# Exhibit 73

**December 9, 2024**

# General Causation Expert Report of KATHLEEN M. GILBERT, PHD

# TCE, PCE, benzene and bladder cancer

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# I. INTRODUCTION

I was asked to provide opinions concerning the role of trichloroethylene (TCE), perchloroethylene (PCE), benzene and other chemicals contributed to the development of bladder cancer in individuals who lived and/or worked at the Camp Lejeune Marine Base in North Carolina between 1953 and 1987. A summary of my qualifications as relevant to this report is described below, while my complete CV can be found in Appendix A. My previous trial and deposition testimony experience has been limited to one previous trial testimony, one hearing and seven depositions in unrelated cases (see Appendix B). If called as a witness, I could and would competently testify to the matters set forth in this report. All of my opinions are expressed to a reasonable degree of scientific certainty, and I reserve the right to amend these opinions should new information be made available to me. I may also provide supplemental opinions regarding this case if requested.

The contaminants of interest at Camp Lejeune site include TCE, PCE, vinyl chloride and benzene. These toxicants were present in the drinking water from approximately 1953 to 1987. My report will focus on TCE since that is the contaminant that has been the nexus of my own research. However, PCE and benzene will be discussed as sources of added toxicity.

# II. BACKGROUND AND QUALIFICATIONS

My qualifications as an expert witness stem from more than 35 years of experience as a scientist conducting bench research in the areas of immunology, immunotoxicology and human health toxicology. I have been funded by the National Institutes of Health (NIH) and the Environmental Protection Agency (EPA) to study the health impacts of adult and developmental exposure to TCE. I also received $1.5 million in grant money from the Arkansas Biosciences Institute for developing an Immunotoxicology Center in Arkansas. Based on my expertise I have been asked to review grants for the National Science Foundation, the EPA, and several study sections of the NIH, including the Superfund Basic Research (ZES1 LWJ-M), and Career Award Applications for the National Institute of Environmental Sciences.

I retired from the University of Arkansas for Medical Science (UAMS) as a tenured NIH-funded Full Professor in 2017. My work at UAMS was preceded by positions at The Scripps Research Institute in La Jolla, CA; the National Institute for Medical Research in London, UK; and Memorial Sloan-Kettering Cancer Center in New York, NY.

I have published 13 book chapters, and over 80 peer-reviewed publications, more than 30 of which are directly concerned with the health effects of TCE or its metabolites. Those publications examined:

- How TCE exposure at different life stages (fetal, early life, or adult) impacts its toxicity.[9-14]
- How TCE exposure impacts the immune system at the disease, tissue, cellular, genetic and epigenetic level.[9, 15-25]
- How co-exposure to other chemicals or life-style risk factors impacts TCE-induced toxicity.[14, 26]
- How TCE causes neurotoxicity.[11, 12]

- How TCE exposure alters the gut microflora in ways that may promote systemic toxicity.[27]
- How mathematical modeling can be used to examine TCE-induced immunotoxicity.[28]

My research on TCE-induced health effects is well regarded in the scientific community. For example, I was asked by the publishers of Springer/Humana Press to edit the 2014 book entitled *Trichloroethylene: Toxicity and Health Risks*, (Springer/Humana Press New York/Heidelberg). Springer/Humana Press has published a series of excellent books on how environmental toxicants impact human health. They are used to provide reliable information for the professional practice of environmental and natural scientists, as well as human and veterinary medicine.

In recognition of my expertise in TCE toxicity and human health I have been asked several times over the years by different federal agencies including the National Research Council, the National Academy of Sciences, and the NIH's National Toxicological Program to review various TCE-related health risk documents. These included: *Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues*, 2006; *USEPA TSCA Workplan Chemical Risk Assessment for Trichloroethylene: Degreaser and Arts/Crafts Uses,* 2013*;* a possible change in listing status for TCE in the *Report on Carcinogens*, 2014; and *DOD's Approach to Deriving an Occupational Exposure Level for Trichloroethylene,* 2019.

Lastly, I was nominated for and subsequently accepted a position as one of the founding members of the Scientific Advisory Committee on Chemicals (SACC) for the EPA from 2017 to 2021. The SACC is tasked with providing independent advice and expert consultation on issues related to the implementation of the Frank R. Lautenberg Chemical Safety for the 21st Century Act which amends the Toxic Substances Control Act (TSCA). We reviewed EPA risk evaluations for 10 chemicals between 2017 and 2021 including: **TCE**, **perchloroethylene** (**PCE)**, carbon tetrachloride, methylene chloride, 1-bromopropane, n-methyl pyrrolidone, 1,4-dioxane, pigment violet 29, and cyclic aliphatic bromide cluster. I was tasked with providing opinions on the EPA's assessment of the human health effects of these chemicals.

For preparing this report I have invoiced my time at the rate of $400/hour. For deposition and trial testimony my hourly rate is $500/hour.

## III. MATERIALS USED TO FORM OPINIONS

During my many years working with TCE and related compounds, I have read hundreds of studies concerning the health outcomes of TCE and PCE exposure, as well as studies that examined the relevant modes of action. My opinions about TCE and PCE toxicity are based in part on that cumulative literature review. Since it would be impossible for me to list all the manuscripts that I have read over the years I have worked on toxicants and human health the references cited in this report should be considered representative rather than exhaustive, and I reserve the right to call upon my exhaustive research and experience with TCE and PCE literature should I be called to testify in this case.

In addition to peer-reviewed published manuscripts, I also relied on the following federal reports:

*US EPA's Toxicological Review of Trichloroethylene* of 2011. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0199tr/0199tr.pdf

*Addendum to the Toxicological Profile for Trichloroethylene* published by the Agency for Toxic Substances and Disease Registry in 2013. https://www.atsdr.cdc.gov/toxprofiles/tce_addendum.pdf

*Morbidity study of former Marines, employees, and dependents potentially exposed to contaminated drinking water at US Marine Base Camp Lejeune;* Agency for Toxic Substances and Disease Registry, April 2018. https://www.atsdr.cdc.gov/sites/lejeune/docs/health_survey_report-508.pdf

*The importance of animals in the science of toxicology: Society of Toxicology Animals in Research Public Policy Statement, 1999* https://www.toxicology.org/pubs/docs/air/AIR_Final.pdf

*US EPA's Final Risk Evaluation for Trichloroethylene (CASRN: 79-01-6)* November 2020 https://www.epa.gov/sites/production/files/2020-11/documents/1._risk_evaluation_for_trichloroethylene_tce_casrn_79-01-6.pdf

*US EPA's Final Risk Evaluation for Perchloroethylene (Ethene,1,1,2,2-Tetrachloro) (CASRN: 127-18-4) December, 2020.* Final Risk Evaluation for Perchloroethylene CASRN:127-18-4 (epa.gov)

ATSDR Assessment of the Evidence for the Drinking Water Contaminants at Camp Lejeune and Specific Cancers and Other Diseases https://www.atsdr.cdc.gov/sites/lejeune/docs/atsdr_summary_of_the_evidence_for_causality_tce_pce_508.pdf

*US EPA's 2023 Trichloroethylene: Regulation Under the Toxic Substances Control Act* https://www.govinfo.gov/content/pkg/FR-2023-10-31/pdf/2023-23010.pdf

*Evaluation of Cancer Incidence Among Marines and Navy Personnel and Civilian Workers Exposed to Contaminated Drinking Water at USMC Base Camp Lejeune: A Cohort Study* http://medrxiv.org/content/early/2024/01/29/2024.01.27.24301873

Contaminated water supplies at Camp Lejeune. Assessing potential health effects *https://nap.nationalacademies.org/download/12618*

Risk Evaluation for Perchloroethylene (Ethene,1,1,2,2-Tetrachloro-) file:///C:/Users/gilbe/Downloads/EPA-HQ-OPPT-2019-0502-0058_CONTENT.PDF

Toxicological Profile for Benzene https://www.epa.gov/sites/default/files/2014-03/documents/benzene_toxicological_profile_tp3_3v.pdf

## IV. PROCESS USED TO FORM OPINIONS

The opinions described in this report are based upon my education, training, and experience as a scientist/toxicologist and are all made to a reasonable degree of scientific certainty. My opinions in this report were arrived at using the same methodology I employ in other projects such as conducting TCE research, preparing peer-reviewed manuscripts, writing research grants and assessing federal regulatory documents. As a matter of course, this includes making decisions about whether a particular toxicant is associated with a specific disease outcome.

In brief, my opinions were based on:

- Thirty-five years of experience as an immunologist, and 25 years of experience as an immunotoxicologist and human health toxicologist. This includes a well-developed understanding of correct scientific principles and methodologies.
- My own extensive peer-reviewed and NIH-funded research on TCE and disease outcomes.
- Experience on the SACC that included my invited professional review of the *EPA 2020 Risk Evaluation for Trichloroethylene*[29] and the *EPA 2020 Risk Evaluation for Perchloroethylene*.[30]

As identified above my causation opinions and methodology for this report are supported in part by the *EPA 2020 Risk Evaluation for Trichloroethylene*, and the *ATSDR (Agency for Toxic Substances Disease Registry) Assessment of the Evidence for the Drinking Water Contaminants at Camp Lejeune and Specific Cancers and Other Diseases*. [29, 31] As discussed in greater detail throughout my report, I independently assessed the sources relied upon by these agencies in reaching my opinions in this case.

To form their causation opinions both agencies used a weight-of-evidence (WOE) approach. The WOE is defined as "*a systematic review method, applied in a manner suited to the nature of the evidence or decision, that uses a pre-established protocol to comprehensively, objectively, transparently, and consistently identify and evaluate each stream of evidence, including strengths, limitations, and relevance of each study and to integrate evidence as necessary and appropriate based upon strengths, limitations, and relevance.*" (https://beta.epa.gov/sites/default/files/2018-06/documents/final_application_of_sr_in_tsca_05-31-18.pdf) The EPA WOE approach integrates data from epidemiological studies, animal studies and mechanistic studies. I also employ a WOE approach in reaching my opinions in this case, which is standard practice of experts, including myself, in my fields of expertise.

To meet the TSCA science standards for the literature reviews included in their reports, EPA used the TSCA systematic review process described in the *Application of Systematic Review in TSCA Risk Evaluations* (https://19january2021snapshot.epa.gov/sites/static/files/2018-06/documents/final_application_of_sr_in_tsca_05-31-18.pdf). This process complements the Risk Evaluation process in that the data collection, data evaluation and data integration stages of the systematic review process are used to develop the exposure and hazard assessments based on reasonably available information.

It is important to note that the conclusions reached by the EPA do not represent the opinion of a single individual or a single agency. As required by TSCA, the EPA 2020 *Final Risk Evaluation*

*for Trichloroethylene* [29] was reviewed by the members of the SACC. This committee contains wide-ranging expertise, including risk assessors, epidemiologists, statisticians, toxicologists, and industry representatives. I was one of the SACC members that participated in the review of TCE. The evaluation was held in a public meeting in which anyone could register to make comments. Cumulatively, dozens of scientists and non-scientists weighed in the evaluation and general consensus was reached regarding TCE causation. For these reasons, it is my opinion that the conclusions reached by the EPA, and which I describe here, are carefully reasoned and scientifically valid.

The causation evaluations conducted by the EPA and ATSDR were based on modified Bradford Hill Criteria. In 1965 Professor Hill gave a talk in which he described nine "viewpoints" to consider while determining disease causation.[32] This list included temporality of exposure, strength of exposure, dose-response determination, plausibility, elimination of alternative explanations, consistency/reproducibility, experiment (e.g. alleviation of toxicity by preventing or removing exposure), coherence, and specificity. These common-sense considerations are not a strict checklist, as recognized by Professor Hill when he said "None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a *sine qua non*." Although the Bradford Hill criteria as a framework for critical thinking continues to be very useful, the field of toxicology has evolved to include new technologies and new fields of study (e.g. molecular toxicology). A modified Bradford Hill approach reflects these changes and is more comprehensive in its inclusion of all applicable data (animal studies, *in vitro* studies and epidemiological studies).

Similar to the EPA and the ATSDR I used a modified Bradford Hill approach to derive my opinions. This means I considered results from epidemiological, animal and mechanistic (sometime *in vitro*) studies. The importance of these three components in risk evaluation will be described below.

With respect to the causation standard I employed in reaching my opinions, I have reviewed the Camp Lejeune Justice Act (CLJA) and am aware the causation standard under the CLJA explains that Plaintiffs in this case must show "the relationship between exposure to the water at Camp Lejeune and the harm is—(A) sufficient to conclude that a causal relationship exists; or (B) sufficient to conclude that a causal relationship is at least as likely as not." *ATSDR Assessment of Evidence*, referenced above, considers the "at least as likely as not" standard to be the functional equivalent of its category for "equipoise and above." Although my opinions in this case are expressed to the higher "more likely than not" standard, the ATSDR's definition of "equipoise and above" served as a guidance for me in this case.

### A. Importance of epidemiological studies

Epidemiological studies examine the direct real-life relationship between exposure to a particular toxicant and its associated toxicity. They are very useful in providing proof of concept that exposure to a particular toxicant is associated with a specific pathology. This approach is obviously relevant for human risk evaluation and circumvents the need to extrapolate animal or *in vitro* exposure levels to human equivalence.

Although very useful for proving causation, epidemiological studies are limited in terms of documenting precise toxicant exposure and disease outcome, and in terms of providing different tissues and cell types for confirmatory mechanistic information. They are also much more likely to examine occupational rather than environmental exposure because the former is far easier to document. And in many cases people are often unaware of their environmental exposures until many years later, if ever. If epidemiology studies do exist and provide relevant information, they are often looked at as the highest potential level of evidence in determining causal relationships. Thus, although human studies provide the highest level of evidence when determining causation, they are often augmented by animal and *in vitro* models, which can circumvent some of the limitations of epidemiological studies.

**B. Importance of animal studies**

As stated in the Society of Toxicology Animals in Research Public Policy Statement: "In the absence of human data, research with experimental animals is the most reliable means of detecting important toxic properties of chemical substances and for estimating risks to human and environmental health." https://www.toxicology.org/pubs/docs/air/AIR_Final.pdf

*"If there is sufficient human data to describe the exposure-response relationship for an adverse outcome(s) that is judged to be the most sensitive effect(s), reference values should be based on human data. If sufficient human data are not available, data from animal studies must be employed with appropriate interspecies and intraspecies extrapolation factors."* https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf

*Animal models have many advantages when examining chemical toxicity*:

- Toxicant exposure is carefully administered and monitored. This makes it easier to evaluate dose-dependent and time-dependent effects. Such studies would obviously be unethical in humans.
- Animal models can evaluate the effects of toxicant exposure on specific life stages (i.e., infants and the elderly). Unlike human exposure, animal models greatly expand the types and numbers of samples (blood and multiple tissues) that can be collected and examined.
- Animal models have advantages over the use of cultured cells, even if human in origin, to study toxicity. For example, any disease with immune system involvement, which includes myriad conditions including bladder cancer, involves multiple cellular interactions. It is currently impossible to mimic the complex interactions that cause immunotoxicity in humans using individual populations of cultured cells. Instead, a sophisticated physiological system, such as can only be found in an intact animal, is needed to recapitulate complex diseases such as cancer.

Mice and humans share over 90 percent of the same genes, and are strikingly similar in terms of anatomy, physiology, and drug metabolism. The vast number of chemicals identified in the environment or introduced as commercial products have never been tested for carcinogenicity in humans. Unlike clinical trials that test chemicals with intended therapeutic value, using human subjects to test the carcinogenicity these other chemicals has long been considered unethical. Animal models provide a crucial avenue for such testing.

Information obtained in mouse models is very often relevant to humans. For example, immune-based cancer vaccines and new cancer therapeutics known as immune checkpoint inhibitors, which are widely used to treat many types of cancer, including bladder cancer, were initially developed in mouse models before being successfully translated into human clinical trials.

Thus, animal models, especially mouse models, recapitulate human systems very well, and are often used to estimate the effects of a toxicant on human health.

