

Using study evaluation to inform evidence integration: Application in a systematic review of hexavalent chromium male reproductive outcomes

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Background

Study evaluation is used in systematic reviews to identify the strengths and weaknesses of the evidence base in a consistent and transparent manner. These evaluations can be used to inform evidence integration by identifying factors that may affect the reliability and interpretability of the results. Here, we describe how this principle was applied in a systematic review of the male reproductive effects of hexavalent chromium [Cr(VI)].

Methods

Literature search and screening: This evaluation of male reproductive effects was conducted as part of a systematic review of the health effects of Cr(VI) exposure. Studies were identified by searching three online databases (PubMed, Web of Science, Toxline) through May 2018. Title/abstract screening followed by full-text screening was used to identify animal studies meeting the following PECO (Population, Exposure, Comparators, Outcomes) criteria:

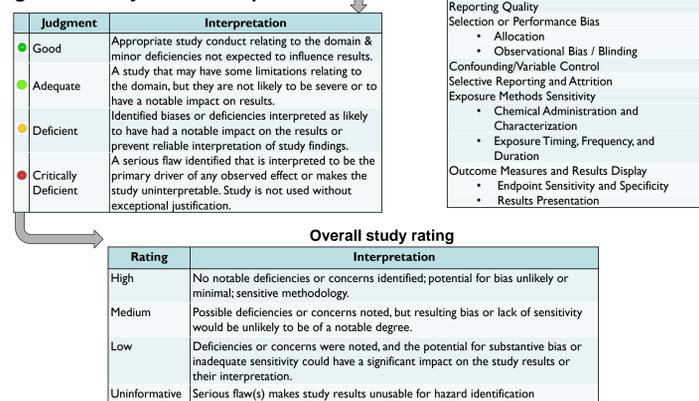
- P:** Nonhuman mammalian animals (whole organism) of any life stage
- E:** Any exposure to Cr(VI) by oral or inhalation routes
- C:** Concurrent vehicle control or untreated control group
- O:** All cancer outcomes; noncancer outcomes in relevant target systems

The literature search identified 23 animal toxicology studies that examined effects on the male reproductive system. Studies included evaluation of:

- Male fertility
- Sperm parameters
- Reproductive hormones
- Reproductive organ weights
- Anogenital distance (AGD)
- Sexual behavior

Study evaluation: Each of these studies was evaluated by at least two independent reviewers for reporting quality, risk of bias, and sensitivity using the domain-based approach outlined in Figure 1. Based on the results of the evaluation, each study was rated overall as *high* confidence, *medium* confidence, *low* confidence, or *uninformative*. Evaluations were performed on an outcome-specific basis, as the utility of a study may vary across outcomes.

Figure 1. Study evaluation process



Evidence synthesis: Evidence was synthesized across studies, using the following considerations to articulate the strengths and weaknesses of the dataset: consistency, biological gradient (dose-response), strength (effect magnitude) and precision, biological plausibility, and coherence. Careful examination was given to the potential impacts of risk of bias and sensitivity on the conclusions. Relevant mechanistic data identified in the literature search was considered as part of the weight of evidence for biological plausibility. Based on this synthesis, the evidence was assigned a conclusion of *robust*, *moderate*, *slight*, *indeterminate*, or *compelling evidence of no effect*.

Results

Table 1. Study evaluation results. These results represent the composite ratings for male reproductive outcomes within each evaluation domain; there were some instances where outcomes within the same study were rated differently due to outcome-specific concerns, in which case an average rating (representative of most outcomes) is shown here. In addition to the 15 studies shown in this table, 8 studies were considered *uninformative* due to serious flaws in the study design (e.g., use of wild-caught animals) or reporting (e.g., data could not be interpreted) and were excluded from consideration.

