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The relationship between study sponsorship, risks of bias, and research outcomes in atrazine exposure studies conducted in non-human animals: Systematic review and meta-analysis

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ABSTRACT

Background: A critical component of systematic review methodology is the assessment of the risks of bias of studies that are included in the review. There is controversy about whether funding source should be included in a risk of bias assessment of animal toxicology studies.
Objective: To determine whether industry research sponsorship is associated with methodological biases, the results, or conclusions of animal studies examining the effect of exposure to atrazine on reproductive or developmental outcomes.
Methods: We searched multiple electronic databases and the reference lists of relevant articles to identify original research studies examining the effect of any dose of atrazine exposure at any life stage on reproduction or development in non-human animals. We compared methodological risks of bias, the conclusions of the studies, the statistical significance of the findings, and the magnitude of effect estimates between industry sponsored and non-industry sponsored studies.
Results: Fifty-one studies met the inclusion criteria. There were no differences in methodological risks of bias in industry versus non-industry sponsored studies. 39 studies tested environmentally relevant concentrations of atrazine (11 industry sponsored, 24 non-industry sponsored, 4 with no funding disclosures). Non-industry sponsored studies (12/24, 50.0%) were more likely to conclude that atrazine was harmful compared to industry sponsored studies (2/11, 18.1%) (p value = 0.07). A higher proportion of non-industry sponsored studies reported statistically significant harmful effects (8/24, 33.3%) compared to industry-sponsored studies (1/11; 9.1%) (p value = 0.13). The association of industry sponsorship with decreased effect sizes for harm outcomes was inconclusive.
Conclusion: Our findings support the inclusion of research sponsorship as a risk of bias criterion in tools used to assess risks of bias in animal studies for systematic reviews. The reporting of other empirically based risk of bias criteria for animal studies, such as blinded outcome assessment, randomization, and all animals included in analyses, needs to improve to facilitate the assessment of studies for systematic reviews.

1. Introduction

Results from animal studies are a critical, and often the only, input to assessing potential harm from exposure to chemicals. However, the lack of reproducibility of findings from animal research has reduced public confidence in the utility of animal experiments (van der Worp et al., 2010) and led to claims that animal research is a waste of financial resources (Macleod et al., 2014). These problems with animal research have resulted in significant debate about how to assess biases in animal studies used in systematic reviews, risk assessments and other regulatory decisions (Woodruff and Sutton, 2011; Rooney et al., 2014; National Academies of Science, 2014). A critical component of systematic review methodology is the assessment of the risks of bias of studies that are included in the review.

Risk of bias occurs when the methodological characteristics of a study produce a systematic error in the magnitude or direction of the results (Higgins and Green, 2011). Bias can shift effect estimates to be larger or smaller. For example, in controlled human clinical drug trials, studies with a high risk of bias (such as those lacking randomization, allocation concealment, or blinding of participants and outcome assessors) produce larger treatment effect sizes, thus falsely inflating the efficacy of the test interventions, compared to studies that have these

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design features (Schulz and Grimes, 2002a; Schulz and Grimes, 2002b). However, biased human studies assessing the harms of drugs are more likely to report smaller estimates of adverse effects (Nieto et al., 2007).

Less is known about methodological risks of bias in animal studies, although a systematic review of instruments for assessing risks of bias in animal studies identified criteria that have been shown empirically to bias effect estimates in animal models (Krauth et al., 2013). For example, analyses of animal studies examining interventions for stroke, multiple sclerosis and trauma have shown that lack of randomization, blinding, specification of inclusion and exclusion criteria, statistical power, and failure to use comorbid animals are associated with inflated effect estimates of pharmaceutical interventions (Bebarta et al., 2003; Crossley et al., 2008; Sena et al., 2010a).

Industry funding for research and industry relationships with academic researchers pose an additional risk of bias. Considerable evidence shows a strong association between industry funding, investigator financial conflicts of interest, and biased outcomes in clinical research, even when controlling for methodological characteristics of the studies (Lundh et al., 2012). There is little evidence regarding the influence of these conflicts of interest on the outcomes of animal research (Krauth et al., 2014; Bennett et al., 2010; Abdel-Sattar et al., 2014). There are conflicting results concerning the association of industry funding and research outcomes among the small cohorts of animal studies that have been examined and further research on the influence of conflicts of interest on animal studies is needed (Bennett et al., 2010; Abdel-Sattar et al., 2014). There is controversy about whether funding source should be included in risk of bias assessments for studies included in systematic reviews (Bero, 2013).

