Trichloroethylene Protocol Introduction: Methods for Preparing the Draft RoC Monograph

Trichloroethylene (TCE) is a volatile chlorinated alkene that is primarily used as a metal cleaner and degreaser. It has been listed in the *Report on Carcinogens* (RoC) as *reasonably anticipated to be a human carcinogen* since 2000. This listing was based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of carcinogenicity from studies in experimental animals, and supporting information from studies on mechanisms of carcinogenesis. Since TCE was listed in the RoC, additional cancer studies in humans have been published. The NTP has proposed that TCE be reviewed for a possible change in its listing status in the RoC because of an extensive database of cancer studies and public health concern due to its pervasiveness in the environment, and presence in food, numerous consumer products, and the workplace.

Background information and goals

The database on TCE exposure and cancer includes several comprehensive reviews of the epidemiologic data, toxicological data, metabolism, genotoxicity, and potential modes of action (IOM 2003, NRC 2006, EPA 2011), and several recent meta-analyses of epidemiologic studies. These reviews have identified non-Hodgkin lymphoma (NHL) and cancers of the liver and kidney as sites of concern in humans. There is site concordance for these cancers in experimental animals. No new studies or reviews were identified to question the RoC 2000 conclusion that there is *sufficient evidence of carcinogenicity from studies in experimental animals (Trichloroethylene substance profile).*

The goal of the cancer evaluation component of the draft RoC monograph is to conduct an independent assessment of the scientific literature while utilizing information from the extensive reviews conducted by other agencies and scientific panels to focus the deliberations. The draft RoC monograph will assess the level of evidence from human cancer studies and evaluate the evidence from mechanistic and other related data such as metabolism, genotoxicity, and immunotoxicity. The cancer evaluation will not re-evaluate the level of evidence of carcinogenicity from studies in experimental animals. The monograph will limit its assessment to NHL, multiple myeloma, chronic lymphocytic leukemia and cancers of the kidney and liver in humans. Studies of multiple myeloma and chronic lymphocytic leukemia (CLL) will also be reviewed because these types of lymphohematopoietic cancers are considered to be within the family of NHL (Goldstein 2010[1]). The original listing established that a significant number of persons in the United States are (or were) exposed to TCE. The exposure data will be updated in the draft substance profile component of the draft RoC monograph on TCE but will not be reassessed in the cancer evaluation component.

Listing recommendation: RoC listing criteria[2]

The purpose of the cancer evaluation component is to assess the scientific evidence, apply the RoC listing criteria to this evidence, and reach a preliminary level of evidence conclusion from studies in humans, and a preliminary recommendation for the listing status in the RoC. Briefly, the RoC listing criteria for the two listing categories (*known to be a human carcinogen* and *reasonably anticipated to be a human carcinogen*) are as follows:

[1] Goldstein, B. 2010. Benzene as a cause of lymphoproliferative disorders. Chemico-Biological interactions 184: 147-150.

[2] The entire listing criteria is available at http://ntp.niehs.nih.gov/go/15209

Known to be a human carcinogen: Sufficient evidence of carcinogenicity from studies in humans.

Reasonably anticipated to be a human carcinogen

- Limited evidence of carcinogenicity from studies in humans, or
- Sufficient evidence of carcinogenicity from studies in experimental animals, or
- Substance belongs to a structurally related class of substances that are listed in the RoC, or
- Convincing relevant information that the agent acts through a mechanism indicating it would likely cause cancer in humans.

Conclusions regarding the carcinogenicity in humans or experimental animals are based on scientific judgment with consideration of all relevant data.

Protocol components

This protocol discusses the methods that will be used to prepare the draft monograph on TCE (primarily the cancer evaluation component) and includes the following sections:

- Part A: Preliminary outline.
- Part B: Methods for evaluating human cancer studies.
- Part C: Methods for evaluating mechanistic and other relevant data, including but not limited to absorption, distribution, metabolism, and excretion; toxicokinetics; genotoxicity; immune effects; and potential mechanisms of carcinogenicity for NHL (and multiple myeloma and CLL), and cancer of the kidney and liver in animals and humans.
- Part D: Methods for updating the exposure information in the substance profile.

