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A meta-analysis of epidemiologic studies of occupationally exposed styrene workers and micronuclei levels

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ABSTRACT

Previous reviews and meta-analyses have reached different conclusions on whether high styrene exposure increase micronucleus frequencies. The most recent meta-analysis reports an increase in these frequencies related to styrene. We update this meta-analysis of micronucleus frequencies with additional studies not previously included, eliminate double counting of study subjects appearing in more than one publication, considered levels of styrene exposures in the analysis, assess publication bias, and examine consistency of findings across studies. Our meta-analysis used the standardized mean difference as the summary statistic since all studies assess the same outcome but use different comparison populations. The primary meta-analysis of the 12 studies of 516 styrene exposed workers and 497 non-exposed comparisons produces a meta-mean difference of 1.19 (95% CI 0.20–2.18, random effects model), but there was substantial heterogeneity across studies (I^2 of 97.47, p-value < 0.001, fixed effect model). We also observed that studies with higher styrene exposure had a higher mean standard difference compared with studies with lower styrene exposures. However, a longitudinal study did not find any association with styrene exposure and micronucleus frequencies. Given the lack of consistency across studies and the equivocal finding on exposure response, these data are insufficient to support a conclusion that an increase in micronucleus frequencies is due to styrene exposure.

1. Introduction

Styrene (C6H5CH = CH2, CAS number: 100-42-5) is a commercially important chemical used in making plastics, latex paints, coatings, insulation, synthetic rubbers and polyesters. In 2012, the annual global styrene capacity was estimated at over 32.7 million tons with 5 million tons produced in the United States [1]. The highest occupational exposures to styrene historically occurred in the reinforced plastic industry [2].

A recent evaluation by IARC concluded that styrene is probably carcinogenic to humans based on limited evidence in humans and sufficient evidence in experimental animals [3]. The National Academy of Sciences (NAS) conducted an independent review of The National Toxicology Program carcinogenicity review for styrene and concluded that styrene can reasonably be anticipated to be a human carcinogen based on limited evidence in humans and sufficient evidence in rodents [4,5]. Both the IARC and NAS reviews included summaries of the genotoxicity literature for styrene which dates from the 1970s. This literature includes studies conducted *in vitro*, *in vivo* and in occupationally exposed humans. While *in vitro* and *in vivo* rodent studies provide an assessment as to whether a chemical of interest has the potential to induce genetic damage, human studies are considered the most relevant for assessing human risk.

These reviews summarize studies for workers occupationally exposed to high levels of styrene and include assessments for biomarkers of exposure such as urinary metabolites of styrene, and biomarkers of biological effect based on genetic damage endpoints. The biological effects biomarkers were primarily evaluated in peripheral blood lymphocytes [2,4,5]. While a few of these biomarkers of biological effect studies include the evaluation of gene mutation, most of studies utilize cytogenetic assays such as micronucleus and chromosomal aberrations. We note that, another cytogenetic endpoint, sister chromatid exchange (SCE), has been widely used in styrene occupational studies and are included in the styrene exposure reviews. However, the fundamental mechanism underlying the induction of sister chromatid exchange (SCE) has been extensively studied and a recent expert review concludes that SCEs are not reflective of genetic damage and thus not biomarkers of biological effect, but rather are biomarkers of exposure [6].

The human study summaries for the genetic endpoints, based on the investigators' conclusions, report both positive and negative results for all the endpoints [2,4]. It should be noted that the methods for

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conducing the studies and analyzing the data for all the genetic endpoints have changed over the last several decades. Summary and critical reviews of the individual styrene human studies have also been conducted [7–10].

There has been a focus on the chromosome aberration and micronucleus endpoints as biomarkers of biological effect in occupational human studies for a variety of exposures and there have been attempts to link increased frequencies of these endpoints to cancer risk in humans. The results of these studies are inconsistent [11–16]. However, a 2007 evaluation by Bonassi et al. does provide some evidence that micronucleus frequency may be a predictive biomarker of cancer risk [12]. Thus, evaluating the evidence the styrene can induce micronuclei, particularly in humans, is relevant to understanding whether styrene can cause genetic damage in humans.

In 1996, Bonassi et al. undertook a meta-analysis of the published human cytogenetic studies for styrene [17]. In their publication, they clearly state that their goal was not to evaluate study quality, but to see if they could find any general quantitative trend between the levels of styrene exposure and the induction of the cytogenetic endpoints including micronuclei. They conducted a meta-analysis which calculated a frequency ratio for the exposed individuals compared to the unexposed individuals. They concluded that there was no association with level of micronucleus induction and styrene exposure.

Because the methodology has changed substantially for the conduct of the micronucleus assay since the first occupational styrene studies were performed in the late 1970s, a recent review and meta-analysis was conducted and published by Costa et al. using only studies in which the cytokinesis-block method was used [18]. This method uses cytochalasin B which allows the cellular nucleus, but not the cell, to divide. By only scoring cells that have two nuclei (binucleates), only those cells which might form micronuclei are scored and this assures that only first division cells are scored, providing a better assessment.