### C. Importance of mechanistic studies

In addition to information obtained from epidemiological studies and animal mod els,mode of action (MOA) evidence is important to a WOE causation determination. Defining MOA (sometimes called mechanism of action or adverse outcome pathway) means identifying key events between exposure and pathology. Mechanistic research elucidates the cellular, biochemical, and molecular basis of chemical toxicity. The EPA's 2014 *Framework for Human Health Risk Assessment to Inform Decision Making* discussed the importance of identifying MOA in the risk assessment process.
https://www.epa.gov/sites/default/files/2014-12/documents/hhra-framework-final-2014.pdf

Understanding the MOA can inform risk assessment decisions by:

(i) Supporting a designation of causation by defining a biologically plausible pathway between exposure and pathology
(ii) Confirming relevance of data in animals or *in vitro* for human health
(iii) Harmonizing evaluations for various health endpoints
(iv) Defining conditions under which a chemical is likely to cause an adverse effect
(v) Helping to generate pharmacologic and non-pharmacologic strategies to counteract the adverse outcomes of chemical exposure.

Lastly, mechanistic information uncovered while studying a specific toxicant can help unravel complex mechanisms of disease onset and progression and define the role of environmental insult in diseases considered idiopathic.

Defining MOA often uses complimentary toxicokinetic and toxicodynamic evaluations.

**Toxicokinetics** includes information about the absorption, distribution, metabolism, and excretion of a toxicant. Toxicokinetic data for many chemicals are determined by using PBPK (physiologically based pharmacokinetic) modeling. This is a widely used and widely accepted mathematical modeling technique for predicting the absorption, distribution, metabolism and excretion of toxicants in humans and other animal species. PBPK models can be used to convert animal exposures into human equivalent exposures.

**Toxicodynamics** defines the processes by which a toxicant, once it has been absorbed, distributed and metabolized, interacts with the body to cause adverse effects. Toxicodynamic data is derived from human, animal and *in vitro* studies. The EPA also uses mechanistic data derived from *in vitro* experiments (some of which use human cell types as targets) when synthesizing evidence for causation.

## V OPINION 1. THE CONTAMINANTS IN THE DRINKING WATER AT CAMP LEJEUNE MORE LIKELY THAN NOT CAUSE BLADDER CANCER

Bladder cancer, also known as urothelial cancer, is the 4th most common cancer in men and 9th most common in women in the Western World. According to the National Cancer Institute the annual incidence is 18.2/100,000. https://seer.cancer.gov/statfacts/html/urinb.html The term bladder cancer refers to several types of cancer that occur when epithelial cells that line the bladder become malignant. The association between bladder cancer risk and having a first-degree relative with bladder cancer (OR=1.8; 95% CI 1.2-2.9) indicates that bladder cancer relies more on environmental than genetic contributions.[33] Exposure to environmental and work-place related chemicals is one of the most important extrinsic risk factor for bladder cancer.[34] Cigarette smoking constitutes one of these environmental risk factors. As will be shown in Table I, exposure to PCE, TCE and benzene constitutes another source of environmental stress that causes bladder cancer.

This causation determination was based on epidemiological studies, animal studies, mechanistic studies, and the conclusions of federal agencies such as the EPA and ATSDR.

### A. Epidemiological studies

I began my epidemiological review of TCE, PCE, benzene and bladder cancer by reviewing those studies identified by the 2017 ATSDR Assessment of the Evidence. I also reviewed the EPA 2020 Risk Evaluation of Trichloroethylene and the EPA 2020 Risk Evaluation of Perchloroethylene which included epidemiological studies that had been published between 2017 and 2020. I also examined the 2007Toxicological Profile for Benzene https://www.epa.gov/sites/default/files/2014-03/documents/benzene_toxicological_profile_tp3_3v.pdf I found the ATSDR's and EPA's methodology for reviewing the epidemiology to be thorough and scientifically-valid based on my years of experience and training as a scientist.[35] https://www.epa.gov/sites/default/files/2015-07/documents/lit-studies.pdf Lastly, I used PubMed (the free online NIH-sponsored database of medical science studies) to identify studies on TCE and PCE published between 2020 and 2024 and for studies on benzene published after 2007.. Any new federal reports or regulatory documents published since 2020 were also included in my literature search.

For inclusion in the epidemiological assessment of bladder cancer shown here studies had to demonstrate (1) a temporal relationship between chemical exposure and negative health effect (i.e. exposure precedes toxicity) and (2) convincing positive associations represented by a Risk Ratio (RR), Odds Ratio (OR), Standardized Mortality Ratio (SMR), or Standardized Incident Ratio (SIR) greater than 1.1, (3) biological plausibility, (4) adult exposure (unless otherwise specified), and (5) exposure durations of more than one month.

Once those basic requirements have been met epidemiological studies were further assessed to best identify those studies with the highest utility. In line with ATSDR standards the studies were evaluated for:

1. **Proper Controls**. Data quality in epidemiological studies requires the use of proper controls. For example, it would be more appropriate if the results obtained from Marines exposed to TCE at Camp Lejeune were compared to non-exposed Marines at another base who might be expected to have a similar fitness baseline as compared to potentially less healthy civilians.

2. **Confounding bias.** All the studies presented here were evaluated for whether possible confounding variables such as exposure to smoking or other risk factors were taken into account.
3. **Exposure assessment**. Most of the occupational exposure studies used some kind of Job Exposure Matrix (JEM) to estimate TCE exposure. As noted, some studies used actual on-site measurements or bio-monitoring to estimate exposure.
4. **Likely exposure.** Since exposure miscalculation can be lessened if the results were observed in the population determined to be the most likely to be exposed to the highest concentrations of toxicant or exposed for the longest duration this data was chosen for representation.
5. **Dose-duration relationship.** Since an exposure-response relationship adds extra weight in risk determination all the studies described here were evaluated for this criterion.
6. **Incidence vs mortality**. Since mortality can be caused by multiple factors unrelated to cancer diagnosis; incidence determinations are less likely to be mischaracterized and are noted here.
7. **Meta-analysis**. A meta-analysis is given extra weight since it represents the statistical analysis of multiple studies. Its pooled approach to data evaluation can lessen the biases and errors of individual studies.

Included in the epidemiological studies table is one of the studies conducted for the ATSDR by Bove *et al.* which examined bladder cancer incidence in both civilians and Marines who were exposed to the contaminated water at Camp Lejeune. In addition to the ORs for the highest or most likely TCE exposure, any reported data concerning the lowest duration or exposure level to cause disease (threshold info) were included in the tables.

**Table I. Epidemiological studies of contaminant exposure and bladder cancer**

| Studies | Type | OR, RR, SIR,SMR | 95% CI | Exposure | Described threshold | | Confounder adjustment | Dose-duration | Incidence vs Death | Meta-analysis |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (ppb) or Duration (mean) | OR + 95% CI | | | | |
| Anttila[36] | Occup. Cohort | TCE 1.51 | 0.18-5.44 | Monitor TCA levels | >20 yrs since first measurement | | | | ✓ | |
| Aschengrau[37] | Environ. Case-Con | PCE 4.03 | 0.65-25.1 | Survey JEM | | | Smoking, other solvents | | | |
| Blair[38] | Occup Cohort | TCE Mortality 2.0 M Incidence 1.7 M | 0.6-6.4 0.6-4.4 | | | | | | | |
| Boice[39] | Occup. Cohort | | | JEM | < 10 years VOC 1.4 | 0.95-2.0 | | | | |
| Bove[40] | Environ. Retro Cohort | VOC Marines 1.2 Civilians 1.18 | 0.94-1.52 0.8-1.75 | Historical measures | 7-10 quarters Marines 1.18 >21 quarters Civilians 1.18 | 0.95-1.46 0.8-1.75 | Smoking, alcohol, age | | ✓ | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Calvert[41] | Occup. Cohort | PCE 2.59 | 1.24-4.76 | JEM | >5 years 4.08 | 2.13-7.12 | | | | |
| Hadkhale[42] | Occup. Case-Con | | | | TCE >129.5 ppm-yr 1.25 M<br>PCE 14-88 ppm-yr 1.3 F<br>Benzene 15 ppm-yr 1.21 F | 1.1-1.4<br>1.0-1.7<br>1.0-1.6 | Age, smoking | | ✓ | |
| Hansen[43] | Occup. Cohort | TCE 1.21 M | 0.91-1.58 | JEM Measured TCA | Average 5.9 years exposure | | | | ✓ | |
| Lynge[44] | Occup Nested Cohort | PCE 1.44 | 1.07-1.93 | JEM Measured | Mean = 10-100 mg/m$^3$ Duration 2-4 years 2.39 | 1.09-5.22 | Smoking Alcohol Age | | | |
| Morgan[45] | Occup. Cohort | TCE Internal cohort 2.71<br>Meta-analysis 1.15 | 1.10-6.65<br>0.78-1.62 | JEM | | | | | | ✓ |
| Pesch[46] | Occup. Case-Con | TCE 1.6 M<br>PCE 1.8 M | 1.0-2.5<br>1.1-3.1 | JEM | | | Age, smoking | | | |
| Raaschou-Nielsen[47 47] | Occup. Cohort | TCE 1.6 | 0.93-2.57 | JEM Historical measures | | | | | | |
| Ruder[48] | Occup. Cohort | PCE 3.15 | 1.51-5.79 | JEM | >5 years 4.31 | 1.85-8.76 | | ✓ | | |
| Sciannameo[49] | Occup. Case-Con | TCE 1.3<br>PCE 1.4 | 1.0-1.72<br>0.62-3.16 | JEM | | | Age, smoking | | ✓ | |
| Shala[50] | Occup. Case-Cohort | Benzene 1.26 | 0.86-1.86 | JEM | <5.5 Yrs 1.18 | 0.74-1.89 | Age, smoking | | | |
| Vlaanderen[51] | Occup. Cohort + Case-Con | PCE Cohort 1.46<br>Case-Con 1.5 | 1.14-1.87<br>0.8-2.84 | | | | Smoking | | | ✓ |
| Xie[52] | Occup. | Benzene 1.63 | | JEM | | | Smoking | | ✓ | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Case-Con | | | | | | | | | |
| Zhao[53] | Occup. Cohort | TCE Incidence 1.98 Mortality 1.27 | 0.93-4.22 0.43-3.73 | JEM | | | Smoking | | Both | |

F: Female; M: Male; VOC: undifferentiated solvents

### B. Animal studies:

Although there are numerous animal studies showing that TCE causes cancer in the closely associated kidney [54] [55] the ability of TCE or the other contaminants to cause bladder cancer specifically has not been tested.

### C. Mechanism of action:

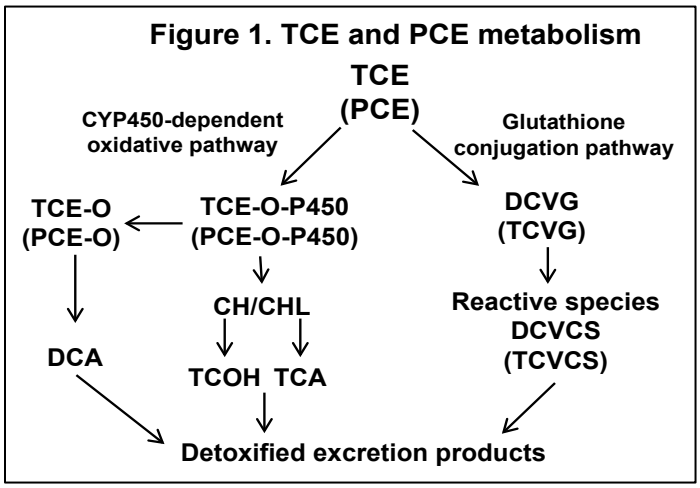
Demonstrating a biologically plausible mechanism is an important part in assigning causation. Consequently, mechanistic information will be included here.

Most, if not all, of the toxicological effects of TCE require its metabolism.[56] [57] Similar to TCE, almost all the adverse effects of PCE can be attributed to it metabolites. Metabolic conversion of benzene is also required for its toxicity, at least in terms of its hepatotoxicity.[58] Since their metabolism is an important part of their toxicity, the discussion of how these TCE, PCE, and benzene trigger cancer growth will be preceded by a brief description of their metabolism.

#### 1. TCE and PCE metabolism

As small lipophilic (fat soluble) chemicals TCE and PCE can easily cross biological membranes regardless of whether exposures occur through a dermal, oral or inhalation route. Following their absorption TCE and PCE rapidly partitions into blood by binding to soluble components such as lipids. Once in the bloodstream TCE and PCE are widely distributed throughout the body but quickly transition to the two main sites of metabolism, the liver and the kidney. Toxicokinetic data indicate that TCE and its metabolites are present in both human blood and urine, and have been found in multiple mouse tissues (blood, urine, kidney, liver, bone marrow, and lymphoid tissues) following TCE exposure. [59] Similarly, PCE is widely distributed in blood and across all tissues tested, including the lung, liver, heart, kidney, and brain. [60] Both TCE and PCE have also been measured in human breast milk.[29, 61]

After transitioning to the liver both PCE and TCE are subject to oxidative metabolism by cytochrome P450s, most predominantly CYP2E1. The first step in the oxidative metabolism of TCE and PCE is the formation of an unstable intermediate TCE-O-P450 or PCE-O-P450 that then leads to the generation of reactive metabolites chloral hydrate (CH) [aka trichloroacetaldehyde hydrate (TCAH)] and chloral (CHL). CH/CHL is reduced by aldehyde dehydrogenases or P450s to trichloroethanol (TCOH) or oxidized to trichloroacetic acid (TCA).




Alternatively, TCE-O-P450 can be converted to TCE-epoxide (TCE-O). TCE-O can form dichloroacetyl chloride (DCAC).trichloracetaldehyde) **(Figure 1**).

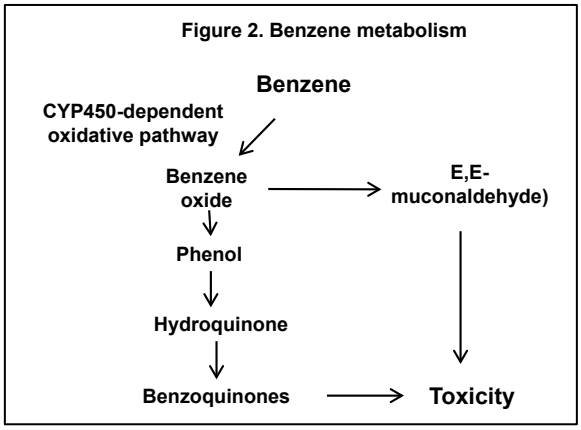
The secondary pathway for TCE and PCE metabolism involves conjugative metabolism with glutathione-S-transferases (GSTs). Conjugation is a process that generally leads to detoxification. However, that is not the case for TCE, PCE and many other halogenated compounds which get converted into harmful reactive metabolites. GSTs conjugates di- and trichlorovinyl-L-glutathione (DCVG and TCVG) form in the liver and then transition to the kidney where they are cleaved into di- or trichloro-vinyl-L-cysteine (DCVC or TCVC). These products can then be enzymatically converted to reactive toxic metabolites such as DCVCS [(1,2-dichlorovinyl)-L-cysteine)] or TCVCS [(1,2-trichlorovinyl)-L-cysteine)]. All metabolites are eventually transformed into detoxified excretion products.

Although the TCE and PCE metabolic pathways are similar, there are differences with regard to rates and tissue distribution that will not be discussed here.

**Benzene metabolism**

Although benzene metabolism has been studied extensively, the steps that lead to different types of toxicity toxicity are not fully understood. However, it is generally believed that both cancer and non-cancer endpoints are caused by one or more of the reactive metabolites of benzene.

Like TCE and PCE benzene is largely dependent on the oxidative pathway mediated by CYP2E1 (**Figure 2**). Some benzene oxide is also reacted with GSH to form S-phenylmercapturic acid.




## 2. TCE, PCE and benzene genotoxicity

Carcinogenesis is a multi-step process that starts with conferring tumor clonality, i.e. the ability of single cells to begin to proliferate abnormally. This transformation is crucial to tumor growth and is quite different from the carefully-controlled and limited proliferation of normal cells. Tumor clonality is thought to require DNA-specific damage known as genotoxicity (which may or may not involve mutagenicity). The National Toxicological Program (NTP has determined that TCE is a “known human carcinogen.” https://ntp.niehs.nih.gov/sites/default/files/ntp/roc/content/

profiles/tricloroethylene.pdf Both the EPA and the International Agency for Research on Cancer (IARC) agree that TCE is carcinogenic to humans.