TCE Protocol Part A:
Preliminary Outline for the Cancer Evaluation Component of the Draft Monograph

As stated in the introduction, the draft RoC monograph on TCE focuses on the relationship between exposure to TCE and NHL, multiple myeloma, CLL and cancers of the kidney and liver. The cancer evaluation component is organized by cancer site, rather than by topic (e.g., human cancer studies, experimental animals studies, etc.), as is the usual convention. The first sections of the cancer evaluation component will discuss studies and data on disposition and toxicokinetics, and on relevant biological effects, e.g., genotoxicity and immune effects, that are useful for the evaluation of all cancer sites. The appendices also contain background information, such as a description of the characteristics and quality evaluation of the human cancer studies and evidence-based tables on biological effects applicable to all targeted cancer sites. Each section on a specific type of cancer will assess both human cancer studies and mechanistic studies relevant for evaluating whether TCE causes that specific cancer. The final section of the draft RoC monograph will contain the NTP preliminary listing recommendation for TCE in the RoC and a summary of the data considered key to reaching that listing recommendation.

The major sections in the cancer evaluation component are as follows:

1 Disposition and toxicokinetics

This section provides an overview of absorption, distribution, and excretion and a more detailed discussion of metabolism in experimental animals and humans.

2 Relevant biological effects

This section assesses the strength of evidence for biological effects induced by TCE (and its metabolites) – primarily genetic and immune – that may play a role in the proposed mechanism of cancer at more than one site. The section discusses the evidence for both humans and experimental animals. Evidence-based tables for these effects will be in Appendices B (genotoxicity) and C (immune effects).

3 Kidney cancer

3.1 Human cancer studies

3.1.1 Overview of the epidemiologic studies evaluating kidney cancer

This section provides an overview of the available studies evaluating kidney cancer, and discusses which studies are considered to be most informative for evaluating this cancer. Details on the literature search, inclusion/exclusion criteria, quality evaluation and strengths and limitations of the studies across studies (not specific for cancer site) are in Appendix D.

3.1.2 Cancer assessment: kidney cancer

This section describes the findings from the individual studies and meta-analyses, evaluates potential confounding specific for kidney cancer, integrates the findings across studies, applies the RoC listing criteria to the body of evidence, and reaches a preliminary recommendation for the level of evidence for kidney cancer from studies in humans.

3.2 Mechanistic and other related data

This section assesses the quality of the mechanistic and other related data, the cohesiveness of the proposed mechanism(s) of action, and the biological plausibility for kidney cancer in experimental animals and humans.

3.2.1 Data relevant for evaluating potential modes of action for kidney cancer

- Studies of kidney toxicity in humans and experimental animals
- Studies of mutations in the Von-Hippel-Lindau tumor suppressor gene

3.2.2 Potential modes of action for kidney cancer

- Mutagenicity
- Cytotoxicity and cellular proliferation
- Other mechanisms, possibly including peroxisome proliferator activated receptor alpha (PPARα) action, alpha 2u-globulin nephropathy, formic acid-related nephrotoxicity, etc.

3.3 Integration across evidence streams (human cancer, studies in experimental animals, and mechanistic studies).

This section integrates the conclusions on the level of evidence in humans and findings from experimental animals (from the current profile in the RoC) and mechanistic studies on kidney cancer.

4 NHL (including multiple myeloma, and CLL)

4.1 Human cancer studies

4.1.1 Overview of the epidemiologic studies evaluating NHL

This section contains an overview of the available studies evaluating NHL, and discusses which studies are considered to be most informative for evaluating this cancer. Details on the literature search, inclusion/exclusion criteria, quality evaluation and strengths and limitations of the studies across studies (not specific for cancer site) are in Appendix C.

4.1.2 Cancer assessment: NHL

This section describes the findings from the individual studies and meta-analyses, evaluates potential confounding specific for NHL, integrates the findings across studies, applies the RoC listing criteria to the body of evidence, and reaches a preliminary recommendation for the level of evidence for NHL from studies in humans.

4.2 Mechanistic and other related data

This section assesses the quality of the mechanistic and other related data, the cohesiveness of the proposed mechanism(s) of action, and the biological plausibility for NHL in experimental animals and humans. Potential modes of action include immunomodulation and genotoxicity.