We update the meta-analyses of micronucleus frequencies of Costa et al. with additional studies not previously included, eliminate populations included in more than one publication, considered levels of styrene exposures in the analysis, assess publication bias, and examine consistency of findings across studies [18]. Our goal was to determine if the published studies provide evidence whether exposure to styrene can or cannot induce genetic damage assessed by micronucleus frequency in humans.

2. Materials and methods

2.1. Search strategy

This review followed guidelines of PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) [19] and MOOSE (Meta-analysis of Observational Studies in Epidemiology) [20]. Studies eligible for inclusion were micronucleus frequencies among styrene exposed workers that were published or identified from January 1, 1975, through February 15, 2018 in PubMed (https://www.ncbi.nlm.nih.gov/pubmed). Eligible studies indexed in PubMed were searched using two search string: (styrene and human and micronucleus) and (styrene and human and micronuclei).

2.2. Selection criteria

All abstracts returned from the PubMed search were reviewed to identify relevant references for evaluating the ability of styrene to induce micronuclei in humans. It was clear from the initial review of the abstracts that many of the papers did not actually contain micronucleus data from human studies and thus they were easily eliminated from further consideration in this first step. The results of this literature search were compared against five literature reviews [7–9,18], to assure that all relevant publications were included. This comparison resulted in the addition of two publications. These publications including micronucleus data were reviewed to identify only those publications using the cytokinesis-block method. We both read and evaluated all studies and extracted from each study the setting and design, exposure estimation, and identified potential confounders. We also examined study strengths and limitations.

2.3. Assessment of study quality

The assessment of methodological quality of each study considered study design, styrene exposure estimates, and the adequacy of the comparison group. Exposure to styrene is highest in the reinforced plastics industry [2]. Many of the studies in this review were done on workers in this industry. In addition, most of the studies examine both external exposure to styrene from industrial hygiene monitoring and internal exposures through biomonitoring. The specific details concerning timing of exposure assessment and the assessment of potentially confounding exposures varied within the studies.

There is normal variability in the frequency of micronuclei. This is true for all assays using the micronucleus endpoint (*in vitro*, rodent *in vivo* and human). A discussion of variability in the standard *in vitro* and rodent *in vivo* genetic toxicology assays can be found in the recent document generated by the OECD providing an overview of the considerations and changes that were made in updating the test guidelines [21]. Laboratory assays used for the micronucleus endpoint, for hazard identification are well controlled to limit variability. However, for humans, there are many factors based on genetic differences, age, lifestyle factors, diet, exercise, co-morbidities, exposure to X-rays, drug usage, use of tobacco products, occupational and home use of other chemicals, which impact the inherent variability in humans, aside from any high level of occupational exposure. The human studies that are being considered in this meta-analysis, in fact, show different degrees of variability within both the worker groups and the comparison groups. There is also a broad range and average of frequencies among the studies included. For instance, Maki-Paakkanen and colleagues [22] found the control frequency to be 12.0 ± 8.0 and the worker frequency to be 14.0 ± 6.0; these were not statistically different. Costa and colleagues reported the controls as a group to have micronucleus frequencies of 2.26 ± 0.20 (females: 3.71 ± 0.41 and males: 1.68 ± 0.18) and the workers to have frequencies of 2.63 ± 0.22 (females: 3.95 ± 0.46 and males: 3.95 ± 0.46) [23]; these were not statistically different. Laffon and colleagues reported 13.91 ± 0.81 for comparisons and 24.63 ± 1.49 for exposed workers [24]. These values, which are well outside the range of values observed in the other studies, were reported as statistically different.

To provide a perspective to these observed micronucleus frequencies, one needs to compare the frequencies with values observed in the literature for large numbers of people. Bonassi and colleagues, as a part of the Human Micronucleus Project study, compiled data submitted from 25 laboratories from 16 countries, using the cytokinesis-block methodology [25]. Although there were differences in methodologies (i.e., culture medium, amount of serum, concentration of Cytochalasin B, scoring criteria etc.), a reference range, based on approximately 7000 subjects was established. The overall median micronucleus frequency for “unexposed” individuals was 6.5 per 1000 cells scored. The interquartile range was between 3 and 12 micronuclei per 1000 cells. Factors which impacted the micronucleus level included age (seen in all but 2 laboratories), and gender (with females having a 19% higher level of micronuclei than males) [25].

Most of the studies that we identified for our review are cross sectional studies comparing frequencies of micronuclei between exposed workers and a comparison group. The cross-sectional design assumes that the potential genotoxic effects of styrene measured through biomonitoring would be immediate and for chronic exposure, they would be in a steady state. A better design would be a longitudinal study where internal exposure and micronucleus frequencies are measured before, during and after exposure to styrene. As done in a previous