Environmental Research 158 (2017) 598–609

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Quantitative meta-analytic approaches for the systematic synthesis of data and hazard identification: A case study of decreased pain sensitivity due to trimethylbenzene exposure

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ARTICLE INFO

Keywords:
Hazard identification
Neurotoxicity
Meta-analysis
Meta-regression
Human health risk assessment

ABSTRACT

Traditionally, human health risk assessments have relied on qualitative approaches for hazard identification, which involves weight of evidence determinations that integrate evidence across multiple studies. Recently, the National Research Council has recommended the development of quantitative approaches for evidence integration, including the application of meta-analyses, to help summarize and evaluate the results of a systematic review. In the meta-analytic approach, a pooled effect size is calculated after consideration of multiple potential confounding factors in order to determine whether the entire database under consideration indicates a chemical is a hazard. The following case-study applies qualitative and quantitative approaches to determine whether trimethylbenzene (TMB) isomers represent a neurotoxic hazard, specifically focusing on pain sensitivity. Following a thorough literature search, the only pain sensitivity studies available for TMBs initially seem discordant in their results: effects on pain sensitivity are seen immediately after termination of exposure, appear to resolve 24 h after exposure, and then reappear 50 days later following foot-shock. Qualitative consideration of toxicological and toxicokinetic characteristics of the TMB isomers suggests that the observed differences between studies are likely due to testing time and the application of external stressors. Meta-analyses and –regressions support this conclusion: when all studies are included and possible confounders (isomer, testing time, laboratory, etc.) are accounted for, the pooled effect sizes are statistically significant, thus supporting that TMBs are a possible neurotoxic hazard to human health. Ultimately, this case study demonstrates how qualitative and quantitative methods can be combined to provide a robust hazard identification analysis by incorporating more of the available information.

1. Introduction

Traditionally, risk assessments have relied on qualitative approaches for evidence integration to inform hazard identification. This involves synthesizing the available data, typically across multiple peer-reviewed studies on a particular outcome or organ system of interest, as well as considering other sources of information (e.g., toxicokinetic or mode of action information). These syntheses evaluate issues such as the consistency, coherence, and biological plausibility of the data, across the set of available studies, to draw inferences regarding causation (National Research Council, 2009, 2011, 2014). However, the NRC recently recommended the development of more systematic approaches for evidence synthesis and integration to improve the consistency and transparency of hazard identification (National Research Council, 2014). While systematic, qualitative approaches to assessing epidemiologic or toxicological data for the purposes of hazard identification will increase the transparency and reproducibility of these decisions, it can sometimes be difficult, and open to subjectivity, to gauge how limitations or differences across studies might impact risk-based decisions in the absence of quantitative comparisons.

Systematic review is a type of literature review that focuses on specific questions and uses clearly defined methods to identify, evaluate, and summarize data from a set of scientific studies (National Research Council, 2014). While systematic literature searches are widely used, systematic syntheses of the identified data are more novel in the basic sciences (i.e., preclinical, toxicological studies) (Vesterinen et al., 2014). Relatedly, recent recommendations (National Research Council, 2014) regarding methods used in the U.S. EPA Integrated Risk Information System (IRIS) have encouraged the development of quantitative methods for hazard identification, including meta-analytical

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http://dx.doi.org/10.1016/j.envres.2017.07.017
Received 20 December 2016; Received in revised form 6 July 2017; Accepted 7 July 2017
0013-9351/ Published by Elsevier Inc.

techniques. Such approaches could be used to summarize the results for particular health endpoints (e.g., an effect estimate using meta-analytic approaches) for sets of studies within and across specific data streams (i.e. epidemiologic, toxicological, and mechanistic data). For example, if it can be reasonably demonstrated that an effect estimate from a meta-analysis does not include the null, the conclusion would be a hazard exists.

The following case study considers exposure to trimethylbenzene isomers (TMBs) and potential effects on pain sensitivity. TMBs are aromatic compounds primarily produced during petroleum refining as a component of the C9 fraction, of which individual TMB isomers (i.e. 1,2,4-, 1,2,3-, and 1,3,5-TMB) make up approximately 45–55% (U.S. EPA, 1994). Uses for TMB isomers include solvents in research and industry, dyestuff intermediate, paint thinner, and as an ultraviolet oxidation stabilizer for plastics (HSDB, 2011a, 2011b, 2012). As such, occupational exposures to 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB are expected to occur in the oil/ gas extraction, special trades and printing and publishing industries, while exposures to the general public can occur with the use of motor vehicles (pumping gas, vehicle emissions) due to the use of the C9 fraction as a component of gasoline. 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB are also present in the soil and groundwater at a number of Superfund sites, and may be associated with contaminated drinking water due to hydraulic fracturing operations (U.S. EPA, 2016).