4.3 Integration across evidence streams (human cancer, studies in experimental animals, and mechanistic studies.

This section integrates the conclusions on the level of evidence in humans and findings from experimental animals (from the current profile in the RoC) and mechanistic studies on NHL.

5 Liver cancer

5.1 Human cancer studies

5.1.1 Overview of the epidemiologic studies evaluating liver cancer

This section contains an overview of the available studies evaluating liver cancer, and discusses which studies are considered to be most informative for evaluating this cancer. Details on the literature search, inclusion/exclusion criteria, quality evaluation and strengths and limitations of the studies across studies (not specific for cancer site) are in Appendix C.

5.1.2 Cancer assessment: Liver cancer

This section describes the findings from the individual studies and meta-analyses, evaluates potential confounding specific for liver cancer, integrates the findings across studies, applies the RoC listing criteria to the body of evidence, and reaches a preliminary recommendation for the level of evidence for liver cancer from studies in humans.

5.2 Mechanistic and other related data

This section assesses the quality of the mechanistic and other related data, the cohesiveness of the proposed mechanism(s) of action, and the biological plausibility for liver cancer in experimental animals and humans. Potential modes of action are as follows:

- Mutagenicity
- PPAR
- Immune effects
- Cytotoxicity and reparative hyperplasia
- Other potential mechanisms including negative selection, glycogen storage, inactivation of GST-zeta, oxidative stress, epigenetic changes

5.3 Integration across evidence streams (human cancer, studies in experimental animals, and mechanistic studies).

This section integrates the conclusions on the level of evidence in humans and findings from experimental animals (from the current profile in the RoC) and mechanistic studies on liver cancer.

6 Final conclusions

This section contains (1) the level of evidence conclusions of carcinogenicity from studies in humans for each of the three cancer sites, (2) the level of evidence conclusion from studies in experimental animals and the data supporting that conclusion (from the substance profile in the current edition of the RoC), and (3) a summary of the integration of the experimental and human data for each cancer site.

Appendices

Appendix A – Literature search strategy for mechanistic and other relevant data

Appendix B - Evidence-based tables on genotoxicity

Appendix C – Evidence-based tables on immune effects

Appendix D – Description and quality evaluation of the human cancer studies

D.1 –Literature search strategy and selection of studies

D.2 – Description of the studies: study characteristics and methodologies

- Text: Overview of studies
- Tables: Study characteristics and methodologies (appendix tables)

D.3 Evaluation of study quality

- Tables: Study quality (similar to appendix tables)
- Text: Discussion of strengths and weaknesses and utility of studies (in general)

TCE Protocol Part B:
Methods for Evaluating Human Cancer Studies

Objectives and Key Questions

As mentioned in the introduction, numerous cancer studies in humans have been published since TCE was first listed in the Report on Carcinogens (RoC). The cancer evaluation component of the draft monograph on TCE will evaluate all relevant epidemiologic studies on TCE exposure and cancer, including those studies previously reviewed for the RoC as well as studies published since that time. The available studies on exposure to TCE and human cancer consist primarily of (1) cohort studies of aircraft and aerospace workers, biomonitoring studies, and studies of TCE-exposed workers in other industries such as electronics, dry cleaners, paperboard or cardboard manufacturers, and TCE producers, (2) hospital- and population-based case-control studies of occupational exposure to TCE, and (3) geographically based studies of environmental exposure to TCE.

The objective of this section is to reach a preliminary level of evidence conclusion [sufficient, limited, or inadequate] for the carcinogenicity of TCE from studies in humans by applying the RoC listing criteria to the body of evidence.

RoC Listing Criteria for Evaluating Carcinogenicity from Studies in Humans

Sufficient evidence of carcinogenicity from studies in humans: indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Limited evidence of carcinogenicity from studies in humans: causal interpretation is credible, but alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded.

Key questions are as follows:

- Which epidemiologic studies should be included in the review?
- What are the methodological strengths and limitations of these studies?
- What are the potential confounders for cancer risk for the tumor sites of interest in these studies?
- Is there a credible association between exposure to TCE and cancer?
- If so, can the relationship between cancer endpoints and exposure to TCE be explained by chance, bias, or confounding?