EPA has classified PCE as *likely to be carcinogenic in humans by all routes of exposur*e. https://iris.epa.gov/static/pdfs/0106tr.pdf The International Agency for Research on Cancer has classified PCE as a Group 2A carcinogen, which means that it is *probably carcinogenic to humans*. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identificatio-Of-Carcinogenic-Hazards-To-Humans/Trichloroethylene-Tetrachloroethylene-And-Some-Other-Chlorinated-Agents-2014 The U.S. National Toxicology Program (NTP) has classified PCE as *reasonably anticipated to be a human carcinogen*. https://ntp.niehs.nih.gov/sites/default/files/ntp/roc/content/profiles/ tetrachloroethylene The National Institute for Occupational Safety and Health (NIOSH) recommends handling PCE in the workplace as if it were a human carcinogen. https://www.cdc.gov/niosh/docs/78-112/default.html

Benzene is classified as a "known" human carcinogen (Category A) under the Risk Assessment Guidelines of 1986. Under the proposed revised Carcinogen Risk Assessment Guidelines (U.S. EPA, 1996), benzene is characterized as a known human carcinogen for all routes of exposure based upon convincing human evidence as well as supporting evidence from animal studies. https://iris.epa.gov/ChemicalLanding/&substance_nmbr=276

The EPA has labeled TCE as a genotoxic chemical, at least with regard to kidney cancer.[29] Although the MOA for PCE-induced cancer appears to be complex a genotoxic MOA is supported by the evidence of kidney-specific genotoxic metabolites as well as evidence of PCE genotoxicity in humans from epidemiological studies.[61] *In vivo* and *in vitro* data from both humans and animals indicate that benzene and/or its metabolites are genotoxic.

Both TCE and PCE have been shown to cause genotoxic events. Sister chromatid exchange was increased in workers chronically exposed to TCE, and in human lymphocytes exposed to TCE oxidative metabolites.[62, 63] Similarly, lymphocytes from workers exposed to TCE demonstrated a highly significant increase in frequency of chromosomal structural aberrations (breaks, gaps, translocation, deletions, inversions) and hyperdiploid cells.[64] A recent review of cancer risk in commercial laundry and dry cleaning industries evidence of DNA damage in blood lymphocytes from PCE, even below set occupational limits.[65] Numerous studies, both *in vivo* and *in vitro* suggests that the TCE and PCE metabolite CH/CHL causes genotoxicity such as mutations and chromosome aberrations.[66] One study found that a significant increase in micronuclei in the peripheral blood lymphocytes of infants sedated by oral exposure to CH/CHL.[67] Chromosomal aberrations (hypo- and hyperdiploidy, deletions, breaks, and gaps) in peripheral lymphocytes and bone marrow cells are the predominant genotoxic effects seen in humans following benzene exposure. https://www.epa.gov/sites/default/files/2014-03/documents/benzene_toxicological_profile_tp3_3v.pdf

The ability of TCE, PCE and benzene to cause genotoxicity means that these chemicals can initiate the carcinogenic process. Although the role of TCE- and PCE- and benzene-induced genotoxicity in causing bladder cancer specifically has not been studied the genotoxicity of TCE has been documented in the physically proximal kidney where these chemicals get metabolized. The reactive metabolites are supposed to be acetylated (and thereby rendered non-toxic) before

they leave the kidney, but it is possible that some reach the bladder where the long exposure duration can cause genotoxic events in the urothelial cells in the bladder.

In addition to their genotoxicity, there is also evidence that the three chemicals can further support cancer development via immunotoxicity. This mechanism will be discussed in Opinion 2. These MOA provide biological plausibility and further support the causal relationship between PCE, TCE and benzene and bladder cancer.

### D. Agency conclusions regarding TCE, PCE, and benzene and bladder cancer:

**TCE:**

**ATSDR**: ATSDR concluded that there was not sufficient evidence for causation.

**USEPA Risk Evaluation of Trichloroethylene (2020).** The USEPA concluded that there was some evidence of association for bladder cancer and high cumulative TCE exposure, but that the reasonably available studies examined multiple sites and did not completely account for potential confounding factors. [29]

**PCE:**

**ATSDR**: Sufficient evidence for causation.[31]

**USEPA IRIS Toxicological Review of Tetrachloroethylene (Perchloroethylene) (2012):** "The epidemiologic evidence provides a pattern associating tetrachloroethylene exposure and several types of cancer, including bladder cancer, non-Hodgkin's lymphoma and multiple myeloma." "In conclusion, the pattern of results from this collection of studies is consistent with an elevated risk for tetrachloroethylene of a relatively modest magnitude. "[68]

**USEPA Risk Evaluation of Perchloroethylene (2020). "**There is some evidence for bladder cancer and multiple myeloma (MM) but results are mixed." [29]

**International Agency for Research on Cancer (IARC): "**The bladder and oesophagus may be target tissues for tetrachlorethylene-induced carcinogenesis in humans; however, there were no studies to suggest mecahnisms underlying these effects." [60]

**Benzene**:

**ATSDR**: ATSDR concluded that there was not sufficient evidence for causation. It should be noted that the evidence for benzene exposure as a risk factor for bladder cancer has been published since the 2017 ATSDR evaluation.

### E. Summary

There is epidemiological evidence that exposure to PCE, TCE and/or benzene can promote the development of bladder cancer. This opinion is supported by the determination by the ATSDR that there was sufficient evidence for causation for PCE-induced bladder cancer. It is also supported by the Bove *et al*. study that actually showed an increase in bladder cancer incidence in the Marines and civilians from Camp Lejeune.[40] In addition, the high-quality meta-analysis

demonstrated a statistically significant association between PCE exposure and bladder cancer.[51] Not all studies found an association between TCE or PCE and bladder cancer. However, the numerous studies that have reported an association between these chemicals and bladder cancer, together with the ATSDR assessment [31], and the results of the meta-analysis provide WOE for a more-likely-than-not causation determination. The connection between bladder cancer and these chemicals has not been studied in animal models. Although there are no MOA studies that address bladder cancer specifically, the ability of TCE, PCE, and benzene to cause genotoxic effects provides biological plausibility.

While scientific studies have yet to demonstrate a level of TCE or PCE exposure below which cancer risk goes to zero, epidemiological studies (Table I) do provide evidence that TCE and PCE, at levels of exposure comparable to and lower than those encountered at Camp Lejeune, can cause cancers, including an increased incidence of bladder cancer.

## VI. ROLE OF IMMUNE SYSTEM IN MEDIATING BLADDER CANCER

Immune dysfunction, encompassing both generalized inflammation and tumor-specific suppression, is one of the biggest mediators of bladder cancer etiology. Many of the intrinsic and extrinsic risk factors identified for bladder cancer such as increased age, smoking, and male gender are associated with T cell immune suppression and/or chronic inflammation. Similarly, there is evidence that in addition to their genotoxic effects TCE and PCE also promote bladder cancer via immunotoxicity.

The immune system is crucial for fighting infection and preventing cancer. The importance of a correctly working immune system in combating cancer cannot be overstated. Cancer treatment is undergoing a renaissance due to the recent profound clinical successes of tumor immunotherapy. Thus, understanding more about how immune system dysfunction occurs holds incredible promise to advance our ability to fight this disease.

Before describing how the immunotoxic effects of TCE can promote tumorigenesis (OPINION 2), the crucial role of the immune system in mediating cancer in general, and bladder cancer specifically, will be discussed.

### A. Summary of Immune System

The immune system is a network of cells and their soluble mediators. The cells of the immune system can be found in the circulation as well as in specific immune organs such as the spleen and lymph nodes. Anything that can trigger a response from the cells of the immune system is called an antigen. Most antigens encountered by humans are harmful foreign molecules such viral proteins, bacterial toxins, and tumor cells.

Like a good security system the immune system is supposed to prevent intruders from accessing your home. The immune system is comprised of both “innate” and “adaptive” components. When the intruders (e.g cancer cells) trigger your security alarm, the Innate system acts as the first stage of protection (dogs barking) while you wait for the police (adaptive immune response) to arrive. Anything that disrupts either of those protective elements can increase cancer cell access.

The innate immune system is comprised of cells such as macrophages, dendritic cells, neutrophils and natural killer cells. These cells are considered somewhat immune non-specific in that they do not have receptors that recognize specific antigens. The cells of the innate immune system travel to the site of injury (e.g. infection or tissue damage) and provide the first line of defense via the production of soluble mediators known as cytokines or chemokines.

The adaptive immune system provides the second and more specific line of defense against antigens. It is comprised primarily of B cells and T cells (which consist of CD4+ T cells and CD8+ T cells). Unlike the cells of the innate immune system, all B cells and T cells have on their surface a unique receptor specific for one particular antigen, such as a specific tumor marker. Antigen-specific B cells and T cells travel to the site of antigen introduction, guided in part by the presence of the innate system soluble mediators. Once the B cells interact with their specific antigen they begin to make antibodies, soluble proteins that bind to and destroy soluble antigens or indirectly act to promote the death of antigen-expressing cells. Antigen-triggered $CD8^+$ T cells, often called cytotoxic T cells, can kill cancer cells expressing tumor antigens or non-cancerous cells infected by intracellular pathogens. Antigen-triggered CD4+ T cells, often called helper T cells, provide crucial support for B cell antibody production. The effector function of activated CD4+ T cells and $CD8^+$ T cells can involve direct cell-to-cell contact or the release of soluble mediators such as cytokines.

## B. Immune System and Tumorigenesis

### 1. Immune surveillance

The innate and adaptive immune system work together to prevent cancer through a mechanism known as **immune surveillance.** Immune surveillance is an ongoing process by which the immune system surveys the body for tumors and then eliminates them. Once a cell such as a bladder cell has been transformed (e.g. by genotoxicity) into a cancer cell and begins proliferating it often upregulates tumor-specific antigens which make it a target for the immune system. Unfortunately, the targeted anti-cancer immune response is not always successful. This is due to bidirectional and sometimes flawed interactions between the immune system and cancer cells known as **cancer immunoediting**. During cancer immunoediting the host immune system shapes tumor growth in three phases, elimination, equilibrium and escape:

#### (a). Elimination

In the elimination phase proliferating cancer cells expressing tumor antigens are destroyed by a competent immune system. This represents a coordinated attack by the innate and adaptive immune system against cells expressing tumor antigens. The cells of the innate immune system can kill tumor cells through the release of soluble mediators (e.g. perforin and granzyme). The adaptive immune response can cause tumor cell killing directly through $CD8^+$ T cell cytotoxic mechanisms or indirectly through different CD4+ T cell-mediated mechanisms or via the generation of natural anti-tumor antibodies.

#### (b). Equilibrium

If not all the tumor cells are destroyed during the elimination phase of cancer immunoediting tumorigenesis may enter into the equilibrium phase. In this phase, which can last months if not years, the immune system prevents the outgrowth of the tumor but is unable to eliminate all the tumor cells. Unfortunately, the selective pressure to evade elimination by the activated immune cells can drive the tumor cells to evolve through mutations or epigenetic alterations into cells with mechanisms that allow them to circumvent immune system control.

**(c). Escape**

The escape phase represents the final phase of tumorigenesis where the tumor cells that have developed to evade the immune system grow progressively and develop into a clinically apparent disease. This involves the establishment of an immunosuppressive microenvironment in the tumor that discourages further destruction by CD4+ and CD8+ T cells.

**2. How immune surveillance goes wrong during development of bladder cancer**

**(a) Chronic inflammation**

In order for the immune system to work correctly to fight infection or prevent cancer the innate and adaptive immune system must maintain a delicate balance of immune activation and deactivation. Temporary inflammation is a crucial part of the immune response to antigen and subsequent tissue repair. The immune response is supposed to be proportional to the threat, and to subside once the threat has been resolved. However, when the immune response is not resolved (cancer is not eliminated) or when the innate immune system is artificially stimulated by extrinsic factors it can lead to a sustained state of **chronic inflammation** that is more harmful than protective. Chronic inflammation has been shown to be a contributing factor to cancer, cardiovascular disease, diabetes and other metabolic diseases, chronic kidney disease, and neurodegenerative disorders.[69]

Chronic inflammation is an important driver of bladder cancer and provides support for tumor progression, metastasis and anti-cancer resistance.[70] Inflammation in bladder cancer is mediated in large part by soluble factors such as IL-6 and TNF-$\alpha$.[70] Both of these soluble factors are considered pro-inflammatory cytokines that are made by macrophages of the innate immune system, and which can also be generated by bladder cancer cells and other cells in the tumor microenvironment (TME). Increased levels of these pro-inflammatory cytokines, whether in the TME or serum correlate with bladder cancer recurrence and progression.[71-73] Both can initiate signaling that promotes tumor proliferation, survival, immune evasion and/or metastasis.[74] The functional importance of these cytokines is underlined by the fact that a treatments that targeted the IL-6 pathway impaired disease progression in a mouse models of bladder cancer.[75] The ability of TCE to increase the levels of these pro-inflammatory pro-tumorigenic cytokines will be described in Opinion 2.

Closely related to inflammation is oxidative stress, which can be either causal or secondary to inflammation. Oxidative stress is considered the cellular imbalance between antioxidants and oxidants [reactive oxygen species (ROS)]. ROS are a byproduct of mitochondrial respiration during normal cell function and can serve as important signaling molecules in normal physiology. However, an imbalance between the formation and destruction of ROS can occur leading to excess intracellular accumulation of ROS, a condition known as oxidative stress. Oxidative stress also plays a major role in cancer development where it can promote initiation, angiogenesis, invasiveness, and metastasis.[76] Oxidative stress generates peroxynitrate which a

mutagenic compound that is thought to cause DNA mutations in proliferating cells. Patients with bladder cancer displayed increased levels of multiple markers of oxidative stress.[70] There are several ongoing experimental and clinical studies involving antioxidants as potential agents against oxidative damage in bladder cancer development and progression by forming preventive or repair systems.[77] The ability of TCE to increase oxidative stress will be discussed in Opinion 3.

**(b) Immunosuppression of adaptive immune response**

In addition to chronic inflammation, the opposite side of the immune spectrum, namely immune suppression, also plays a crucial role in the development of bladder cancer. In the case of bladder cancer, the activity or inactivity of intratumor CD4+ T cells appears to predict tumor growth. [78] The importance of a functioning immune system in battling bladder cancer is underlined by the fact that the predominant treatment for bladder cancer is intravesical instillation of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG).[79] Although BCG appears to have multiple anti-tumor effects, the efficacy of BCG treatment has been most clearly linked to activation of the adaptive immune response including increased tumor infiltration of CD4+ T cells, increased T cell cytotoxic activity and increased systemic production of IFN-γ. [80] The ability of TCE to induce immune suppression will be discussed in Opinion 2.

The functionally ineffectiveness of anti-tumor T cells in patients with bladder cancer may be linked in part to their inability to secrete significant levels of IFN-γ in response to tumor antigen stimulation. IFN-γ. IFN-γ is a pleiotropic cytokine that is primarily secreted by CD4+ T cells and CD8+ T cells. Although it has some pro-tumorigenic effects, IFN-γ also has many anti-tumor effects. It can: (a) act a cytotoxic cytokine that initiates tumor cell death, (b) increase the ability of macrophages and dendritic cells to activate anti-tumor T cells, and (c) inhibit the angiogenesis needed for tumor growth.[81] [82] [83] The success of *Mycobacterium bovis* BCG immunotherapy in the treatment of bladder cancer has been associated with increased production of systemic IFN-γ. [80]. The ability of TCE to alter levels of IFN-γ will be discussed in Opinion 2.