Atrazine (6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is used as an herbicide. Atrazine is commonly found in drinking water in the United States. The EPA has concluded that "atrazine is an endocrine disruptor" (Agency, 2007) but not that atrazine affects amphibian sexual development (Agency, 2010). As of 2013, the EPA has not altered these conclusions (Agency, 2013). Atrazine studies are a good topic for an analysis of funding bias because concerns have been raised about the influence of industry sponsorship on the design and results of studies examining the effects of atrazine on reproductive and developmental outcomes (Hayes, 2004).

The objective of this study is to determine whether industry research sponsorship is associated with the methods, conclusions, or results of animal studies examining the effect of exposure to atrazine on reproductive or developmental outcomes. We test three specific hypotheses. First, we hypothesize that industry sponsored studies will be less likely to have conclusions indicating harm from atrazine than non-industry sponsored studies. Second, we test the hypothesis that industry sponsored studies will be less likely to report statistically significant results indicating harm from atrazine than non-industry sponsored studies. Third, we test the hypothesis that industry sponsored studies will have smaller effect estimates of harm than non-industry sponsored studies. In addition, we compare the methodological risks of bias of industry sponsored vs. non-industry sponsored studies to determine if there are differences in the methods of the studies.

2. Methods

We searched for studies that addressed the following question: "Does exposure to atrazine have adverse reproductive or developmental effects in non-human animals"? We searched for studies that had non-human animal subjects that were exposed to any dose of atrazine during any life stage. Exposure levels of atrazine were classified and adverse outcomes were grouped as described below.

2.1. Inclusion/exclusion criteria

Articles were included if they met the following criteria: (1) study conducted using whole animals; (2) original research, defined as a study that presented original data and did not specifically state that it was a review; (3) atrazine compared to no exposure or control (eg, vehicle or some other exposure); (4) contains at least one group receiving only atrazine exposure; and (5) reports results data for at least one developmental and/or reproductive health outcome.

Studies were excluded if they met any of the following criteria: (1) pharmacokinetic or pharmacodynamic studies; (2) editorials, letters to the editor, commentaries, abstracts, unpublished reports, systematic reviews, meta-analyses; (3) studies comparing only different doses of atrazine; (4) studies in which atrazine was present in all the comparison groups; (5) in vitro-analysis; (6) studies with no comparison groups.

Abstracts and article titles were first screened for inclusion. The full text of each article was then discussed by two authors who made a final decision about inclusion.

2.2. Search strategy

There we no language restrictions for the search. We searched Medline from January 1, 1966 to June 26th, 2013 using a search term combination containing the following MeSH terms, text words and word variants:

(*atrazine*) *AND* (*animal** *OR preclinical OR* "*pre-clinical*" *OR mice OR rats OR rabbits OR dog OR dogs OR monkey OR monkeys OR* "*animal experimentation*"[*MeSH Terms*] *OR* "*models*, *animal*"[*MeSH Terms*] *OR* "*invertebrates*"[*MeSH Terms*] *OR* "*Animals*"[*MH*] *OR* "*animal population groups*"[*MeSH Terms*]) *NOT* (*humans*[*mh*] *NOT animals*[*mh*:*noexp*]) *AND* (*health effect OR health effects OR toxic OR toxicity OR toxicities OR efficacy OR efficacies OR toxicology OR safety OR harm** *OR drug effects*[*sh*] *OR therapeutic use*[*sh*:*noexp*] *OR adverse effects*[*sh*] *OR poisoning*[*sh*] *OR pharmacology*[*sh*:*noexp*] *OR chemically induced*[*sh*]) *AND eng*[*la*] *NOT review*[*pt*] *NOT systematic review** *NOT meta-analysis*[*pt*].

We also searched, between May 1 and July 30, 2013 the following toxicology databases for articles that met our inclusion criteria:

DART http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?DARTETIC

EPA Science Inventory http://www.epa.gov/gateway/science/

NIOSHTIC2 http://www2.cdc.gov/nioshtic2/Nioshtic2.htm

Toxline http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE

USEPA Health and Environmental Studies Online http://hero.epa.gov/

TSCA Test Submissions: http://www.ntis.gov/products/ots.aspx.

We identified 11 additional citations that were not in Medline. Nine could not be obtained even after contacting the authors. Two went on to full text screening. We searched the reference lists of all articles that met the inclusion criteria and identified one additional reference. Of the 3 additional references identified, one did not meet the inclusion criteria after full text screening.

2.3. Data extraction

2.3.1. Single-coded data collection

DK collected the following characteristics from each included study:

2.3.2. Study citation information

Title of the study, month of publication, year of publication, and journal name.

2.3.3. Author affiliation

Author(s) affiliation(s) was obtained from the article and classified into (1) industry, if all authors were employed by industry (2) non-industry, if no author was employed by industry, or (3) combined, if at least one author was employed by industry and at least one author was not employed by industry. If a single author had affiliations with industry and non-industry sources, the study was coded as "combined".

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