Although other potential neurotoxic effects of TMB exposures have been reported (U.S. EPA, 2016), pain sensitivity in animal toxicology studies was the most widely studied neurotoxic endpoint from this small database, and data were available following acute (< 1 day), short-term (~ 4 weeks), and subchronic (~ 13 weeks) exposure durations. Additionally, the TMB pain sensitivity database includes studies reporting potentially discordant results across studies of different durations and test chemicals (i.e., either mixtures of TMBs or individual isomers), thus allowing for an examination of the extent to which quantitative approaches may help integrate ostensibly incongruent evidence and improve conclusions regarding the potential for TMB-induced neurotoxicity. Given the characteristics of the database for this endpoint, this case-study focused on pain sensitivity data available from animal toxicology studies to demonstrate quantitative techniques that can inform hazard identification. This focused case study illustrates the utility of meta-analytical methods on a small set of studies to provide insights and an increased confidence in the application of such methods to future assessments with more complex, larger databases.

2. Methods

2.1. Literature search and data extraction

A publicly available, comprehensive literature search was performed in support of the IRIS Toxicological Review of Trimethylbenzenes (TMBs) (U.S. EPA, 2016): https://hero.epa.gov/hero/index.cfm/project/page/project_id/2375. Briefly, two separate literature searches (December 2011 and February 2016) for TMB isomers (i.e, 1,2,4-TMB [pseudocumene], 1,2,3-TMB [hemimellitine], or 1,3,5-TMB [mesitylene]), its metabolites, or defined mixtures containing TMB isomers (e.g., C-9 fraction, white spirit) and any health effect were carried out in multiple databases (PubMed, Web of Science, TOXLINE); additional targeted literature searches pertaining to specific modes of action, quantitative methods support, and guidance documents were performed and are reported in the results. (see Fig. 1 for details on the search and screening criteria). A total of 3565 references were located; after screening references for inclusion based on publication in a non-relevant journal (e.g., chemistry or physics) title/abstract (n = 3342 excluded) and then full-text screening (n = 73 excluded), a total of 150 references were cited in the Toxicological Review.[2] Of these 150 references, 38 animal toxicology studies were cited, including 15 neurotoxicological studies. From this subset of neurotoxicological studies, six references (Table 1) were identified for the current analysis, based on the following criteria:

- a duration of exposure most relevant to hazard identification for potential lifetime health effects[3] (i.e. either subchronic or short-term exposure; no chronic studies were identified and all acute studies were excluded due to a general incomparability in findings from these exposure scenarios to effects that might result from repeated, longer term exposures);
- investigation of effects on thermal pain sensitivity in laboratory animals (note that this endpoint was the basis for the risk estimates in the recently completed IRIS Toxicological Review for TMBs, although only qualitative methods were used to determine hazard);
- and exposure to a test agent for which the proportions of TMBs and other constituents were defined (e.g., studies on Aromatol or Farbasol, the compositions of which were not defined, were excluded).

From these six studies (see Table 1), dose-response data on thermal pain sensitivity, measured as latency to paw lick (in seconds) on the hotplate test (Ankier, 1974), was extracted from peer-reviewed publications. In addition, information on aspects of the experimental design such as exposure duration, exposure route, exposure concentrations, number and type of animals, time of testing, parameters (e.g., temperature) for performing hot plate measurements, randomization and blinding procedures, and use of additional manipulations was extracted. Finally, the research laboratory at which the experiments were performed was extracted, given that the majority of studies on this endpoint were conducted by the same research group.

2.2. Meta-analysis

A meta-analysis was performed to determine whether subchronic or short-term exposure to individual TMB isomers or the C9 fraction resulted in statistically significant pooled estimates of effect. When the pooled effect size is larger than zero and the 95% confidence intervals exclude zero, it can reasonably be inferred that a hazard exists (National Research Council, 2014).

A random effects model was chosen *a priori* to calculate pooled effect sizes across all included comparison groups. In order to include as many datasets as possible, effect sizes were based on the standardized difference of means, calculated as the difference in means of the control and exposure group divided by the pooled variance (see Supplementary material for more details). Individual comparison groups represented effects observed in different dose groups across studies, thus it was not expected that there was one “true” effect size, and that a fixed effects model would be inappropriate. The use of a random effects model corresponds to the assumption that the calculated effect sizes are drawn from a population of normally distributed of “true” effect sizes (Borenstein et al., 2007) and accounts for intra-study (sampling error) and inter-study (differences in true effect size) variability (Vesterinen et al., 2014). Multiple measures were calculated to characterize heterogeneity between studies: τ^2 as an estimate to “total” heterogeneity, I^2 as a measure of the amount of the total heterogeneity that is attributed to the heterogeneity across the individual group's “true” effects (Viechtbauer, 2010), and chi-squared p-value for the test of heterogeneity (Cochran, 1954; Viechtbauer, 2010).

[2] Note, this total does not include additional (n = 35) references included in the Toxicological Review at the request of the Scientific Advisory Board during external peer review or references from a third literature search on related alkylbenzene compounds, none of which were relevant to this case study.

[3] While this case study focused on potential health effects with a lifetime of exposure, this criterion would not be appropriate if the review encompassed potential hazards after acute exposure (e.g., for an accidental exposure).