## VII. OPINION 2. TCE, PCE AND BENZENE CAUSE IMMUNOTOXICITY THAT CAN PROMOTE CANCER DEVELOPMENT

### A. Epidemiological Studies

Table II lists the epidemiological studies that have examined the ability of TCE to cause immunosuppression and/or inflammation. These were all cross-sectional studies, some of which compared TCE-exposed workers to unexposed workers, and some of which compared unexposed workers to those that had been diagnosed with TCE-induced hypersensitivity disease. The studies evaluated a variety of immunological endpoints best represented as specific values such as μg/ml of a serum immunoglobulin or percentages of specific cell types. Consequently, the data was not presented as Odds Ratios which is better suited to comparing yes/no disease incidence in two or more populations. In some cases, the endpoints in a published study were presented as bar graphs or as box and whisker plots. This is not unusual for this kind of data but necessitated describing the relative differences between controls and TCE-exposed rather than presenting actual number values.

| Table II Epidemiological studies of TCE and immunotoxicity | | | | | |
|---|---|---|---|---|---|
| **Study** | **Endpoints** | **TCE exposure estimates (ppm)** | | | **Other** |
| | | Controls <0.03 | <12 (mean=5.2) | >12 (mean=38.4) | |
| Lan *et al*. [1] | Total T cells<br>CD4+ T cells<br>CD8+ T cells<br>B cells<br>NK cells | **Per ml blood**<br>1356±374<br>675±200<br>544±216<br>227±133<br>467±279 | **Per ml blood**<br>1310±391<br>664±220<br>508±175<br>194±99<br>370±148 | **Per ml blood**<br>**1124±346 (p=0.0001)**<br>**577±192 (p =0.004)**<br>**430±150 (p=0.007)**<br>**152±68.1 (p=0.001)**<br>**282±145 (p=0.002)** | N=80 Exposed, N=96 Controls<br>Only selected workers from factories that used TCE but had no or negligible levels of other chlorinated solvents |
| Hosgood *et al*. [2] | Effector memory CD4+T cells<br>Naïve CD4+ T cells<br>Naïve CD8+ T cells | **Per ml blood**<br>225±93<br>283±126<br>216±117 | **Per ml blood**<br>**183±55 (p=0.014)**<br>293±142<br>212±101 | **Per ml blood**<br>**184±89 (p=0.001)**<br>**236±113 (p=0.017)**<br>**152±93 (p=0.0001)** | Same cohort as Lan *et al*, 2013 |
| Lee *et al.* [3] | Serum IgG<br>Serum IgM<br>Effector memory CD4+T cells | **mg/ml**<br>10.99±2.97<br>1.18±0.820.<br>$10^3$ cells/μl blood<br>224.9 + 92.9 | **mg/ml**<br>**9.24±1.66 (p=0.008)**<br>**0.76±0.34 (p=0.0008)**<br>$10^3$ cells/μl blood<br>**181.8** + 56.7 **(p=0.042)** | **mg/ml**<br>**8.9±2.15 (p=0.002)**<br>**0.71±0.37 (p=0.002)**<br>$10^3$ cells/μl blood<br>184.5 + 86.1 | Same cohort as Lan *et al*, |
| Lehmann[4] | Cord blood T cell cytokine production | | **Maternal exposure PCE < 7.3 μg/m³ indoor air**<br>**IFN–γ:** 3.6 % of cord-blood T cells | **Maternal exposure PCE 7.3 μg/m³ indoor air**<br>**IFN-γ: 2.6 % of cord-blood T cells** | Environmental exposure in indoor air. Adjusted for smoking and other VOC exposures. |
| Zhang *et al*. [5] | Serum IgG<br>Serum IgM | N/A<br>N/A | **Decreased**<br>**Decreased** | **Decreased**<br>**Decreased** | Box and whisker plots; no values, but indication of statistical significance |
| | | **Controls** | **TCE-induced disease** | | |
| Li *et al*. [6]<br>Compared patients with TCE-induced hypersensitivity to controls with no disease or exposure | Neutrophils<br>Eosinophils<br>Basophils | **$10^9$/L**<br>3.53±1.08<br>1.1±1.7<br>0.18±0.21 | **$10^9$/L**<br>**7.75±6.8 (p=0.021)**<br>**0.14±0.19 (p=0.013)**<br>**0.02±0.01 (p=0.002)** | | **Saw clearly doubled increase in TNF-a - producing CD4+ T cells: just bar graphs, no values.** |
| Kamijima *et al*. [7]<br>Compared patients with TCE-induced hypersensitivity to controls | Serum levels of TNF-α | % more than 3 SD above mean control values<br>4% | % more than 3 SD above mean control values<br>**79% (p=0.01)** | | N=28 with disease, N=48 Controls<br>Measured end of shift TCA levels, but no estimates for TCE |
| Jia et al. [8] | Serum levels of:<br>IL-1β<br>TNF-α<br>IL-6 | | | | **Significantly increased serum levels of all three cytokines represented as bar graphs and dot plots** |

Bolded values are significantly different from controls. P-values were provided for these values.

The epidemiological studies described how TCE exposure generated immune suppression as indicated by (a) decreased serum levels of IgG and IgM, and (b) decreased blood levels of lymphoid populations including CD4+ T cells, CD8+ T cells, B cells and NK cells. Although this report is focused on adult exposure it is interesting to note that *in utero* exposure to TCE (267 ppb) in contaminated well water statistically increased the childhood incidence of leukemia, kidney/urinary tract infections and lung/respiratory infections.[84] Aside from markers of immune suppression indications of inflammation such as significantly increased levels of TNF-$\alpha$, IL-1$\beta$ and IL-6 were found in workers exposed to TCE.

Studies which looked at the effects of PCE on the immune system were relatively few compared to TCE and focused primarily on models of atopy and hypersensitivity rather than immune suppression. However, one study showed that maternal exposure to environmental levels of PCE (7.3 $\mu g/m^3$ in indoor air) significantly reduced the percentage of IFN-$\gamma$-generating cells from neonatal T cells in cord blood. [4] Similar to TCE, exposure to PCE increased serum markers of oxidative stress and proinflammatory cytokines (i.e. TNF-a) in dry-cleaning workers.[85]

A recent systemic review provided new evidence that human exposure to benzene causes immunotoxicity.[86] Based on multiple human studies the authors concluded that benzene increases proinflammatory makers such as TNF-$\alpha$ and IL-6, while suppressing the adaptive immune system, particularly targeting CD4+ T cells and B cells. They proposed that the carcinogenic effects of benzene are related to impaired immunosurveillance.

### B. Animal studies:

Inhalation exposure to TCE in mice has been shown to enhance susceptibility to pulmonary infection, an appropriate endpoint for immune suppression.[87] Similarly, mice exposed to TCE in drinking water showed significantly decreased cell-mediated immunity and bone marrow stem cell colonization.[88] High-dose exposure to TCE for 4 weeks caused a 70% decrease in antigen-induced antibody production in Sprague-Dawley rats. [89] PCE has similarly been shown to have immunosuppressive effects: a single 3-hr inhalation exposure to PCE decreased bactericidal activity by 9% and doubled mortality in mice with an experimental respiratory streptococcus infection.[90] Lastly, benzene exposure can also suppress the T cell-mediated aspects of immune surveillance; inhaled benzene reduced the cytolytic capacity of T cells and increased the lethality of tumor inoculation.[91]

Like the findings in humans, studies in animal models have shown that TCE can alter the number, function and/or phenotype of lymphoid cell population in blood, spleen and thymus. [88, 89, 92]

Although these chemicals can cause immunosuppression in animal models, they can also cause inflammation. Mice sensitized to dermal TCE exposure showed dramatic increases in the levels of IL-6, IL-1$\beta$ and TNF-$\alpha$ in the kidney. [93] The functional significance of these TCE-induced inflammatory mediators was evident when a TNF-$\alpha$ inhibitor was shown to relieve liver damage in TCE exposed mice.[94] PCE has similarly been shown to increase expression of IL-6 in a mouse macrophage cell line. [95]

### C. Mechanism of Action for Immunotoxicity:

The function of immune cells, similar to almost all cells, is largely mediated via gene expression. We and others have shown that TCE-induced changes in IFN-γ levels reflect a corresponding change in expression of *ifng*. [21, 96] We have linked the TCE-induced change in gene expression to at least two mechanisms: (a) binding to T cell proteins in a way that triggers transcription factor activation and downstream gene expression, and (b) inducing long-term epigenetic alterations that help control gene expression.[15, 17 18 9, 19, 20] Both of these TCE mechanisms have subsequently been confirmed in humans. [6, 97, 98, 99]

The fact that TCE generates epigenetic alterations in both humans and animal models provides a mechanism to explain why TCE effects can remain even after exposure cessation.[10] Along these lines occupational TCE exposure was found to increase aging in terms of epigenetic alterations in lymphocytes by two years compared to chronological aging [100] The effects of PCE exposure on epigenetics has not been investigated. However, my former colleague Dr. Sarah Blossom recently showed that exposure to CH/CHL *in vitro* altered the methylation of CD4+ T cell genes, most importantly the gene for IFN-γ.[101] Since CH/CHL is a metabolite of both TCE and PCE, this finding provides further evidence that these toxicants can mediate immunotoxicity via epigenetic alterations. Benzene can also mediate epigenetic changes. Workers exposed to benzene at 1.35 ppm demonstrated increased epigenetic changes in their peripheral blood lymphocytes.[100] A transcriptomic analysis of peripheral blood lymphocytes from workers exposed to benzene at >1 ppm identified DNA methylation changes in genes that mediate the immune response and pro-inflammation.[102]

### D. **Federal and International Agency Conclusions**:

**USEPA Risk Evaluation of Trichloroethylene (2020): "**Overall, immunotoxicity in the form of both autoimmunity and immunosuppression following TCE exposure are supported by the weight of evidence." The regulatory values derived from a mouse study of immunosuppression [87] were used by the EPA to evaluate the potential risk to workers by TCE exposure**.** [29]

**USEPA RISK Evaluation of Perchloroethylene (2020**): "Despite some lack of precision and uncertainties in the results of immunotoxicity studies, evidence suggests PCE exposure may lead to immunotoxicity."

### E. Summary

TCE, PCE and benzene have been shown to alter several immune parameters in humans and animal models that are also seen in bladder cancer. They are remarkably similar in their ability to suppress aspects of the adaptive immune response while stimulating pro-inflammatory cytokines such as IL-6 and TNF-α. Studies in animal models confirm these chemicals cause immunosuppression and chronic inflammation. All three chemicals can alter the epigenetics of peripheral lymphocytes in human; a finding that provides biological plausibility for their immunotoxicity. Since both immunosuppression and inflammation are crucial mediators of bladder cancer, the ability of PCE,TCE and benzene to promote these processes provides a clear mechanism by which they can cause bladder cancer.

The ATSDR studies of Camp Lejeune by Bove *et al*. did not include any immune endpoints. However, it is my opinion that it is more-likely-than-not that immunotoxicity would occur at the monthly median levels of TCE contamination found in the drinking water at Camp Lejeune. The EPA has designated the immune system as the most sensitive non-cancer endpoint as evidenced by their focus on immune system endpoints for the derivation of regulatory values.[29] In mouse models levels of TCE below that of occupational exposure cause functional immune system disruption.[10] This strongly suggests that the chemical exposure at Camp Lejeune was sufficient to trigger immunotoxicity that promoted development of bladder cancer.

## VIII. OPINION 3. CONTAMINANT-INDUCED OXIDATIVE STRESS CAN AUGMENT IMMUNOTOXICTY IN PROMOTING BLADDER CANCER

### A. Background

As mentioned above, oxidative stress appears to be important in bladder cancer etiology. [70] [77] Thus, the ability of TCE, PCE and benzene to induce oxidative stress represents another MOA by which they can mediate bladder cancer.

As mentioned above, an imbalance between the formation and destruction of ROS can lead to a condition known as oxidative stress. Excess ROS can cause cell damage through harmful reactions with proteins, lipids and DNA. Elevated levels of ROS disrupt cellular homeostasis and contribute to the loss of normal cellular functions, thereby promoting the initiation and progression of various types of cancer.[103] Aside from promoting cell transformation, reaction oxygen species can also promote tumor development by suppressing tumor-specific T cells.[104]

### B. TCE- PCE- and benzene-induced oxidative stress

An exposure-dependent association between TCE exposure and systemic markers of oxidative stress has been reported in a mouse model.[105] Oxidative stress has been implicated as an intermediate in DCVC-induced DNA damage following TCE exposure. [106]

Indicators of oxidative stress in TCE-exposed mice include increased levels of lipid peroxidation-derived aldehydes such as malondialdehyde, and higher serum iNOS and nitrotyrosine.[107] Signs of TCE-induced oxidative stress have been found in multiple tissues including liver[108], placenta[109], gut[110], embryonic heart[111], and kidney [112] and spleen.[113] Thus far, TCE-induced oxidative stress has been associated with kidney damage [112], skin hypersensitivity [114], functional neurotoxicity [115], autoimmune disease[116], liver cancer[117], and hematological cancers.[118] DNA damage mediated by TCE-induced oxidative stress has been reported as a mechanism for TCE genotoxicity in liver cells.[8]

Oxidative stress is not just a by-product of TCE exposure; it appears to be important in mediating TCE toxicity; anti-oxidant treatments protect against cardiac toxicity, dermal hypersensitivity, and autoimmunity caused by TCE exposure. [116, 119, 120]

Less is known about the role of PCE- and benzene-induced oxidative stress in their pathology. However, they both have been shown to stimulate this pro-cancer process. PCE exposure

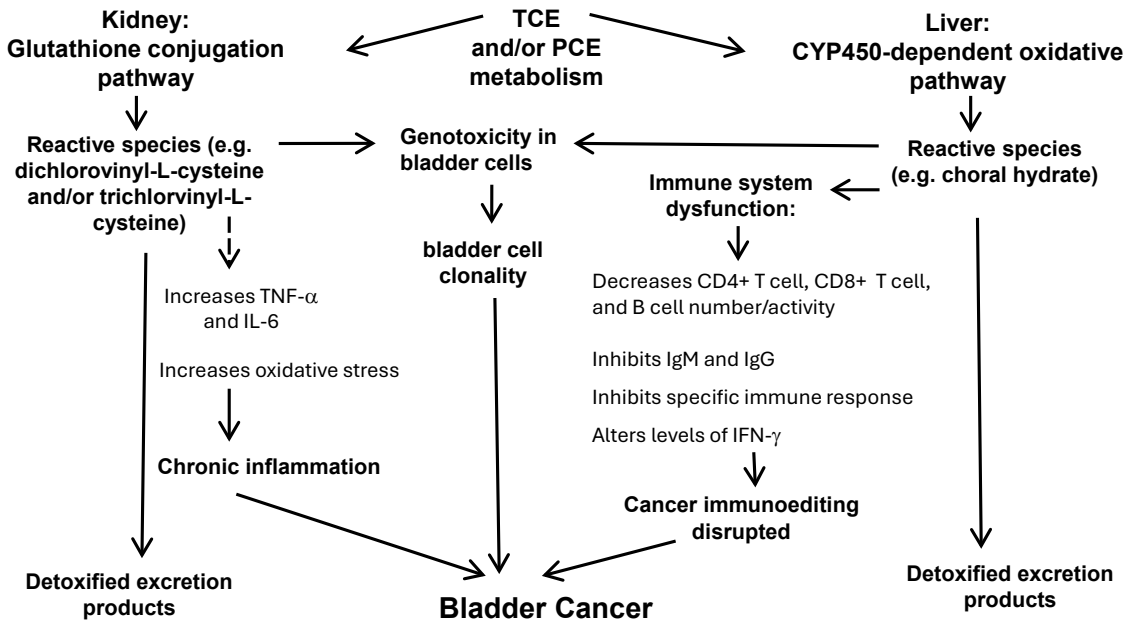
increased oxidative stress markers in the serum and peripheral blood cells of dry-cleaning workers.[85, 121] 8-Oxo-2'-deoxyguanosine (8-Oxo-dG) is one of the major products of DNA oxidation, and concentrations of 8-Oxo-dG within a cell are a measurement of oxidative stress. Peripheral blood lymphocytes from workers exposed to benzene showed a dose-dependent increase in the levels of 8-Oxo-dG.[122]

Since the toxic effects of oxidative stress do not appear to be cell specific, it is more likely than not that the oxidative stress induced by TCE, PCE and benzene could promote bladder cancer indirectly by effects on lymphocytes (immunotoxicity) or more directly by effects on bladder epithelial cells.

## Summary: Model of TCE- and/or PCE-induced bladder cancer

Taken together, it seems likely that TCE- PCE- and benzene-induced oxidative stress represents another mechanism, together with genotoxicity, chronic inflammation, and inhibitory effects on T cells in  a multi-component process by which these contaminants can cause bladder cancer. Since more is known about the role of TCE and PCE metabolites in causing toxicity these two chemicals were included in a single model of bladder cancer etiology ( Figure 2). There is still not enough information concerning the mechanisms by which benzene mediate its toxicity.  Even though the steps leading to these effects are still being delineated, it seems likely that the benzene-induced endpoints of oxidative stress, immune suppression and chronic inflammation work together in a model similar to TCE and PCE to promote bladder cancer.