Steps in the Cancer Evaluation Process:

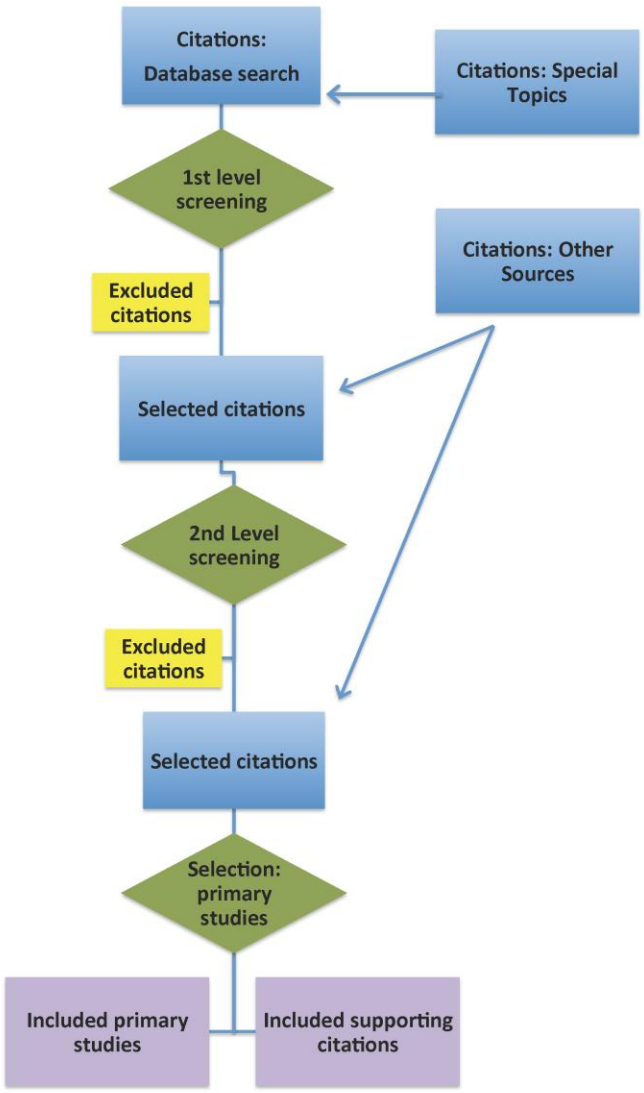
The steps for conducting the human cancer evaluation are outlined below. The procedures and guidelines for conducting each step are described in Sections 1 through 4 of Part B of this protocol.

1. Selection of the literature included in the human cancer evaluation (Section 1)
2. Systematic extraction of data from the epidemiologic studies (Section 2)
3. Assessment of the quality of the individual epidemiologic studies (Section 3)
4. Assessment of the level of evidence of carcinogenicity (sufficient, limited, or inadequate) of TCE from studies in humans (Section 4)

1 Section 1: Selection of the literature included in the human cancer evaluation

This section discusses procedures to identify and select literature relevant for the human cancer evaluation, including the literature search strategy and inclusion and exclusion criteria. This literature includes the primary epidemiologic studies, which form the basis for the cancer evaluation, and supporting literature (e.g., included supporting citations) that may be relevant for interpretation of the studies. The first step in the process is to develop a literature search strategy and associated inclusion/exclusion criteria to identify the relevant literature, and the second step is to select the primary epidemiologic studies from this database. Figure 1-1 is a schematic of the process, which is described in detail below.

Figure 1-1. Literature identification and selection process



The identification of the relevant literature for the cancer evaluation includes strategies for searching for citations and inclusion/exclusion questions for selecting the relevant citations from the searches.

1.1 Literature search strategy

TCE is used primarily as a metal degreaser and is widely used in industries such as metal fabricating and working, electronics, the aerospace industry, and dry cleaning. These exposure scenarios are used to develop search terms in the literature search strategy. The following approaches for identifying literature will be employed.

