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Hypothesis-driven weight of evidence analysis to determine potential endocrine activity of MTBE

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ABSTRACT

Endocrine-related endpoints in animals have been reported to respond to high doses of methyl tertiary-butyl ether (MTBE), however, a systematic and transparent evaluation of endocrine potential has not been published. Resolving whether MTBE exhibits endocrine activity is important given regulatory and public interest in endocrine disrupting substances and their potential for causing adverse effects in humans or wildlife. A weight-of-evidence (WoE) analysis was conducted, focusing on hypotheses related to the potential for MTBE to interact with estrogen, androgen, and thyroid pathways, and steroidogenesis. To reach scientifically justified conclusions based on the totality of evidence, this WoE procedure involved a semi-quantitative relevance weighting of each endpoint for each hypothesis and systematic consideration of each endpoint in various study designs. This procedure maximized use of an extensive body of relevant and reliable literature on MTBE with evidence supporting or opposing a given mode of action hypothesis. Evaluating the strength and consistency of observations from many MTBE studies also provided a way to assess whether high doses used in experiments with MTBE confound identification of direct endocrine system responses. Based on results of studies using mammalian and fish models and *in vitro* screening assays, this WoE assessment reveals that MTBE lacks direct endocrine activity.

1. Introduction and background

Methyl tertiary-butyl ether (MTBE, CAS RN 1634-04-4) is an additive in gasoline used to increase the efficiency of combustion and bring associated environmental benefits. MTBE also has minor uses as a solvent in closed systems. Endocrine-related responses in rodents have been observed at high doses of MTBE, and endocrine activity has been theorized as a mode of action (MoA) responsible for some of these effects (Bird et al., 1997; McGregor, 2006; Cruzan et al., 2007). However, to date, there has been no comprehensive, detailed, and systematic review of the database on MTBE that weighs the evidence with attention focused on specific endocrine pathways. Given regulatory and public interest in endocrine active substances and their potential for causing adverse effects in humans or wildlife, resolving whether MTBE exhibits endocrine activity is important. The goal of this paper is to determine if MTBE acts through an endocrine MoA. To achieve this we used a robust and transparent WoE process. The WoE analyses performed were focused on specific hypotheses addressing the estrogen, androgen, and thyroid hormonal pathways and steroidogenesis, including an analysis of studies related to aromatase, the steroidogenic P450 enzyme that converts androgens to estrogens. Studies pertaining to both mammalian and non-mammalian organisms were considered and used in the WoE assessment if relevant to any of the hypotheses.

A WoE approach has been in the health risk assessment lexicon for many years but may have different meanings to different people (Weed, 2005). The US Environmental Protection Agency Endocrine Disruptor Screening Program (US EPA EDSP) has developed guidance for using a WoE approach to evaluate and integrate all relevant scientific and technical information from Tier 1 screening tests to draw conclusions about the need for further testing (US EPA, 2011). Others further recommend objective, systematic and structured hypothesis-driven approaches for WoE evaluations (Rhomberg et al., 2010; Bars et al., 2011, 2012; Borgert et al., 2011a,b, 2014). The WoE approach chosen for this analysis is semi-quantitative, based on ranking of the relevance of responses following a pre-defined framework that should be blind to the study outcomes, with an assessment of the reliability of data applied to each endpoint and study (Borgert et al., 2011a,b, 2014).

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Briefly, this involved systematic consideration of each endpoint observed in one or more study designs, and a semi-quantitative weighting of the relevance of each type of response to a given hypothesis, to reach scientifically justified conclusions based on the totality of the evidence. It is important to emphasize that, unlike some approaches that weigh relevance of an effect observed strictly on the basis of type of study design (e.g., *in vivo* vs. *in vitro*), this analysis is aimed at discerning which endpoints are the best predictors of mode of action. Study purpose, study design and the ability of the design to meet that intended purpose are also carefully considered in terms of reliability. In the end, conclusions were based on a simple classification system of pro- or anti-hormone effects, and then evidence for (or against) direct interaction with the endocrine system versus secondary effects of toxicity is discussed in more detail. Indirect effects of systemic toxicity, unrelated to a primary endocrine mode of action, are an important consideration for regulatory classification of any chemical, including MTBE.

This evaluation is timely since the US EPA has included MTBE on a list of chemicals to be screened under the EDSP. Listing is based on potential exposure and other statutory considerations, but does not imply that the US EPA suspects those chemicals of interacting with endocrine systems of humans or other species. Tier 1 of the EDSP uses a battery of assays to determine if a chemical has the potential to interact with the endocrine system. Identifying adverse effects is not the focus of the Tier 1 battery, and no single assay alone is sufficient to determine if there is the potential for the chemical to interact with the endocrine system. Further testing of those chemicals that show potential to interact would be needed to identify adverse effects and, with an understanding of relevant environmental exposure, quantify the risk.