**Figure 1. Model for TCE and/or PCE-induced bladder cancer**



Lastly, the ability of TCE and PCE to promote bladder cancer may be linked to their ability to cause kidney toxicity. Both TCE and PCE cause kidney damage. As noted by the EPA : "*Overall, based on effects seen in multiple studies in both animals and humans, kidney toxicity following PCE exposure is supported by the weight of the scientific evidence.*"[30] Similarly the EPA stated: "*The kidney is one of the more sensitive targets of TCE, with toxicity resulting from conjugative metabolites such as DCVC. Both animal and human studies have observed induction of kidney toxicity (e.g., damage to renal tubules and nephropathy) and progression of existing kidney disease. Overall, kidney toxicity following TCE exposure is supported by the weight of evidence."*[29] There is considerable evidence that kidney disease is associated with increased incidence of bladder cancer.[123-125] Although the mechanism is unknow the strong link between kidney toxicity and bladder cancer provides another mechanism by which TCE and PCE promote bladder cancer.

# IX. OPINON 4. LEVELS OF TCE AND OTHER CONTAMINANTS AT CAMP LEJEUNE WERE HAZARDOUS TO HUMAN HEALTH

When assessing the likelihood that a toxicant causes pathology, duration, temporality, and concentration are usually considered. These will be addressed regarding the Camp Lejeune plaintiffs.

## A. TCE exposure duration

A long-term cohort cancer incidence study by Bove *et. al* compared Marines and civilians who had lived at Camp Lejeune with those that had lived at Camp Pendelton that did not have contaminated drinking water. [40] Both civilians and Marines showed an increase in the incidence of bladder cancer. The Marines in the study served an average of 18 months on the base. The non-military personnel worked at the base for an average of 29 months.

Although the ATSDR did not study the exposure duration at Camp Lejeune required to cause disease, they did say that epidemiological data did not contradict a minimum duration of 30 days.[31] They went on to say; "Moreover the results from the Camp Lejeune mortality studies suggest that a 30 day minimum duration requirement may be appropriate since the elevated risks for some of the disease evaluated were observed for exposure durations of 1-3 month."

A recent evaluation of the Camp Lejeune data similarly concluded that based on the concentrations of contaminants at the base, just one month of exposure was sufficient to increase the likelihood of developing cancer.[126]

All of the plaintiffs in this case were exposed to the contaminants at Camp Lejeune for longer than one month.

### TCE Disease latency

When evaluating causality, the time between exposure and the onset of disease is another factor to consider. When discussing the limitations of the ATSDR's 2024 examination of cancer incidence among Camp Lejeune personnel, Dr. Bove noted that the median age of personnel at the end of the follow-up examination was only 57 years.[40] Dr. Bove went on to note that for the cancers best known for their associated with occupational TCE exposure (e.g. NHL, and

cancers of the kidney and liver) the median ages at diagnosis are 67, 64 and 65 years, respectively.

Bladder cancer is a slow developing cancer and can take decades to present in many patients. For example, researchers have found that the latency period after first exposure to a carcinogen and bladder cancer diagnosis can exceed 26 years.[127]The average age of people when they are diagnosed with bladder cancer is 73. https://www.cancer.org/cancer/types/bladder-cancer/about/key-statistics.html Thus, it is likely that the incidence of bladder cancer and other cancers for the people at Camp Lejeune would have increased if the follow-up had been extended.

A long lag time between chemical exposure and disease diagnosis is not unusual. A meta-analysis of three Nordic cohort studies examined risk of cancer following 5.5 – 6.3 years of occupational TCE exposure. Even after a lag period of 20 years, TCE exposure increased the risk of liver cancer (SIR 2.09; 95% CI 1.34-3.11).[43] Similarly, many of the increased cancers diagnoses in the Marines and civilians that lived or worked at Camp Lejeune were detected in the cohort studies after significant lag periods.

### TCE exposure level

The ATSDR reconstructed the VOC concentrations in the drinking water at Camp Lejeune.[128] The agency reported the levels of TCE at the Hadnot Point water treatment plant reached a maximum average of 783 μg/L, compared to a maximum measured value of 1,400 μg/L, during the time period between August 1953 and December 1984. The Holcomb Boulevard water-distribution contained maximum concentrations of TCE of 32 μg/L prior to 1972 and an estimated maximum concentration of 66 μg/L between 1972 and 1985. Levels of PCE at the Tarawa Terrace water treatment plant reach a maximum monthly level of 215 μg/L, and a maximum monthly level of 183 μg/L, and exceeded the EPA MCL (5 μg/L) from November 1957 and February 1987. These levels of TCE and PCE in the drinking water were hazardous to human health and known to cause cancer.

In addition to reviewing the ATSDR modeling reports that are publicly available I have reviewed the summary tables of Plantiff's expert Morris Maslia. These levels are hazardous to humans generally and have been shown to cause bladder cancer.

There is no one methodology that clearly defines what concentration of TCE, PCE or benzene causes bladder cancer. The epidemiological studies in Table I provide evidence that TCE, PCE and benzene cause bladder cancer. However, most of these studies did not attempt to define the minimum exposure or minimum duration to cause bladder cancer. Exposure-duration studies conducted to assess hazards are confined to drug clinical trials. It would be unethical to conduct similar studies using known toxicants. Instead, we are left estimating exposure levels from epidemiological studies that used often ill-defined occupational exposure data. Thus, many of the epidemiological studies in Table I compared results from TCE-exposed to non-TCE exposed, or devised relative exposure classifications (e.g. low, medium, high) without defining specific concentrations. However, there have been some epidemiological studies that examined environmental exposures and described specific exposure levels and durations. For example, Bove *et. al* noted that increased incidence of bladder cancer at Camp Lejeune was associated with exposures of 7-10 quarters for Marines and > 21 quarters for civilians.[40] A second cohort study showed an increase in bladder cancer (OR 2.39, 95% CI 1.09-5.22) following an occupational exposure to 10-100 mg/m$^3$ PCE for 2-4 years. [44]

Although values obtained from epidemiological studies are given the most weight, a second way to discuss the relationship between human exposure levels and increased bladder cancer incidence involves the use of regulatory values such as the oral slope factor. While not directly related to causality, such data points can be referenced in an overall evaluation of the likelihood that a particular exposure caused a disease outcome. Regulatory values are derived by the EPA based on cumulative exposure-response data from epidemiological and animal studies. They are designed to estimate future risk from a chemical in order to inform mitigation decisions. They were not developed to determine causation in a specific individual. Using estimates of future risk does not fit an analysis for determining causation for individuals who already have cancer because the risk of someone who has cancer getting cancer is of course 100%. However, when kept in proper context, these values can be referenced to confirm that a known chemical exposure could be expected to increase the incidence of a specific pathology. Predictions based on oral slope factors probably underestimate cancer risk since real accuracy would require defining the full level of exposure (e.g., involving multiple routes of exposure) and the additive effects of co-exposure to other chemicals with their own slope factors. In addition, with some exceptions for certain cancers such as kidney cancer, oral slope factors are generally generic rather than organ-specific.

The oral cancer slope factor is usually expressed in mg/kg/day and is used to estimate additional cases of cancer per million people chronically exposed to a particular concentration of a chemical for a lifetime. Because of a potential genotoxic MOA for kidney cancer the EPA used the low-dose linear default non-threshold assumption for the derivation of the cancer slope factors for TCE. The oral slope factor for TCE is $4.6 \times 10^{-2}$ mg/kg/day and respectively. Active military personnel are estimated to ingest from 4 to 10 L/day depending on temperature and activity level and to weigh a default 80 kg.[1] As explained in Opinion 5, TCE exposure based on drinking water ingestion should be at least doubled to estimate a more accurate total exposure that takes into account dermal and inhalation exposure from the same water.

To estimate the increased cancer risk from chemical exposure, the oral cancer slope factor for a specific chemical is multiplied by the lifetime exposure (in mg/kg/day). An example of its use will be provided here using TCE levels of 783 μg/L which represent the maximum monthly average at the Hadnot Point water-treatment plant between August 1953 and December 1984.[128] Using default values of 4- 10 L/day of water a Marine's exposure to TCE would be between 0.0783 mg/kg/day (0.783 mg/L x 2 (accounting for dermal and inhalation x 4 liters/day/80 kg) to 0.1958 mg/kg/day (0.783 mg/L x 2 (accounting for dermal and inhalation x 10 liters/day/80 kg). If you multiplied that by the oral slope factor for TCE (0.046 per mg/kg/day) you would obtain values ranging from 0.0036 to 0.0090 which means you could expect between 3,600 to 9,000 extra cancers per million people for individuals chronically exposed to that level of TCE. Those values are for TCE alone and do not take into account co-exposure effects from the other contaminants. They also predict total cancers, not bladder cancer specifically.

The reconstructed values for the drinking water contaminants at Camp Lejeune did not identify a monthly average for benzene. However, we can use the level of benzene (720 μg/L) detected in a supply well at Camp Lejeune and the benzene oral cancer slope factor $1.5 \times 10^{-2}$ to $5.5 \times 10^{-2}$ to predict extra cancers. If we multiply the concentration (0.72 mg/L) X 4 L and divide by a default 80 kg we end up with 0.036 which when multiplied by the slope factor of $1.5 \times 10^{-2}$ would predict an extra 540 cancers/million people for individuals chronically exposed to that concentration of benzene. Alternatively, we can multiply the concentration ().72 mg/L) x 10 liters and divide by default 80 kg to end up with 0.09 which when multiplied by the slope factor

[1] *See* https://www.epa. gov/sites/default/files/2015-11/documents/OSWER-Directive-9200-1-120-ExposureFactors.pdf.

of 5.5 x 10 $^{-2}$ would predict an extra 4,950 cancers per million people. Once again those predictions are not specific for bladder cancer and would need to be adjusted for duration of exposure.

A third way to get perspective on TCE exposure dose and pathology involves the new Existing Chemical Exposure Limit (ECEL) for TCE proposed by the EPA on October 31,2023. The ECEL was published in the Federal Register as a proposed rule concerning *Trichloroethylene: Regulation Under the Toxic Substances Control Act*.[2] The EPA is proposing a new ECEL for TCE of either 4.0 ppb or 1.1 ppb for occupational inhalation exposures as an 8-hour time-weighted average (TWA). Only exposures equal to or below the ECELs are considered free from an unreasonable risk for chronic cancer and non-cancer and acute non-cancer inhalation endpoints.

The proposed 1.1 ppb (0.0059 mg/m$^3$) ECEL is approximately 100,000 times lower than the OSHA PEL of 100 ppm for an 8-hour TWA. This reflects the EPA's commitment to using new findings and recent advances in modeling and scientific interpretation of toxicological data to update regulatory guidelines for environmental hazards.

Using the default air intake values (15.2 m$^3$/day for adult males) provided by the ATSDR[3] it is possible to estimate TCE exposure based on the ECEL of 0.00037 mg/kg/day for an 8-hour day. [15.2 m$^3$/day divided by 24 hours = 0.63 m$^3$ air intake per hour; 0.63 m$^3$ air/hour x 0.0059 mg/m$^3$ = 0.0037 mg/hour x 8 hours/80 kg = **0.00037 mg/kg/day**]. In contrast, a Marine's exposure to 366 μg/L TCE in the Camp Lejeune drinking water with 4 L ingestion rate/80 kg = **0.018 mg/kg/day**. Thus, even with a conservative drinking water rate of 4 L/day, the Marine at Camp Lejeune would have been exposed on a daily basis to a level of TCE almost 50-times higher than the level thought not to be unsafe. This level of TCE exposure would need to be adjusted upward to reflect inhalation and dermal exposure.

### Summary

It is more-likely-than-not that the levels and duration of the TCE, PCE and benzene exposure at Camp Lejeune were sufficient to promote the development of bladder cancer.

## XI. OPINION 5. THE LIKELIHOOD THAT THE CONTAMINANTS IN THE DRINKING WATER AT CAMP LEJEUNE CAUSED BLADDER CANCER WAS INCREASED BY AGGREGATE EXPOSURE VIA INHALATION AND DERMAL ROUTES, AND BY CUMULATIVE CO-EXPOSURE

There is considerable evidence that the toxic response to a chemical such as TCE by one route of exposure can be augmented by exposure to the same chemical by a different route of exposure or by co-exposure to another chemical. This is an important consideration since cumulative and aggregate exposure assessments more realistically depict real-world exposures in both occupational and environmental settings. This is certainly true at Camp Lejeune. Aggregate exposure assessments are used when an individual is exposed to a single contaminant via different routes.

[2] *See* https://www.govinfo.gov/content/pkg/FR-2023-10-31/pdf/2023-23010.pdf.
[3] *See* https://www.atsdr.cdc.gov/hac/phamanual/appg.html.

### A. Aggregate exposure to TCE, PCE and benzene

On April 18, 2024, Michal Freedhoff, the EPA Assistant Administrator for the Office of Chemical Safety and Pollution Prevention, signed the following document: *Action: Final Rule Title: Procedures for Chemical Risk Evaluation Under the Toxic Substances Control Act (TSCA).* https://www.epa.gov/system/files/documents/2024-04/prepubcopy_frl-8529-02-ocspp_fr_doc_aa_esignature_verified.pdf *In this document the EPA noted that the inclusion of all exposure pathways relevant to the chemical substance was needed for an accurate risk assessment*. The EPA also proposed additional regulatory text to ensure that EPA would no longer exclude from the scope of TSCA risk evaluations exposure pathways that are addressed or could in the future be addressed by other EPA-administered statutes and regulatory programs (e.g. Clean Water Act). This document underscores the EPA belief that accurate TSCA risk evaluations should consider all possible routes of exposure in evaluating a hazard. TSCA is designed to evaluate risk to people from occupational and consumer exposures. However, the principle also holds true for human environmental exposure such as Camp Lejeune.

At Camp Lejeune risk evaluations have focused on TCE exposure from ingesting contaminated drinking water. However, when estimating risk from TCE it is important to also consider inhalation and dermal exposure resulting from the use of the TCE-containing drinking water for cleaning and bathing. Results from PBPK modeling and from human experimental samples have shown that inhalation and dermal exposure from TCE-contaminated water is at least equal to that from ingestion. [129, 130] A 2024 study by Rosenfeld et al. used new methodology to quantify cancer risk for the Marines who had lived at Camp Lejeune between 1953 and 1986.[126] They estimated that most of the increased cancer risk (59%) was in fact due to inhalation from the contaminated drinking water. This suggests that, at the very least, one should double the ingestion exposure of TCE to estimate total TCE exposure at Camp Lejeune. Similarly, Health Canada has determined that for PCE the contribution of the dermal route is equivalent to that of the oral ingestion. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/water-quality/drinking-water/canadian-drinking-water-guidelines.html

Breathing indoor air contaminants in Camp Lejeune's buildings due to vapor intrusion is another potential pathway of exposure to shallow groundwater contaminants. Volatile chemicals such as TCE and PCE in contaminated shallow groundwater can evaporate and move upward through the ground surface into indoor air of overlying or nearby buildings—this process is called vapor intrusion. At this time ATSDR is evaluating about 150 buildings at Camp Lejeune. https://www.atsdr.cdc.gov/ sites/lejeune/Vapor-Intrusion-PHA.html Although the values obtained will reflect current rather than historical levels of contaminants in the groundwater, they may at least provide some insight into the contribution of this exposure pathway to total exposure.

### A. Cumulative co-exposure to TCE and PCE and benzene

Cumulative exposures are of particular interest when conducting community-based assessments that need to take into account exposure to multiple chemical stressors such as the

case at Camp Lejeune. Co-exposure to one or more chemicals with additive or synergistic effects that target the same system may promote disease at concentrations that would be harmless for any of the chemicals alone.

It is widely acknowledged that many instances of environmental contamination involve concurrent exposures to a mixture of compounds that may induce similar effects over exposure periods ranging from short-term to lifetime. This is certainly the case at Camp Lejeune.

The assessment of chemical mixtures is an area of active scientific investigation. The ATSDR is committed to better defining the impacts of chemical mixtures.[4] To carry out this legislative mandate, the ATSDR's Division of Toxicology has developed and coordinated a mixtures program that includes *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity.[5] The ATSDR has identified 16 Final or Draft Interaction Profiles describing chemical mixtures of human health concerns. Four of the Interaction Profiles contain TCE. One of these four mixtures contains TCE and PCE, and the other contains TCE and vinyl chloride.