1. Literature searches of three scientific databases – PubMed, Scopus, and Web of Science – using a pre-determined range of search terms. Search terms for potential TCE exposure, e.g., terms related to exposure scenarios and terms specific for TCE are combined (using "and") with search terms for epidemiologic studies and with search terms for the outcome, i.e., cancer. The specific search terms are listed in the table below. (See Figure 1-1, Citations: Database.)

Substance-specific search terms[3]	**Epidemiologic search terms**	**Cancer search terms**
((trichloroethylene) OR TCE) OR trichloroethene) OR "acetylene trichloride") OR "ethylene trichloride")) OR "79-01-6") OR ((degreas* OR aircraft OR aerospace OR aircraft-maintenance OR (metal manufact*) OR (electr* AND manufact*)) AND work*)) OR chlorinated solvents OR trichloroethylene[MeSH] (for PubMed only)	(epidemiolog* OR case-control OR cohort OR case-report OR case-series OR workers OR workmen) OR Meta-analysis [publication type] (for PubMed only)	(cancer OR tumor OR NHL OR lymphoma* or "lymphohematopoietic cancer" OR "multiple myeloma" OR "chronic lymphocytic leukemia" OR CLL)

1. Full-text searches of a Quosa-based database of case-control studies on occupational exposure (general) using the term TCE or its synonyms. (See Figure 1-1, Citations: Database.)
2. Searches of a pre-determined standard list of general sources including U.S. and international government agency reports, authoritative reviews and related reports (e.g., International Agency for Research on Cancer, U.S. Environmental Protection Agency, Agency for Toxic Substances and Disease Registry, European Union, National Academy of Sciences, National Institute for Occupational Safety and Health) to identify any additional primary epidemiologic studies together with supporting reviews and material that may be relevant for the interpretation of the primary studies. (See Figure 1-1, Citations: Other Sources.)

[3] Note: No search terms were developed for dry-cleaning workers because it is unlikely that substantial numbers of them were frequently exposed to sufficient amounts of TCE (NRC 2006) and thus these studies are not included in the review.

3. Citation searches from articles, reports, and reviews identified above to identify any additional primary studies or other relevant literature. (See Figure 1-1, Citations: Other Sources.)
4. Additional literature searches may be conducted on special topics or issues. Examples include searches for information on co-exposures found in the different occupational settings, or information on exposure measures, each of which would require different search terms.

The scientific database search strategies will be saved in Scopus, Web of Science, and PubMed, respectively, which automatically send out weekly notifications concerning newly identified citations using the saved search strategy.

1.2 Selection of relevant literature

Citations retrieved from literature searches will be uploaded to web-based systematic review software and screened using pre-defined inclusion and exclusion criteria (see below). Multi-level screening of the literature identified from the searches is conducted (see Figure 1-1); the initial screening is based on titles and abstracts only (Level 1), and subsequent screening is based on full-text PDFs (Levels 2 and 3).

Literature is screened at each level by two reviewers using inclusion/exclusion criteria for each level as listed below. The objective of Levels 1 and 2 is to identify literature that is useful for the cancer evaluation section, including primary research studies, reviews, and studies on relevant issues (such as confounders) related to cancer evaluation of TCE. In general, the inclusion and exclusion criteria are similar at each level, but because screening of the literature at Level 1 is done using titles and abstracts, the "bar" for excluding literature is very high; a more detailed review of the studies for inclusion/exclusion is conducted at Level 2 using the full text article. The objective of the Level 3 review is to select the primary epidemiologic studies that will be discussed in the cancer review, as described below.

Inclusion/exclusion criteria: Level 1 (titles and abstracts) and Level 2 (full text)

The following criteria will be applied to the selection of citations at Level 1 for further review at Level 2:

(1) Studies of TCE (but not its metabolites or members of its chemical class) and human cancer that potentially provide information related to answering the key questions for the review of human cancer and exposure to TCE published in any year.

(2) Relevant information includes, but is not limited to, epidemiologic studies, descriptive studies, pooled analyses, meta-analyses, reviews, letters to editors, exposure-assessment studies (for use in epidemiologic studies), exposure-validation or relevant epidemiologic studies of biomarkers, and information on co-exposures or potential confounders and other special topics of relevance to the evaluation.