| Reference | Study description | | | Study evaluation | | | | | | | | | |
|-----------------------|-----------------------------------------------|------------------------------------------------|--------------------|-------------------|------------------------|---------------------------|--------------------------------|-----------------------------|------------------------------|----------------------------|----------------------------------------------------|-------------------------|--------------------|
| | Species/Strain | Exposure life stage and duration | Route of exposure | Reporting quality | Test animal allocation | Blinding of investigators | Confounding / variable control | Reporting or attrition bias | Characterization of exposure | Utility of exposure design | Sensitivity, specificity, and usability of results | Presentation of results | Overall confidence |
| NTP 1997 | Mouse (BALBC) | Reproductive Assessment by Continuous Breeding | Diet | G | G | NR | G | G | G | G | G | G | High |
| NTP 1996a | Mouse (BALBC) | Adult males and females; 3, 6, or 9 weeks | Diet | G | G | G | G | A | G | G | G | G | High |
| NTP 1996b | Rat (Sprague-Dawley) | Adult males and females; 3, 6, or 9 weeks | Diet | G | G | G | G | G | G | G | G | A | High |
| NTP 2007 | Rat (F344/N), Mouse (B6C3F1, BALB/c, C57BL/6) | Adult males and females; 3 months | Drinking water | G | G | A | G | A | G | G | G | G | High |
| Elbetieha et al. 1997 | Mouse (Swiss) | Adult males and females; 12 weeks | Drinking water | G | NR | NR | D | A | D | G | G | A | Low |
| Bataineh et al. 1997 | Rat (Sprague-Dawley) | Adult males; 12 weeks | Drinking water | D | A | NR | D | D | D | G | A | G | Low |
| Yousef et al. 2006 | Rabbit (NZ white) | Adult males; 10 weeks | Oral gavage | G | A | NR | D | D | D | G | G | D | Low |
| Li et al. 2001 | Rat (Wistar) | Adult males; 6 weeks | Oral feeding | D | NR | NR | D | A | D | A | A | A | Low |
| Rasool et al. 2014 | Mouse (strain not reported) | Adult males; 30 or 60 days | Oral (unspecified) | D | A | NR | D | A | D | G | D | D | Low |
| Wang et al. 2015 | Rat (Sprague-Dawley) | Adult males; 4 weeks | Drinking water | G | A | NR | G | G | A | G | D | A | Low |
| Kumar et al. 2017 | Rat (Wistar) | F1 offspring; GD 9-14 | Drinking water | A | NR | NR | G | D | D | A | A | D | Low |
| Al-Hamood et al. 1998 | Mouse (BALBC) | F1 offspring; GD 12-PND 20 | Drinking water | G | D | NR | D | D | D | G | G | D | Low |
| Glaser et al. 1986 | Rat (Wistar) | Adult males; 18 months | Inhalation | A | A | NR | G | A | A | A | D | A | Low |
| Glaser et al. 1985 | Rat (Wistar) | Adult males; 28 or 90 days | Inhalation | A | A | NR | G | A | A | G | D | A | Low |
| Kim et al. 2004 | Rat (Sprague-Dawley) | Adult males; 90 days | Inhalation | G | A | NR | G | A | A | A | A | D | Low |

Abbreviations: Gestation day (GD); Postnatal day (PND); Good (G); Adequate (A); Deficient (D); Not Reported (NR)

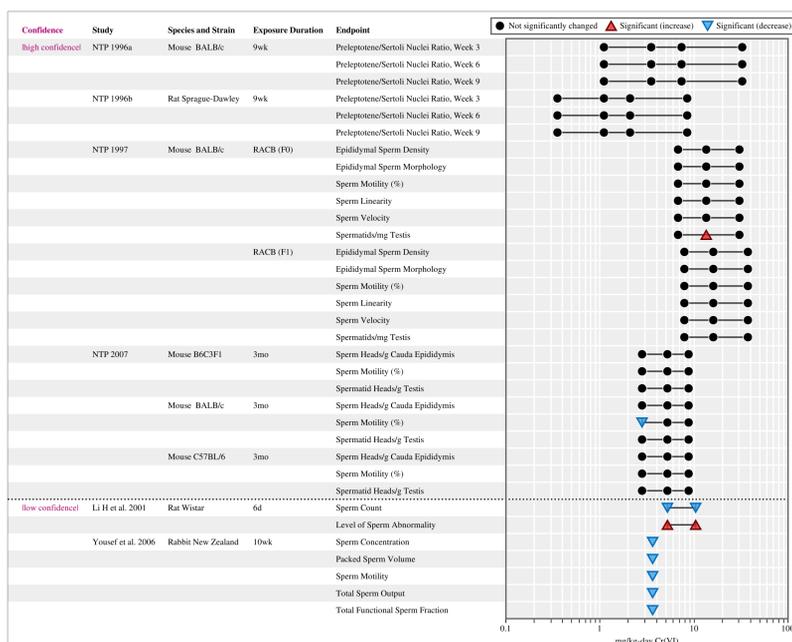


Figure 2. Summary of effects on sperm. Data is shown for all studies for which the ingested dose of Cr(VI) could be calculated. Decreased sperm count, motility, and viability were also observed in the *low confidence* study by Kumar et al. 2017, but the ingested dose of Cr(VI) could not be calculated based on the reported information.