The main focus on endocrine activity is not intended to imply that this should be viewed as the most important toxicological concern for MTBE. Acknowledging that many study endpoints can be affected by multiple modes of action is key to conducting an objective and scientifically sound WoE analysis (Rhomberg et al., 2010). The appearance of increased (or decreased) hormone-dependent cell growth, changes in hormone-dependent organ weights and histology, *in vitro* hormone receptor-ligand changes, altered hormone concentrations, include some of the observations that may collectively imply the potential for endocrine activity when there is sufficient strength and consistency of the responses. However, none alone is sufficient evidence of an endocrine mode of action, and these may also be affected by non-endocrine mechanisms, for example altered induced or diminished catalytic activity of liver enzymes that metabolize steroid hormones.

An objective evaluation of potential endocrine activity must also consider the fact that many toxicology studies administer doses of chemicals that far exceed possible occupational, consumer, or environmental exposures. This conservative approach to testing chemicals has some justification, but one must also always keep in mind that excessive doses of any chemical increase the probability of systemic toxicity and effects on endocrine endpoints mediated indirectly by other effects. Typical concentrations of MTBE in air and water are many orders of magnitude below the exposures tested in MTBE toxicology studies using experimental animals. Likewise, tissue concentrations of MTBE measured in human populations when MTBE was in widespread use are also many orders of magnitude below the concentrations tested in *in vitro* toxicology studies.

2. Methods

2.1. Literature search

National Library of Medicine search engines, PubMed and Toxnet, were used with the following keywords: methyl t(ertiary)-butyl ether, MTBE, tert-butyl methyl ether, 2-methoxy-2-methylpropane and toxicity. Open peer-reviewed literature was supplemented with additional unpublished and other 'grey' literature resources in comprehensive MTBE reviews (ATSDR, 1996; McGregor, 2006), as well as studies listed in the European Union risk assessment report (EU, 2002) and updated in the REACH submission (ECHA, 2012).

Maximal use of all existing relevant and sufficiently reliable information was a goal. Consistent observations in multiple studies and multiple species with different experimental designs can reduce unknown bias or confounding and increase support for or against a given mode of action hypothesis (Boobis et al., 2008). Risk assessors and others increasingly acknowledge that all of these types of studies can have merits, whether or not they were conducted with strict adherence to standard guidelines and good laboratory practices (GLP) (McCarty et al., 2012; Batke et al., 2013). All studies were first considered for relevance and then for reliability, if endpoints measured were deemed sufficiently relevant.

An MTBE study did not have to have an endocrine focus in order to be considered relevant. Results of reproduction/fertility and prenatal developmental toxicity studies that encompass different life stages, and also studies that provide carcinogenicity information on endocrine organs were all considered relevant to a consideration of endocrine activity (OECD, 2012). All endpoints associated with the endocrine pathways addressed by the hypotheses were considered relevant, including, for example, subchronic, or chronic general toxicology studies reporting gonadal, thyroid or hormone-dependent weights of organs or histopathology. If an MTBE study had an endocrine organ endpoint related to any of the hypotheses then it was included in the initial literature review.

A few studies were eliminated from consideration after the initial review of literature. For example, a study with exposure to gasoline vapor condensate with and without MTBE was not included due to the confounding effects of exposure to various gasoline components (Benson et al., 2011). Three *in vitro* studies (Li and Han, 2006; Li et al., 2007, 2009) using cells from a rodent endocrine organ, specifically mouse or rat testis, had no measured endpoints that could be related to the hypotheses so were also excluded before reliability scoring.

2.2. Data quality assessment

Considerable toxicity testing of MTBE was conducted during the 1980s (Duffy et al., 1992) before adoption of study protocols and guidance for evaluating the potential for endocrine activity. Some study designs were generally similar to a standard test protocol, but relatively few were similar enough to qualify as guideline studies in the context of current methods for evaluating endocrine activity. While it is appropriate to have greater confidence in studies that have employed standardized and validated test methods and were conducted according to GLPs (Becker et al., 2009; Borgert et al., 2011b; McCarty et al., 2012), a non-guideline study could still be considered reliable if the methods were sufficiently well-documented and the results transparently and thoroughly reported. A prevailing objective was to retain as many sufficiently reliable studies as possible for the WoE analysis.

The data quality discussion about primary, secondary and tertiary validity in Borgert et al. (2011a and supplement) was consulted, as were other consensus opinions on principles and processes for judging reliability and quality of study designs and data (Klimisch et al., 1997; Schneider et al., 2009; Tluczkiewicz et al., 2013). Sufficient transparency in the documentation (secondary validity) and classification by Klimisch score became one of the most important criteria, insofar as adequate documentation of study details is necessary for making further judgments about study and specific endpoint reliability for the WoE analysis.

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