1.3 Selection of primary epidemiologic studies

As mentioned in the introduction, the database on TCE exposure and cancer includes several comprehensive reviews (IOM 2003, NRC 2006, EPA 2011) of the epidemiologic data. These reviews are used to focus the RoC evaluation on specific cancer endpoints (NHL, and cancer of the liver and kidney) and on primary epidemiologic studies with information specific for TCE exposure at the individual level. For example, some types of studies had few or no data to evaluate potential exposure to TCE at the individual level (such as geographical, ecologic workers or studies on

exposure to mixed solvents) or were of occupations (such as dry cleaner) with insufficient numbers of workers who were frequently exposed to sufficient amounts of TCE (NRC 2006); these studies will be excluded from the RoC evaluation. The ORoC will select primary epidemiologic studies for the cancer evaluation from Level 2- references that meet the criteria listed below in Bullets 1 to 3.

Inclusion/exclusion criteria: Level 3 (full text)

The following criteria will be applied to the selection of citations for further review at Level 3:

(1) The publication is a peer-reviewed, primary research study on potential exposure to TCE and human cancer (NHL, kidney cancer, or liver cancer).

(2) The study reports a risk estimate (or information to calculate a risk estimate) for cancer; descriptive studies will not be included in the evaluation.

(3) The publication is a peer-reviewed, primary research study that provides information specific for potential exposure to TCE at the individual level.

Any primary epidemiologic study (such as geographical studies) on potential exposure to TCE that were retrieved from the literature search strategy and not included in the cancer evaluation, and the reason for their exclusion, will be identified in the monograph. Information from multiple publications relating to the same study population may be included in the draft monograph, but the publications will be counted as one study.

"Included supporting citations" (see Figure 1-1) refers to other literature (such as studies on co-exposures, potential confounders or exposure assessments, reviews, or meta-analyses) that may help inform the evaluation of the primary studies and that will be cited in the monograph.

2 Section 2: Systematic extraction of data from the epidemiologic studies

Two independent reviewers will extract data (such as methods and findings) from the individual studies into a database in a systematic manner using standardized instructions and questions. The database contains "fields" that are specific for the different types of extracted information (such as study population characteristics, exposure assessment, analytical methods, and results). The instructions (questions or guidelines) describe the specific type of information that should be summarized or entered into each field. The fields will be used to populate tables used in the monograph.

For the cohort and case-control studies, the reviewer will usually extract data from the latest published follow-up or update for each cancer endpoint included in the study. Other relevant information (such as exposure data or re-analyses) from earlier and related publications on the same or overlapping study population will also be included in the review if these publications provide unique or additional data to inform the cancer evaluation of the primary study under review.

Quality assurance and quality control of data extraction and database entry will be accomplished by (1) double-checking of each data entry by the two independent reviewers and (2) flagging any discrepant entries and resolving them by mutual discussion in reference to the original data source.

3 Section 3: Assessment of the quality of the individual epidemiologic studies

Each primary study will be systematically evaluated for its ability to inform hazard identification. Studies that will be given the most weight in the evaluation of study quality are those that provide the most valid (i.e., low risk of systematic error or biases) and precise (i.e., low risk of random error) risk estimates, and that are adequate (e.g., sufficient power and adequate range of exposure) to detect an effect. Study limitations and characteristics will be taken into account in evaluating the reported cancer findings. In addition, studies should accurately report their findings and apply appropriate analytical methods for calculating risk estimates. The procedures (questions and guidelines) for evaluating the different components of study quality are described in Sections 3.1 to 3.5, below. Similar questions and guidelines have been used for the review of other chemicals (*ortho*-toluidine and pentachlorophenol and byproducts of its synthesis) evaluated by the RoC but are adapted to be specific for the review of TCE. The guidelines state characteristics of ideal studies, and the studies are evaluated in the context of how each study element approaches (or departs from) these ideals. As part of its toxicological review of TCE, the U.S. EPA conducted a systematic review of study quality using set criteria for the epidemiologic studies on exposure to TCE, which discussed some of the key issues for evaluating study quality (EPA 2011). The RoC concurs with this approach and has integrated some elements of EPA's study evaluation criteria into its guidelines, when appropriate, as discussed below.