Summary of effects in high vs. low confidence studies

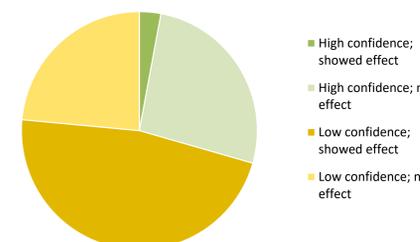


Figure 3. Incidence of outcomes indicative of male reproductive effects across high and low confidence Cr(VI) animal studies. One *high* confidence study observed decreased testis weight, but otherwise *high* confidence studies found no evidence of male reproductive effects. Comparative, male reproductive effects was frequently observed in *low* confidence studies.

Integration of evidence

It was concluded that animal toxicology studies along with supportive data from mechanistic studies provide *slight* evidence that Cr(VI) is a male reproductive toxicant. The rationale for this conclusion is documented in an evidence profile table (Table 2). Relatively severe male reproductive effects were observed across multiple *low* confidence studies and are supported by mechanistic evidence. However, similar effects were not observed in *high* confidence studies, and concerns were raised about the potential impact of bias on the interpretation of the results in *low* confidence studies. Fertility (ability to produce offspring) was not affected in any studies but this did not affect overall conclusions, since rodents can remain fertile after large reductions in sperm count.

Table 2: Evidence profile table for Cr(VI) male reproductive effects

| | Studies [confidence] | Factors that increase strength | Factors that decrease strength | Summary of findings |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fertility and Fecundity | NTP 1997 [high] Bataineh et al. 1997 [low] Elbetieha et al. 1997 [low] Al-Hamood et al. 1998 [low] | | • Only study that observed an effect is considered <i>low</i> confidence • No effects observed in <i>high</i> confidence studies | No effects on male fertility (ability to produce offspring) were observed across studies in rats or mice, although one <i>low confidence</i> study in rats observed decreased fetal viability following paternal exposure (Elbetieha et al. 1997). |
| | Sperm evaluation | NTP 1996a [high] NTP 1996b [high] NTP 1997 [high] NTP 2007 [high] Kumar et al. 2017 [low] Li et al. 2001 [low] Yousef et al. 2006 [low] | • Dose-response gradient • Biological plausibility (mechanistic evidence of oxidative stress, effects on blood-testis barrier, and altered meiosis) | • No effects observed in <i>high</i> confidence studies • Studies that observed effects were all considered <i>low</i> confidence |
| Histopathology | NTP 2007 [medium] Kumar et al. 2017 [low] Li et al. 2001 [low] Rasool et al. 2012 [low] Wang et al. 2015 [low] Glaser et al. 1985 [low] Kim et al. 2004 [low] | • Coherence with effects on sperm • Dose-response gradient • Biological plausibility (mechanistic evidence of oxidative stress and effects on blood-testis barrier) | • No effects observed in <i>high</i> confidence studies • Studies that observed effects were all considered <i>low</i> confidence | No histopathological effects were reported in the <i>high confidence</i> study in rats and a variety of mouse strains by NTP 2007, whereas three <i>low</i> confidence studies in rats and mice observed histopathological changes in the testis and seminiferous tubules. |
| | Organ weight | NTP 1996a [high] NTP 1996b [high] NTP 1997 [high] NTP 2007 [high] Al-Hamood et al. 1998 [low] Bataineh et al. 1997 [low] Elbetieha et al. 1997 [low] Kumar et al. 2017 [low] Yousef et al. 2006 [low] Wang et al. 2015 [low] Kim et al. 2004 [low] Glaser et al. 1986 [low] | • Coherence with decreased testosterone | • Unexplained inconsistency • Most studies that observed effects were considered <i>low</i> confidence |
| Hormones | Yousef et al. 2006 [low] Kumar et al. 2017 [low] | • Consistency • Biological plausibility (mechanistic evidence of decreased steroidogenesis) | • Few studies • Only <i>low</i> confidence studies available | Decreased testosterone was observed in rabbits exposed as adults, and decreased testosterone and gonadotropins were observed in F1 rats that had been exposed during gestation. |
| | Sexual behavior | Bataineh et al. 1997 [low] Yousef et al. 2006 [low] | • Consistency | • Few studies • Only <i>low</i> confidence studies available |
| AGD | Kumar et al. 2017 [low] | • Coherence with decreased testosterone | • Single study • <i>Low</i> confidence | Decreased AGD was observed in F1 rats, which is consistent with the observation of decreased testosterone in these animals. |

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