The EPA is similarly committed to studying mixtures as a means to inform risk assessment. The EPA's 2020 *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* was designed to generate a consistent Agency approach for evaluating data on the chronic and subchronic effects of chemical mixtures. It is a procedural guide that emphasizes broad underlying principles of the various science disciplines (toxicology, pharmacology, statistics) necessary for assessing health risk from chemical mixture exposure.. https://assessments.epa.gov/risk/document/&deid=20533 The EPA defines mixtures as any combination of two or more chemical substances regardless of source. In some instances, complex mixtures consist of multiple compounds that are generated simultaneously as byproducts from a single chemical such as degradation of PCE or TCE. Mixtures can also consist of unrelated chemicals which because of inadequate but proximal disposal processes end up contaminating the same water supply. Both of those definitions apply to the contamination at Camp Lejeune.

The chemicals in a mixture can interact in a manner that is additive, less than additive (e.g. antagonistic) or greater than additive (e.g. synergistic). The term additive describes the situation when the combined effects of two or more chemicals equal the sum of the effects of the chemicals acting independently. Response addition has often been used for the risk assessment of mixtures of carcinogens.[131]

Because of the complexity of considerations that must be undertaken to develop a chemical mixtures health risk assessment, the EPA notes that it is not practical to recommend a clear listing of default procedures that covers all cases. The Agency describes different approaches to a quantitative health risk assessment of a chemical mixture, all of which are outside the scope of this report. However, such general considerations for mixtures assessment such as functional and chemical similarities and similar toxicodynamics make it likely that coexposure to the contaminants at Camp Lejeune induced additive toxicity and carcinogenicity.

The ATSDR has been mandated to determine the health impact of exposure to combinations of chemicals (https://www.atsdr.cdc.gov/mixtures/assessment.html ). To carry out this legislative mandate, ATSDR's Division of Toxicology has developed and coordinated a mixtures program that includes *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint

[4] *See* https://www.atsdr.cdc.gov/mixtures/assessment.html.
[5] *See* (https://www.atsdr. cdc.gov/interactionprofiles/ip02.html).

action, and methodological development for assessment of joint toxicity (https://www.atsdr.cdc.gov/interactionprofiles/ip02.html). The ATSDR has identified 16 Final or Draft Interaction Profiles describing chemical mixtures of human health concerns. Four of the Interaction Profiles contain TCE. One of these four mixtures contains TCE and PCE. It should be noted that that study of toxic co-exposures is still relatively new. However, I, as well as other, have demonstrated that co-exposure can alter TCE-induced toxicity and gene expression.[26, 132, 133] Although these particular co-exposure studies didn't examine TCE in combination with PCE they provide proof of concept that co-exposure effects should be considered when assessing TCE toxicity.

Rosenfeld *et al*. examined the combined cancer risk from co-exposure to the different contaminants in the drinking water at Camp Lejeune.[126] They predicted that the cancer risk from TCE exposure would be increased by an additive effect from vinyl chloride.

A qualitative co-exposure evaluation of the cancer risk posed by the chemicals in the drinking water at Camp Lejeune is beyond the scope of this report. However, I will briefly discuss why co-exposure to the contaminants at Camp Lejeune are expected to increase the likelihood of TCE-induced bladder cancer.

As described in EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (mixtures guidance) (U.S. EPA, 2000), evidence for toxicological similarity exists along a continuum but may include the following: (1) structural similarity, (2) effects on the same target organ, and (3) similar toxicodynamics.

Trichloroethylene Perchloroethylene

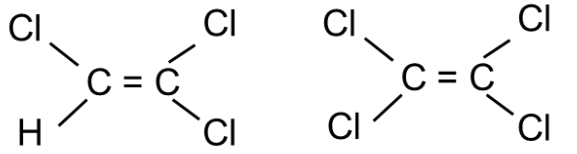



**Figure 3. Structure of TCE and PCE**

In terms of structural similarity TCE and PCE both contain 2 carbon molecules with three or four chloride molecules respectively (**Figure 3**).

They have qualitatively similar metabolic pathways in both rodents and humans. [134] This includes oxidative metabolism in the liver and conjugative metabolism in the kidney, largely, but not solely dependent on the same enzyme, CYP2E1.[135] The conjugation pathway converts both chemicals into toxic and reactive metabolites in the kidney.

Unlike PCE and TCE, benzene is not chlorinated; its structure consists of six carbon atoms forming hexagonal ring with one hydrogen molecule attached to each. Benzene metabolism, although different in many ways, is similar to that of PCE and TCE in that it involves an oxidative pathway in the liver mediated by CYP2E1. Assigning specific metabolites of benzene to specific pathological processes is still being defined.

Although not as obviously similar as PCE and TCE, benzene has important functional parallels to the other two chemicals. Perhaps most importantly, all three cause immunotoxicity. This immunotoxicity shares several important endpoints, namely induction of pro-inflammation and suppression of T cell-mediated adaptive immunity. This confluence means that these three chemicals have the capacity to promote many types of cancer, including bladder cancer, in

which chronic inflammation and immunosuppression play important roles in etiology. Their ability to target the immune system is underlined by the fact that the ATSDR has declared that there is sufficient evidence that all three can cause NHL, a classic lymphoid cancer.[31] In addition, three can generate genotoxic effects and all three induce oxidative stress, processes that are important in the generation of many cancers, including bladder cancer.

**Summary**

There is evidence that TCE, PCE and benzene can increase the incidence of bladder cancer. There is also considerable evidence that they share cancer-causing MOA such as induction of genotoxicity, immunotoxicity and oxidative stress. Thus, it is more-likely-than-not that they have an additive effect on the development of bladder cancer, and that the levels and duration of their co-exposure at Camp Lejeune caused bladder cancer. This likelihood takes into account the fact that estimating total exposure to toxicants at Camp Lejeune cannot be based solely on drinking water ingestion but should also take into account inhalation and dermal exposure from the contaminated drinking water, and perhaps inhalation of indoor air contaminated by vapor intrusion.

**Signature**. I hold all of the above opinions to a reasonable degree of scientific certainty. I reserve the right to supplement and/or review my opinions as presented in this report as new information becomes available.

12-5-24

Kathleen M. Gilbert, PhD Date

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# Appendix A

# KATHLEEN M. GILBERT, PHD
## IMMUNOTOXICOLOGIST

(501) 249-4552
gilbertkathleenm4@gmail.com
240 West 75th Street, Apt 1B, New York, NY 10023
800 Shire Court, Fort Collins, CO 80526

**SUMMARY**: Although initially trained as a molecular immunologist with an emphasis on immune tolerance and autoimmune disease, Dr Gilbert has for the last 25 years focused her research on the immunotoxicity of trichloroethylene (TCE). She has authored over 30 articles examining the immunotoxicity of TCE or related products, and co-edited a book entitled *Trichloroethylene: Toxicity and Health Risks*, Springer, New York/Heidelberg. She is a member of the US EPA's Scientific Advisory Committee on Chemicals (SACC) which has thus far reviewed risk evaluations for ten high-priority chemicals, including TCE, that were identified as part of the Toxic Substances Control Act. Dr. Gilbert has provided risk assessments for human exposure to TCE and other chemicals including perchloroethylene, vinyl chloride, and benzene. Dr. Gilbert is a long-standing member of the Society of Toxicology.

## EDUCATION:

Occidental College, Los Angeles, CA, BS, Biology, 1976
Tulane University, New Orleans, LA, PhD, Immunology, 1980

## PROFESSIONAL EXPERIENCE:

Postdoctoral Research Associate: Sloan Kettering Institute, New York, NY
1980 -1982
Research Associate, Sloan-Kettering Institute, New York, NY
1982 -1985
Visiting Worker: National Institute for Medical Research, London, UK
1985 -1987
Senior Research Associate: Department of Immunology, The Scripps Research Institute, La Jolla, CA,1987 -1991
Assistant Member (Assistant Professor): The Scripps Research Institute, La Jolla, CA
1991 -1994
Assistant Professor: Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR
1995 – 2001
Associate Professor: Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR
2001 – 2010
Professor (tenured), Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR
2010- 2017 (Retired)

Secondary Appointments at UAMS: Dept. of Pediatrics, 2002-2017

Dept. of Pharm. and Toxicology, 2010-2017

Adjunct Professor, Dept. of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, 2017-Present

## TEACHING/MENTORING

**Teaching Medical Students at UAMS**

| Course | Total Hours | Level | Years |
|---|---|---|---|
| *Medical Microbiology-Immunology-Parasitology*<br><br>Now called *Disease and Defense* | Three-five 1-hour lectures/year<br><br>Three 3-hour Patient-Oriented Problem Solving sessions/year | 2nd year<br><br>1st year | 1995 – 2011<br><br>2011- present |

**Teaching Graduate Students at UAMS**

| Course | Total Hours | Level | Years |
|---|---|---|---|
| Immunology | six 1.5-hour lectures/year | 1st year | Course Director, 1995 - 2002<br>Lecturer, 1995 - 2017 |
| Advanced Immunology | three 2-hour lectures/year | 2nd-3rd year | 1995 -1997 |
| Molecular Mechanisms of Immunology | four 2-hour lectures/year | 2nd-3rd year | 1998 - 2017 |
| Current Topics in Immunology | two 1-hour lectures/year | 1st-5th year | 1995 - 2017 |
| Biochemical Methods | two 1.5-hour lectures/year | 1st-2nd year | 2000 - 2002 |
| Methods in Pharmaceutical Sciences | one 1.5-hour lecture/year | 1st-2nd year | 2001 |
| Molecular & Translational Toxicology | three 1.5-hour lectures/year | 2nd -3rd year | 2002 - 2017 |
| Systems Toxicology | three 1.5-hour lectures/year | 2nd and 3rd year | 2009 - 2017 |

## MENTORING

Dr Gilbert has served as a dissertation/thesis committee chair or member for 22 graduate students at UAMS. She has served as mentoring committee chair or member for 8 junior faculty members at UAMS, and has mentored 21 other undergraduates, graduate students and faculty members as part of INBRE grants or Honors in Research Programs.

# RESEARCH/SCHOLARLY WORK

**Most Recent Research Support**

| Title | Funding Agency | Dates | Direct Costs | Role | Effort |
|---|---|---|---|---|---|
| **Extramural** | | | | | |
| Developmental Programming of TCE-induced autoimmune disease R01ES021484 | NIEHS | 12/1/12 – 11/30/17 | $1,372,000 | Co-Principal Investigator | 30% |
| Gender Supplement to R01ES021484 | NIEHS | 9/15/14-10/31/16 | $99,724 | Co-Principal Investigator | 0% |
| Determining how trichloroethylene alters $CD4^+$ T cell function: 1R01ES017286 | NIEHS | 1/07/10 - 12/31/14 | $675,000 | Principal Investigator | 40% |
| Trichloroethylene toxicity and remediation | Competitive grant funded by Organic Compounds Property Contamination class action settlement (CV 1992-002603) | 7/01/08-6/30/15 | $735,000 | Principal Investigator | 25% |
| Training program in the pathophysiology of renal disease: 5T32DK061921 (PI: Portilla) | NIDDK | 2006-2011 | $603,335 | Faculty Mentor | 0% |
| **Intramural** | | | | | |
| Developing an Immunotoxicology Center in Arkansas | Arkansas Biosciences Institute | 7/01/02 – 6/30/17 | $1,500,000 | Principal Investigator | 10% |

**Past Research Support**

| Title | Funding Agency | Dates | Direct Costs | Role | Effort |
|---|---|---|---|---|---|
| **Extramural** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Screening disinfection by-products for their ability to promote autoimmunity | Environmental Protection Agency | 10/01/01 -9/31/04 | $500,000 | Principal Investigator | 25% |
| Mechanisms of chlorinated ethylene-induced autoimmunity, Proposal No. R826409 | Environmental Protection Agency | 3/25/98 - 3/24/01 | $278,356 | Principal Investigator | 25% |
| Cyclin-dependent kinase inhibitors mediate T cell anergy: MCB-9817191 | National Science Foundation | 3/15/99 - 3/14/02 | $230,660 | Principal Investigator | 25% |
| The use of G1 blockers in a novel system of immune intervention | National Arthritis Foundation | 1/1/02 – 12/21/04 | $270,000 | Principal Investigator | 25% |
| Use of tributyrin as a novel system of immune intervention, Proposal No. KG071598 | Arthritis Foundation, Arkansas Chapter | 7/15/98 - 10/15/99 | $31,280 | Principal Investigator | 25% |
| Mechanism of Toxicant-induced T cell Suppression; Grant No. 187-B | American Cancer Society | 2/1/96 - 1/31/97 | $15,000 | Co-principal investigator | 25% |
| A Novel System of Immune Intervention: Grant No. J246 | R.W. Johnson Pharmaceutical Research Institute | 10/1/92 - 9/30/94 | $129,032 | Principal Investigator | 35% |
| Mechanisms of renal tubular epithelial cell injury (PI: S. Shah), PO1-DK-58324 | NIDDK | 2001-2006 | | Core Director | 5% |
| Suppressor B cells | Arthritis and Rheumatism Council, UK, Project Grant | 1986-1987 | $45,000 | Principal Investigator | 100% |
| Effect of Th cell Tolerance on Cell Cycle Components: Proposal No. MCB-9308198 | National Science Foundation | 6/1/93 - 5/31/95 | $18,000 | Principal Investigator | 25% |
| A Novel System of Immune Intervention: Proposal No. 96-B-37 | Arkansas Science and Technology Authority Award | 1/31/96 - 1/30/97 | $33,050 | Principal Investigator | 30% |
| Mechanism of B cell suppression | International Union Against Cancer | 1985-1986 | $16,000 | Principal Investigator | 100% |
| National Research Service Award | USPHS | 1980-1983 | | Postdoctoral Fellow | 100% |
| Comparison of the effects of methyl palmitate and glucan on tumor growth | Cancer Association of Greater New Orleans | 1978 | $900 | Graduate Student | 100% |

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| **Intramural** | | | | | |
| Interventive Therapy for Type I Diabetes | Children's University Medical Group | 7/1/05-6/31/07 | $39,500 | Principal Investigator | 10% |
| Inactivating autoreactive T cells by HDAC inhibitors | Sturgis Charitable Trust | 2/01/10 – 9/30/10 | $24,970 | Principal Investigator | 0% |

**Manuscript review activities**

| Journal | Year Started |
|---|---|
| *Journal of Immunology* | 1994 |
| *Cellular Immunology* | 1994 |
| *Blood* | 2000 |
| *Toxicology and Applied Pharmacology* | 2006 |
| *American Journal of Transplantation* | 2007 |
| *Biochemical Pharmacology* | 2007 |
| *Endocrine* | 2009 |
| *International Journal of Environmental Sciences* | 2008 |
| *Libertas Academica* | 2009 |
| *Toxicological Sciences* | 2009 |
| *Journal of Environmental Science and Health* | 2010 |
| *International Journal of Nephrology and Renovascular Disease* | 2010 |
| *Transplant Immunology* | 2008 |
| *Toxicology* | 2011 |
| *BMC Pharmacology* | 2011 |
| *ISRN Immunology – Editorial Board* | 2011 |
| *Chemical Research in Toxicology* | 2012 |
| *Toxicology Research* | 2012 |
| *Journal of Immunotoxicology – Ad hoc Editor* | 2012 |
| *Environmental and Molecular Mutagenesis* | 2013 |
| *International Journal of Molecular Sciences* – Guest Editor for special issue on "Environmental Toxicants and Autoimmune Disease | 2013 |
| *Drug Design, Development and Therapy* | 2015 |
| *BMC Medical Genomics* | 2015 |
| *Expert Review of Gastroenterology & Hepatology* | 2015 |
| *Environmental Health Insights* | 2015 |
| *Neurotoxicology* | 2015 |
| *Annals of Neurology* | 2016 |
| *PLOS ONE* | 2016 |
| *Inhalation Toxicology* | 2016 |

**Grant review activities**

| Agency | Study Section | Years |
|---|---|---|

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|---|---|---|
| National Science Foundation | Signal Transduction and Regulation | 1996 |
| National Science Foundation | Biocomplexity | 2000 |
| Environmental Protection Agency | Experimental Toxicology | 2007 |
| National Institutes of Health | Superfund Basic Research (ZES1 LWJ-M) | 2009-2015 |
| National Institutes of Health | Systemic Injury by Environmental Exposure (SIEE) | 2016 |
| National Institutes of Health | Digestive, Kidney and Urological Systems (DKUS-A) | 2009 |
| National Institutes of Health | Immunology (IMM-E) | 2009 |
| National Institutes of Health | Infectious Disease and Microbiology (IDM-C) IFG | 2009 |
| Wellcome Trust | | 2006 |
| RJ Reynolds Foundation | External Research Program | 2006<br>2009 |
| Louisiana Board of Regents | | 2007 |
| UAMS Medical Research Endowment Grant awards<br>Hornick Grant<br>UAMS Pilot Study Grants<br>UAMS Center for Clinical and Translational Research<br>UAMS Bridging Grants<br>ACHRI Children's University Medical Group awards | | 1999-2017 |
| National Institutes of Health | Environmental Health Sciences Review Committee for T32 applications | 2013 |