3.1 Reporting quality questions

Is there adequate documentation and reporting of the (1) description of the selection and follow-up of the population, (2) methods to assess exposure and disease, (3) analytical methods, and (4) cancer findings?

3.2 Analyses of biases: a priori *questions and guidelines*

The application of the RoC listing criteria to the body of studies on TCE includes an analysis of whether any association observed between exposure to TCE and cancer can be explained by chance, bias, or confounding. The first step in the assessment is to evaluate the study methods to determine whether there is a potential for bias. Biases in observational studies are often classified into three major categories: (1) selection bias, (2) information bias, and (3) confounding (Rothman *et al.* 2008).[4] Studies with a lower potential for bias are generally considered to be the most informative for the cancer evaluation. However, the presence of a potential bias in a study does not necessarily mean that the findings of the study should be disregarded. Therefore, an important step in the process of evaluating biases is to determine the probable impact of the potential biases on study results—that is, the magnitude of distortion and the direction in which each bias is likely to affect the outcome of interest. This step is reflected in the second part of the questions (below) and is analyzed in the assessment of the level of evidence (Appendix D).

Questions and guidelines for evaluating methods used to select the study population and obtain information on exposure and disease are provided in Sections 3.2.1. and 3.2.2. The approach for evaluating confounding, which is a key issue in the cancer evaluation of TCE, is discussed in Section 3.3.

[4] Rothman K, Greenland S, Lash T. 2008. *Modern Epidemiology, 3rd Edition*. New York: Lippincott, Williams, and Wilkins, 851 pp.

3.2.1 Selection and attrition

Studies will be evaluated for the potential for selection or attrition bias. The questions are somewhat different depending on the study design. The questions and guidelines will be used to identify studies in which there is probable concern for selection bias, and to determine whether this concern would lead to an over- or under-estimation or indeterminable direction of the risk estimate.

Questions

- In cohort studies, are the unexposed subjects and exposed subjects from the same underlying population? If not, what information is available to estimate the potential direction and relative magnitude of distortion from the bias? Is there any evidence of a healthy worker hire effect?[5] If so, what is the direction and relative magnitude of the distortion from the bias on the risk estimate?
- In case-control studies, are controls selected from the same underlying population as the cases using similar inclusion/exclusion criteria? If not, what is the likely direction and relative magnitude of distortion from the bias?
- In case-control studies, is there any evidence that the methods used to identify and select the controls and/or cases are related to exposure to TCE? If so, what information is available to estimate the direction and magnitude of distortion from any potential bias?
- Is there any evidence for self-selection or that refusal to participate in the study is related to both exposure and disease status? If so, what information is available to estimate the direction and magnitude of distortion from the bias?
- Is the ascertainment of vital status at the end of the follow-up period in cohort studies adequate? Is there any evidence to suggest that there is systematic bias in ascertainment, i.e., that completeness of follow-up is related to both exposure and disease status? If so, is it possible to predict the direction and magnitude of distortion from the bias?
- Is there any evidence of a healthy worker survivor effect or left truncation in cohort studies? If so, were appropriate analyses performed to control for the potential bias? Is it possible to predict the direction and relative magnitude of distortion from any uncontrolled (residual) bias?

Guidelines

In cohort studies, the exposed and unexposed groups should ideally be similar in all respects except for exposure to TCE. Occupational cohorts should consist of all potentially exposed employees within a given plant or exposure setting (employed over a specified period of use of TCE) compared with similar unexposed employees from within the same plant or setting (i.e., internal controls) to minimize the healthy worker effect and other differences between exposed and unexposed groups. When external referents are used in, e.g., SMR or SIR studies, local (or regional) mortality or incidence rates are generally, but not always, preferable to national rates.

Systematic biases may be introduced if the length and completeness of follow-up differ between exposed and unexposed groups and are related to the outcome of interest. Ideally, the total loss to follow-up should be less than approximately 5% over the duration of the study observation period. Overall, studies should have more than 80% to 90% total follow-up, although incidence studies may have greater loss to follow-up than mortality studies. Statistical power may be reduced in studies having a high percentage of all subjects (regardless of exposure and disease status) lost to follow-up.

[5] The healthy worker effect can also be considered as a confounder.

