
- Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev* 33(3):378–455.
- Reason for exclusion:* Review (references examined).
- Weselak M, Arbuckle TE, Foster W. 2007. Pesticide exposures and developmental outcomes: the epidemiological evidence. *J Toxicol Environ Health B Crit Rev* 10:41–80.
- Reason for exclusion:* Review (references examined).
- Young HA, Mills PK, Riordan DG, Cress RD. 2005. Triazine herbicides and epithelial ovarian cancer risk in central California. *J Occup Environ Med* 47(11):1148–1156.
- Reason for exclusion:* Endpoint(s) beyond the scope of present review .

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