## PUBLICATIONS (Peer-reviewed):

- Byrum, S.D., Washam, C.L., Patterson, J.D., Vyas, K.K., **Gilbert, K.M**., and Blossom, S.J. Continuous Developmental and Early Life Trichloroethylene Exposure Promoted DNA Methylation Alterations in Polycomb Protein Binding Sites in Effector/Memory CD4$^+$ T Cells**.** *Frontiers in Immunology* Aug 28;10:2016, 2019.
- Khare, S., Gokulan, K., Williams, K., Bai, S., **Gilbert, K.M.**, and Blossom, S.J., Irreversible effects of trichloroethylene on the gut microbial community and gut-associated immune responses in autoimmune-prone mice, *Journal of Applied Toxicol*ogy 39:209 2019.
- Blossom, S.J**.** and **Gilbert, K.M**. Epigenetic underpinnings of developmental immunotoxicity and autoimmune disease. *Current Opinion in Toxicology* 10:23-30, 201, 2018

- Blossom, S.J., Fernandes, L., Bai, S., Chare, S., Gokulan, K., Yuan, Y., Dewall, M., Simmen, F.A., and **Gilbert, K.M**., Opposing actions of developmental toxicity and high-fat diet coexposure on markers of lipogenesis and inflammation in autoimmune-prone mice, *Toxicological Science*, 164:313-327, 2018.
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- Frye, R.E., Rose, S., Wynne, R., Bennuri, S.C., Blossom, S., **Gilbert, K.M**., Heilbrun, L., and Palmer, R.F., Oxidative stress challenge uncovers trichloracetaldehyde hydrate-induced mitoplasticity in autistic and control lymphoblastoid cell lines, *Science Report* 7:4478, 2017.
- **Gilbert, K.M**., Bai, S., Barnette, D., and Blossom, S.J. Exposure cessation during adulthood did not prevent immunotoxicity caused by developmental exposure to low-level trichloroethylene in drinking water. *Toxicological Science* 157: 429-437, 2017
- Meadows, J.R., Parker, C., **Gilbert, K.M**., Blossom, S.J., and Dewitt, J.C., A single dose of trichloroethylene given during development does not substantially alter markers of neuroinflammation in brains of adult mice. *Immunotoxicology* 14:95-102, 2017.
- **Gilbert, K.M.**, Blossom, S.J., Erickson, S.W., Broadfoot, B., West, K., Bai, S. and Cooney, C.A. Chronic exposure to trichloroethylene increases DNA methylation of the ifng promoter in $CD4^+$ T cells. *Toxicology Letters*, 260:1-7, 2016.
- **Gilbert, K.M.**, Blossom, S.J., Erickson, S.W., Reisfeld, B., Zurlinden T.J., Broadfoot, B., West, K., Bai, S. and Cooney, C.A. Chronic exposure to water pollutant trichloroethylene increased epigenetic drift in $CD4^+$ T cells. *Epigenomics*, 8:633-649, 2016.
- **Gilbert, K.M.**, Reisfeld, B., Zurlinden T.J., Kreps, M.N., Erickson, S.W. and Blossom, S.J. Modeling toxicodynamic effects of trichloroethylene on liver in mouse model of autoimmune hepatitis. *Toxicology and Applied Pharmacology*, 279:284-293, 2014.
- **Gilbert, K.M**., Woodruff, W. and Blossom, S.J., Differential Immunotoxicity induced by two different windows of developmental trichloroethylene exposure, *Autoimmune Diseases.* Epub 2014
- **Gilbert, K.M**. Autoimmunity Hepatitis. *Encyclopedic Reference of Immunotoxicology,* Vohr, Hans-Wener (Ed), Springer Publishing Co., 2013.
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- Fontenelle, B. and **Gilbert, K.M.,** n-Butyrate anergized effector CD4+ T cells independent of regulatory T cell generation or activity, *Scandinavian Journal of Immunology*, 76:457-463, 2012.
- **Gilbert, K.M.**, Nelson, A.R, Cooney, C.A., Reisfeld, B., and Blossom, S.J. Epigenetic alterations may regulate temporary reversal of $CD4^+$ T cell activation

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- **Gilbert, K.M.,** Przybyla, B., Pumford, N.R., Han, T, Fuscoe, J., Schnackenberg, L.K., Holland, R.D., Doss, J.C. MacMillan-Crow, L, and Blossom, S.J. Delineating liver events in trichloroethylene-induced autoimmune hepatitis, *Chemical Research in Toxicology* 22: 623-632, 2009.
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- **Gilbert, K.M.,** Pumford, N., and Blossom, S.J. Environmental contaminant trichloroethylene promotes autoimmune disease and inhibits T cell apoptosis in MRL+/+ mice. *Journal of Immunotoxicology* 3:263-267, 2006.
- Blossom, S.J. and **Gilbert, K.M**. Ability of environmental toxicant trichloroethylene to promote immune pathology is strain-specific. *Journal of Immunotoxicology* 3:179-188, 2006.
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- **Gilbert, K.M**., Whitlow, A., and Pumford, N.R. Environmental contaminant and disinfection by-product trichloroacetaldehyde stimulates T cells *in vitro*. *International Immunopharmacology* 4:25-36, 2004.
- **Gilbert, K.M**., Boger, S. and Fifer, E.K.. Butyric acid derivative induces allospecific T cell anergy and prevents graft-versus-host disease. *Immunopharmacology and Immunotoxicology* 25:13-27, 2003.
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- **Gilbert, K.M**., Griffin, J.M., and Pumford, N.R. Trichloroethylene activates $CD4^+$ T cells: potential role in an autoimmune response. *Drug Metabolism Review*. 31:901-916, 1999.
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**Book Chapters:**

- **Gilbert, K.M**. *Trichloroethylene and Autoimmunity in Human and Animal Models* in Trichloroethylene: Toxicity and Health Risks, Edited by **Gilbert, K.M** and Blossom, S.J. Springer. 15-36, 2014.
- Wartenberg, D. and **Gilbert, K.M**. *Trichloroethylene and Cancer* in Trichloroethylene: Toxicity and Health Risks, Edited by **Gilbert, K.M** and Blossom, S.J. Springer. 171-185, 2014.
- Cooney, C.A., and **Gilbert, K.M**. *Toxicology, Epigenetics and Autoimmunity* in Toxicology and Epigenetics, Edited by Saura C. Sahu. Wiley-Blackwell Inc. 241-252, 2012.
- **Gilbert, K.M**. Clonal anergy of peripheral T lymphocytes. In Chemical Immunology (R.D. Granstein, ed.) Karger Publishing, Basel, Switzerland. 92-109, 1994.
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## Platform presentations

- **Gilbert, K.M.** Autoimmune-prone versus normal mice as models for toxicant-mediated autoimmune disease. In Workshop: Strengths and weaknesses of mice as a model for humans in studies of immunological effects of drugs and chemicals , Society of Toxicology, San Diego, March, 2015.
- **Gilbert, K.M**. Trichloroethylene exposure and epigenetic alterations in T cell function. In Prenatal Programming and Toxicity IV, Boston, MA, October, 2014
- **Gilbert, K.M**. Autoimmune disease triggered by trichloroethylene is associated with epigenetic alterations in $CD4^+$ T cells." In Environmental Health 2013, Science and Policy to Protect Future Generations, Boston, MA, March, 2013
- **Gilbert, K.M**. Chronic exposure to water pollutant trichloroethylene promotes autoimmune hepatitis and induces epigenetic alterations in $CD4^+$ T cells. In Symposium entitled "Role of Environmental Exposures in the Development of Autoimmune Disease", The American Association of Immunologists Annual Meeting, Boston, MA, 2012.
- **Gilbert, K.M**. Environmental pollutants that trigger immune dysfunction and promote autoimmune disease. Keynote speaker at FDA's National Center for Toxicological Research Office of Women's Health Research Update Program, August 10, 2012.
- **Gilbert, K.M**. Trichloroethylene-induced autoimmunity; dependence on metabolism and genetic susceptibility, In Workshop entitled "Autoimmunity

versus systemic hypersensitivity: commonalities useful for toxicity testing", Society of Toxicology Annual Meeting, 2011.

- **Gilbert, K.M**., Rowley, B., Hennings, L., and Blossom, S.J. Co-exposure to mercury accelerates autoimmunity induced by trichloroethylene, NCTR Women's Health Research Workshop, Little Rock, September, 2010.
- **Gilbert, K.M**., Rowley, B., Hennings, L., and Blossom, S.J. Mice exposed to a binary mixture of immunotoxicants developed unique autoimmune effects not induced by single exposure, 49th Society of Toxicology Annual Meeting, Salt Lake City, Utah, March, 2010.
- **Gilbert, K.M.,** Przybyla, B., Pumford, N.R., Han, T, Fuscoe, J., Schnackenberg, L.K., Holland, R.D., Doss, J.C. MacMillan-Crow, L, and Blossom, S.J. Combining transcriptomics and metabolomics to delineate immunotoxicity of trichloroethylene, NSF Advance Program Planning meeting, Petit Jean, AR, March, 2009.
- **Gilbert, K.M.,** Yeung, S., Nelson, A., and Przybyla, B., Susceptibility factors in trichloroethylene-induced autoimmunity, South Central Society of Toxicology annual meeting, NCTR, Jefferson, AR, September, 2008.
- **Gilbert, K.M**., Whitlow, A.B., and Pumford, N.R., Environmental toxicant associated with the development of autoimmune disease stimulates T cell signaling, Environmental Factors in Autoimmune Disease, NIEHS, Durham, NC, February, 2003.
- **Gilbert, K.M**., Whitlow, A.B. and Pumford, N.R. Environmental contaminant associated with induction of autoimmune disease stimulates T cells via Schiff base formation, American Association of Immunologists Annual Meeting, New Orleans, LA, April, 2002.
- **Gilbert, K.M**., Fecher, N.B., Freeman, J.P., Wahid, R. and Fifer, E.K. Potential clinical use of butyric acid prodrugs to induce antigen-specific T cell inactivation. American Association of Immunologists Annual Meeting, Seattle, May, 2000.
- **Gilbert, K.M**., M.L. Thoman, K. Bauche, and Weigle, W.O., TGF-β1-induced tolerance in antigen-specific naïve T cells, The 9th International Congress of Immunology, San Francisco, July, 1995.
- **Gilbert, K.M.** and Weigle, W.O. Use of G1a blockers to induce antigen-specific T cell anergy, New Strategies for Selective Immune Suppression, Cambridge Healthtech Institute, Waltham, MA, October, 1994.
- **Gilbert, K.M.,** and Weigle, W.O., Activated B cells which express CTLA-4 counter-receptors are tolerogenic. American Association of Immunologists Annual Meeting, Anaheim, April, 1994.
- **Gilbert**, **K.M.** and Weigle, W.O., Activation does not reverse B cell tolerogenicity, American Association of Immunologists Annual Meeting, May, Denver, CO, 1993.
- **Gilbert, K.M.,** Hobbs, M.V. Ernst, D.N., and Weigle, W.O., Heterogeneity in the ability of different antigen presenting cells to tolerize Th1 and Th2 clones, American Association of Immunologists Annual Meeting, 1992.
- **Gilbert, K.M.** Effects of tolerance induction on cell cycle progression by the Th1 and Th2 clones, 18th Annual Conference of the La Jolla Immunologists, San Diego, CA, 1991.

- **Gilbert, K.M**. Hoang, K.D. and Weigle, W.O., Tolerized high density Th clones lose bystander helper activity, American Association of Immunologists Annual Meeting, April, 1989.
- **Gilbert, K.M.** and Weigle, W.O. B cell activation by T helper cells. MidWinter Conference of Immunologists, Asilomar, CA., 1988.
- **Gilbert**, **K.M.** and Hoffmann, M.K. cAMP as a 2nd messenger in antibody production by B cells. American Association of Immunologists Annual Meeting, Anaheim, April, 1985.

- **Sessions Chaired:**

- "Epigenetics" as Presented at the 51st Annual Society of Toxicology meeting, San Francisco, CA, March, 2012.
- Platform Session "Developmental Immunotoxicology, Host Resistance and Genomics, Society of Toxicology Meeting, Seattle, 2008.
- Platform Session "Immunotoxicity" Society of Toxicology Annual Meeting, San Diego, CA, 2006.
- Co-chaired Symposia "T cell inactivation and apoptosis", American Association of Immunologists Annual Meeting, San Francisco, April, 1998.
- Co-chaired Immunology session for American Society of Microbiology, South Central Branch, Little Rock, AR, 1995
- Co-chaired Symposium "T cell tolerance and anergy", American Association of Immunologists Annual Meeting, Anaheim, CA, April, 1994.
- Co-chaired Symposium "T cell regulation," American Association of Immunologists Annual Meeting, May, Denver, CO, 1993.

- **Other Invited Presentations:**

- "Environmental pollutants as triggers of autoimmune disease" Distinguished Speaker, Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, April 22, 2015.
- "Trichloroethylene as a trigger of autoimmune disease." In Society for Women's Health Research Autoimmune Disease Roundtable, Washington, D.C., October 5, 2012.
- Trichloroethylene-induced autoimmunity; dependence on metabolism and genetic susceptibility, In Workshop entitled "Autoimmunity versus systemic hypersensitivity: commonalities useful for toxicity testing, Society of Toxicology Annual Meeting, 2011.
- Developing an immunotoxicology center in Arkansas, Arkansas Biosciences Institute Fall Research Symposium, Little Rock, AR, September, 2010.
- Co-exposure to mercury accelerates autoimmunity induced by trichloroethylene, International Congress of Toxicology, Barcelona, Spain, July, 2010.

- Developing an immunotoxicity center in Arkansas, Arkansas Children's Hospital Research Institute Board, May, 2010.
- Environmental pollutants as triggers for autoimmune disease, South Central Branch ASM, Nicholls State University in Thibodaux, LA, November 2009.
- Contribution of environmental pollutants to autoimmune disease, Jones Eye Center Seminar Series, Little Rock, AR, June 2008.
- Combining transcriptomics and metabolomics to delineate immunotoxicity of trichloroethylene, Society of Toxicology Annual Meeting, Seattle, WA, March, 2008.
- Histone deacetylase inhibitors block MAP kinases to induce tolerance in Th1 cells, The University of Pennsylvania School of Medicine, Department of Pathology and Laboratory Medicine, Division of Transplantation Immunology Seminar Series, Philadelphia, PA, May 16, 2007
- Examining the genetic susceptibility to the immunotoxicity of a trichloroethylene metabolite, Society of Toxicology Annual Meeting, Charlotte, NC, March, 2007.
- Distinguished Speaker: Environmental contaminant and Superfund chemical trichloroethylene promotes autoimmune disease and inhibits T cell apoptosis", Bench to Bedside Symposium: Immunomodulation by Environmental Factors: The role of the Environment in Autoimmune Disease, Center for Immunopathology and Microbial Pathogenesis, Morgantown, West Virginia, December, 2006.
- Environmental contaminant trichloroethylene promotes autoimmune disease and inhibits T cell apoptosis, Society of Toxicology Annual Meeting, San Diego, CA, March, 2006.
- Contribution of environmental contaminants to autoimmune disease, Workshop on Lupus and the Environment: Disease Development, Progression and Flares, NIEHS, Washington, DC, September, 2005.
- The environmental toxicant trichloracetaldehyde promotes activation and inhibits apoptosis of mature T lymphocytes by inhibiting fas ligand expression, 12$^{th}$ International Congress of Immunology, Montreal, Canada, 2004.
- Butyric acid derivative induces allospecific T cell tolerance, Arthritis Foundation Research Conference, Keystone Resort, Colorado, June, 2003
- Why you should go with the flow; new research applications for flow cytometry, Okie-Arkie Bi-State Meeting, Little Rock, Arkansas, April, 2003
- Novel G1 blocker inhibits antigen-specific T cell response and prevents graft-versus-host disease, Arkansas Biosciences Institute Fall Research Symposium, Little Rock, AR 2002
- Autoimmunity, Research Council, College of Medicine, UAMS. Research Retreat I, Advancing collaborative research and funding strategies, Little Rock, AR, 2001
- Why you should go with the flow, Pharmaceutical Sciences Seminar, UAMS, 2001
- T cell anergy linked to alterations in cell cycle regulatory proteins. American Association of Immunologists Annual Meeting, San Francisco, April, 1998.
- G1 blockers induce anergy in CD4$^{+}$T cells, American Association of Immunologists Annual Meeting, New Orleans, LA., June, 1996.

- T cell tolerance as immunotherapy, Hematology Oncology Research Seminar Series, Arkansas Cancer Research Center, November, 1995
- Activated B cells which express CTLA-4 counter-receptors are tolerogenic, American Association of Immunologists Annual Meeting, Anaheim, CA, April, 1994.
- Use of G1 blockers to induce Th cell anergy, Glaxo Institute for Molecular Biology, Geneva, Switzerland, January, 1994.
- T cell tolerance, American Association of Immunologists Annual Meeting, Anaheim, CA, April, 1992.
- Effects of tolerance induction on cell cycle progression by the Th1 and Th2 clones, Annual Conference of La Jolla Immunologists, La, Jolla, CA, November, 1991.

## Other abstracts/posters presentations:

- Zurlinden, TJ, **Gilbert, KM**, and Reisfeld,B. A computational approach for characterizing subtle changes in DNA methylation in $CD4^+$ T cells. FutureTox III, Arlington, VA, November, 2015.
- **Gilbert, KM**, Cooney, C , Broadfoot, B., Chandler, G. and Blossom, SJ. Long-term exposure to water pollutant trichloroethylene increased plasticity of DNA methylation in *Ifng* promoter and induced non-monotonic *Ifng* expression in effector/memory $CD4^+$ T cells, Gordon Conference Cellular & Molecular Mechanisms of Toxicity, Andover, NH, 2015.
- **Gilbert, KM**, Cooney, C., and Blossom, S. Autoimmune disease triggered by trichloroethylene is associated with epigenetic alterations in $CD4^+$ T cells, $52^{nd}$ Annual Society of Toxicology meeting, San Antonio, Texas, March, 2013.
- **Gilbert, KM**, Nelson, A., Cooney, C., and Blossom, S. Subchronic trichloroethylene exposure alters epigenetic processes in $CD4^+$ T cells, $51^{st}$ Annual Society of Toxicology meeting, San Francisco, CA, March, 2012.
- Blossom, SJ, Melnyk, **Gilbert, KM,** and James, J. Postnatal trichloroethylene modulates redox status and oxidative stress in mouse hippocampus, $51^{st}$ Annual Society of Toxicology meeting, San Francisco, CA, March, 2012.
- Blossom, SJ, Melnyk, S, **Gilbert, KM**, James SJ. Differential Expression of Neuroimmune Mediators Following Postnatal Exposure to Trichloroethylene. The $27^{th}$ International Neurotoxicology Conference; Environmentally Triggered Neurodevelopmental Disorders: Focus on Endocrine Disruption and Sex Differences in Autism, ADHD, and Schizophrenia, Research Triangle Park, NC**.** October 30-November 2, 2011.
- Blossom SJ, Melnyk S, **Gilbert KM**, James SJ. Altered Redox Status and Oxidative Stress in Hippocampus of Mice Postnatally Exposed to Trichloroethylene, Arkansas Biosciences Institute Fall Research Symposium, September 21, 2011.
- Blossom SJ, Melnyk S, **Gilbert KM**, James SJ. Maternal and early life trichloroethylene exposure modulates gene expression of chemokines and

neurotrophins in the brain, 50th Annual Society of Toxicology meeting, Washington DC. March 10, 2011.

- Blossom SJ, Melnyk S, **Gilbert KM**, James SJ. Neuroimmune dysregulation with developmental exposure to trichloroethylene in a mouse model relevant to neurodevelopmental disorders, Arkansas Biosciences Institute Fall Research Symposium, Little Rock, AR. September 29. 2010.
- **Gilbert, K.M.,** Boger,S., Fifer, and Price, P. T cell tolerance induced by novel G1 blocker is mediated by cyclin-dependent kinase inhibitor p21cip1, 12th International Congress of Immunology, Montreal, Canada, 2004.
- Blossom, S.J., and **Gilbert, K.M**. Trichloroethylene-induced autoimmunity, Environmental Factors in Autoimmune Disease, NIEHS, Durham, NC, February, 2003.
- **Gilbert, K.M**., DeLoose, A., and Jackson, S.K. n-Butyrate-induced Th1 cell anergy associated with $p21^{Cip1}$ inhibition of MAPK pathway. American Association of Immunologists Annual Meeting, New Orleans, LA, April, 2002.
- **Gilbert, K.M**., Jackson, S.K., and DeLoose, A. Th1 cell anergy is associated with increased levels of both $p21^{Cip1}$ and $p27^{Kip1}$ . American Association of Immunologists Annual Meeting, Orlando, FL, April, 2001.
- Brand, K.A., **Gilbert, K.M**., Yingyun, C., E. Kim Fifer, E.K., Synthesis of butyric acid derivatives as immune response modulators. Western Region Merck Pharmacy Research Seminar, Denver, CO, June, 2001.
- Jackson, S.K. and **Gilbert, K.M**. Anergy induction in Th1 cells increases expression of cyclin-dependent kinase inhibitors. American Association of Immunologists Annual Meeting, Seattle, May, 2000.
- **Gilbert, K.M**. and Blossom, S.J. B cells from autoimmune BXSB mice are hypo-responsive to signals provided by CD4+ T cells. American Association of Immunologists Annual Meeting, Seattle, May, 2000.
- Griffin, J.M., **Gilbert, K.M**., and Pumford, N.R. Trichloroethylene accelerates an autoimmune response in MRL+/+ mice at doses similar to human exposure levels. Society of Toxicology, New Orleans, La, March, 1999.
- Fecher, N.I.P., **Gilbert, K.M.** Wahid, R. and Fifer, E.K., Butyric acid prodrugs as modulators of immune response. Western Region Merck Undergraduate Pharmacy Research Seminar", University of Colorado School of Pharmacy, Denver, CO, June, 1999.
- Blossom, S. Chu, E.B., Weigle, W.O. and **Gilbert, K.M**. B cells from autoimmune BXSB mice express CD40 ligand. American Association of Immunologists Annual Meeting, San Francisco, April, 1998
- Griffin, J.M., **Gilbert, K.M.,** and Pumford, N.R. Cytochrome P450 2E1 activation of trichloroethylene initiates a $Th_1$ T cell response in MRL+/+ mice. Linking Environmental Agents and Autoimmune Diseases, National Institutes of Environmental Health and Safety, 1998.
- Griffin, J.M., **Gilbert, K.M**., and Pumford, N.R. Acceleration of an autoimmune response in MRL+/+ mice exposed to trichloroethylene at doses similar to human exposure levels. South Central Society of Toxicology, 1998.
- Griffin, J.M**.,** Wong, J., Blossom, S.M., Jackson, S.K., **Gilbert, K.M**., and Pumford, N.R., Immunomodulation induced by trichloroethylene-in the

autoimmune prone MRL+/+ mice. Society of Toxicology, Seattle, WA, March, 1998.

- Blossom, S. and **Gilbert, K.M.** B cells from autoimmune BXSB mice express CD40 ligand. UAMS Student Research Day, Won second prize for best graduate student presentation, April, 1997.
- Blossom, S., Chu, E.B., Weigle, W.O., and **Gilbert, K.M**. , Role of CD40L+ B cells in autoimmune BXSB mice, SLE Foundation annual meeting, National Institute of Health, November, 1997.
- Griffin, J.M., **Gilbert, K.M**., and Pumford, N.R. Trichloroethylene-induced autoimmunity in MRL/++ mice, South Central Chapter of the Society of Toxicology, Jefferson, AR, November, 1997.
- Blossom, S., **K.M. Gilbert**, Increased expression of costimulator molecules on antigen presenting cells in the BXSB mouse model of systemic lupus erythematosus, American Association of Immunologists Annual Meeting, New Orleans, LA, June, 1996.
- Blossom, S. and **Gilbert, K.M**. Irregular expression of costimulator molecules on antigen presenting cells in the BXSB mouse model of systemic lupus erythematosus, American Society of Microbiology, South Central Branch, Little Rock, AR, November, 1995.
- Weigle, and **Gilbert, K.M**., Th1 cell anergy and G1a blockade. American Association of Immunologists Annual Meeting, May, Denver, CO, 1993.
- **Gilbert**, **K.M.,** Ernst, D.N., Hobbs, M.V., and Weigle, W.O. Cell cycle progression by tolerized Th1 and Th2 clones. American Association of Immunologists Annual Meeting, Anaheim, April, 1992.
- Rothermel, A.L., Ernst, D.N., Hobbs, M.V., Weigle, W.O., and **Gilbert, K.M.** Ability of tolerized Th1 and Th2 clones to stimulate B cell activation and cell cycle progression. American Association of Immunologists Annual Meeting, Anaheim, April, 1992.
- Rothermel, A.L., **Gilbert, K.M.** and Weigle, W.O., Induction of polyclonal B cell proliferation by activated T helper cells and their lymphokines, American Association of Immunologists Annual Meeting, April, 1989.

# LEADERSHIP

### Examples of Leadership Roles at UAMS

| Role | Responsibility | Affiliation | Years |
|---|---|---|---|
| Director | Arkansas Center for Environmental Exposure Research: ACEER was initiated in 2002, and since then received over 3 million dollars in extramural grant support from the NIH and other sources, and 1.6 million dollars in intramural support from the Arkansas Biosciences Institute. It provided salary and/or infrastructure support to recruit and/or retain 10 faculty members and 20 undergraduate and graduate students in 5 institutions around the state, thus enabling them to work together on common issues concerning environmental contamination and remediation. | UAMS/ACHRI | 2002 - 2017 |
| President | Women's Faculty Development Caucus (WFDC) | UAMS | 2005 - 2007 |
| Co-Director and then on organizing committee | **UAMS Graduate School Career Day**. Current graduate students, and undergraduates from all over Arkansas and surrounding states visit UAMS to hear about our graduate program, and to learn about career opportunities for PhDs. | UAMS | 2002 - 2017 |

# PROFESSIONAL RECOGNITION/ADVOCACY FOR WORK WITH TCE

- 2005: Participated in National Institutes of Health Workshop on Lupus & the Environment; Disease Development, Progression and Flares. I represented the work done on solvents and autoimmune disease at this workshop organized by the NIH to develop grant funding on the subject of environmental pollutants and the development of lupus.
- 2006: Consulted with the Committee on Human Health Risks of Trichloroethylene, National Research Council during the development of document entitled *Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues*,
- 2007: Consulted with Pew Charitable Trust Environmental Working Group for "Kid-Safe Chemicals Act of 2008" bill before US Congress.

- 2008: Featured in *The Autoimmune Epidemic* by Donna Jackson Nakazawa, Simon & Schuster, New York
- 2008: Featured in "The Scariest Health Threat You've Never Heard Of" in *Glamour*, September
- 2009: Participated in the EPA Science Advisory Board's review of the Trichloroethylene (TCE) health assessment
- 2013: Served on an expert panel that reviewed EPA TSCA Workplan Chemical Risk Assessment for Trichloroethylene: Degreaser and Arts/Crafts Uses
- 2014: Served on a TCE Information Group tasked with assisting the NTP Office of the Report on Carcinogens as they considered a possible change in listing status for TCE in the Report on Carcinogens. Specifically, we were asked to provide comments on whether the information concerning the immunotoxicity of TCE provided biological plausibility for TCE-related cancers.
- 2014: Worked with the Arkansas Department of Environmental Quality and the Arkansas Department Health to identify TCE-contaminated sites in Northern Arkansas, and to identify toxicant-induced autoimmune disease in people living near the sites
- 2017: Selected as a permanent member of the Scientific Advisory Committee on Chemicals (SACC) (Toxic Substances Control Act), for the US Environmental Protection Agency. The SACC is tasked with providing independent advice and expert consultation on issues related to the implementation of the Frank R. Lautenberg Chemical Safety for the 21st Century Act which amends the Toxic Substances Control Act. The first 10 chemicals reviewed included trichloroethylene, tetrachloroethylene (perchloroethylene), carbon tetrachloride, methylene chloride, 1-bromopropane, n-methyl pyrrolidone, 1,4-dioxane, pigment violet 29, asbestos, and cyclic aliphatic bromide cluster.
- 2019: As requested by the National Academy of Sciences reviewed a report prepared by the National Academies Board on Environmental Studies and Toxicology concerning their review of the *DOD's Approach to Deriving an Occupational Exposure Level for Trichloroethylene*

## LITIGATION HISTORY

| Case | Law Firm | Contribution | Outcome |
|---|---|---|---|
| Jodelle L. Kirk v Schaeffler Group USA Inc.: FAG Bearings, LLC, 2016<br>United States District Court<br>Western District of Missouri,<br>Cause No 3:13-cv-0503 | Humphrey, Farrington, & McClain | Wrote expert report on TCE<br><br>Deposed<br><br>Testified at trial as to general and specific causation | Plaintiff awarded $20.6 million dollars |
| Hostetler, et al. vs Johnson Co, Inc, et al. 2018<br>United Stated District Court<br><br>Northern District of Indiana<br>Cause No. 3:15-CV-226JD-MGG | Taft Stettinius & Hollister LLP | Wrote expert report on TCE general causation increasing risk for autoimmune diseases and cancer<br><br>Deposed | Settled in 2023 |
| Asher, et al. v Raytheon Technologies Co, et al.<br>Huntington Superior Court<br>State of Indiana<br>Cause No. 35D-01-2006-CT-000338<br><br>Emergency hearing to determine health risks of the drinking water in Andrews, Indiana, 2020 | Taft Stettinius & Hollister LLP | Wrote Affidavit concerning human health effects of vinyl chloride and cis-1,2-Dichloroethylene in water supply<br><br>Testified at remote hearing<br><br>Deposed 4-16-24 | Emergency action was denied |
| Millman, Powell and Powell vs Raytheon Technologies F/k/a United Technologies et al. Corporation, 2021<br><br>Northern District of Indiana<br>Cause No.:1:16-cv-00312-HAB-SLC | Taft Stettinius & Hollister LLP | Wrote expert report on human health effects of trichloroethylene, vinyl chloride, benzene, and cis-1,2-Dichloroethylene<br><br>Performed risk assessments for 3 plaintiffs<br><br>Opinioned that toxicant exposure contributed to development of liver cancer and trigeminal neuralgia in 2 plaintiffs<br><br>Deposed | Ongoing |

| | | | |
|---|---|---|---|
| Houlihan vs United Technologies Corporation, 2019<br>Huntington Superior Court<br>State of Indiana<br>Cause No. 35C01-1803-CT-000144 | Taft Stettinius & Hollister LLP | Wrote expert report on the ability of trichloroethylene, vinyl chloride and benzene to promote immunotoxicity and cancer<br><br>Deposed | Ongoing |
| Funderburk et al. vs Johnson Controls, Inc. and TOCON Holdings, LLC, 2021 | Taft Stettinius & Hollister LLP | Wrote expert report concerning the human health effects of trichloroethylene, perchloroethylene, vinyl chloride and cis-1,2-Dichloroethylene<br><br>Performed risk assessments for 94 plaintiffs<br><br>Deposed 8-30-23 | Ongoing |
| Taylor et al. v Schaeffler Group., et al (Case No.:20AO-CC0341) | Humphrey, Farrington & McClain Independence, Missouri | Trichloroethylene general causation<br><br>Deposed 5-17-23 | |
| Preliminary research, 2022 | Mueller Law Offices, Austin Texas | Generated report on epidemiological studies linking trichloroethylene exposure and human health | |
| Preliminary research, 2022 | Romanucci & Blandin, Chicago, IL | Performed risk assessments for 19 plaintiffs | |

# Appendix B

## *Fee Schedule*

**Kathleen M. Gilbert, PhD**
Immunotoxicologist

(501) 249-4552
gilbertkathleenm4@gmail.com

240 West 75th St, Apt. 1B, New York, NY 10023
800 Shire Court, Fort Collins, CO 80526

- Hourly charge for document and record review: $400
- Hourly charge for consulting over the phone: $250
- Hourly charge for affidavit/report writing: $450
- Hourly charge for deposition: $500
- Hourly charge for trial testimony: $500