In nested and population-based case-control studies, controls and cases should be selected from the same underlying population (or cohort) and should be representative of the population (or cohort) from which they were selected. Controls should be free of any diseases related to TCE exposure; the use of controls with diseases related to TCE exposure would bias toward the null. Ideally, participation rates should be high and should be similar for cases and controls, although it is recognized that participation rates in population-based case-control studies are often lower than those in a hospital-based study.

3.2.2 Information (observation) bias

Studies will be evaluated for their adequacy in measuring exposure and disease endpoints, including missing data and the probability of misclassification of exposure and disease. The questions and guidelines will be used to identify studies in which there is "probable" concern for information bias, and to determine whether this concern would lead to an over- or under-estimation or indeterminable direction of the risk estimate. They will also be used to determine the quality of the exposure characterization (limited, adequate, good).

Questions

- What method was used to assign exposure to subjects according to their potential for TCE exposure? Does the method permit exposure assessment for individual subjects or only for exposure groups? Is the exposure measure qualitative, semi-quantitative, or quantitative? Are errors (if any) in classifying exposure similar (i.e., non-differential misclassification) or different (i.e., differential misclassification) across study groups? If there is evidence for misclassification of exposure, what is the direction and relative magnitude of distortion from the bias?
- Are exposure data missing for the cases and controls or cohort members? Were missing data imputed, and if so, how was this done and are these methods adequate?
- What is the level of confidence that the study was able to identify and classify subjects accurately and completely with respect to cancer endpoints? Was disease assessed similarly across study groups? If disease was misclassified, is it possible to predict the direction and relative magnitude of distortion from the bias?

Guidelines: exposure assessment

One of the most important aspects of a study is the ability to characterize exposure at the individual level. The ideal would be to have quantitative estimates of exposure to TCE and relevant co-exposures for each individual that are based on a job-exposure matrix (JEM) or expert assessment that link the subject's occupational history (e.g., job or department titles, task descriptions, duration of employment, calendar years worked) with data on relevant exposure monitoring or on production methods or applications that are calendar-year specific. Exposure estimates using multiple metrics (such as cumulative, peak, average intensity) improve the quality of the assessment. Some studies assessed exposure to TCE by using biological markers of either concentration of trichloroacetic acid (TCA) in urine or TCE in blood (Axelson *et al.* 1994[6]; Anttila *et al.* 1995; Hansen *et al.* 2001). Urinary TCA (U-TCA) is a non-selective marker because other

[6] Axelson O, Seldén A, Andersson K, Hogstedt C. (1994). Updated and expanded Swedish cohort study on trichloroethylene and cancer risk. J Occup Med 36: 556-562. Anttila A, Pukkala E, Sallmen M, Hernberg S, Hemminki K. (1995). Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. J Occup Environ Med 37: 797-806. Hansen J, Raaschou-Nielsen O, Christensen J, Johansen I, McLaughlin J, Lipworth L, Blot W, Olsen J. (2001). Cancer incidence among Danish workers exposed to trichloroethylene. J Occup Environ Med 43: 133-139.

chlorinated solvents besides TCE are also metabolized to TCA. As noted by EPA, U-TCA may be a useful marker in occupational settings where TCE is the only exposure but not in settings where exposure is to mixed solvents (EPA 2011). The half-life of U-TCA is approximately 100 hours, thus U-TCA represents roughly the weekly average of exposure from all sources and routes, including skin absorption (EPA 2011).

Misclassification of exposure in cohort studies is almost always non-differential and usually results in an underestimation of the risk estimate. In general, exposure is better characterized in most occupational cohort studies than in population-based case-control studies. Potential misclassification of exposure can be reduced in studies conducted in geographical areas with industries associated with TCE and that assess exposure using a JEM or expert assessment of information on tasks and jobs collected via detailed occupational questionnaires and interviews. In-person interviews are preferred over mailed or phone interviews, and information obtained from the subject is preferred over information from proxies. Ideally, exposure assessment and interviewers should be blinded to the status of cases and controls. Of these, the blinding of the investigators conducting exposure assessment is considered the most important; blinded in-person interviews are not usually feasible depending on the health of the subject with cancer.

Recall bias in case-control studies in which occupational exposure is assigned based on job titles is less likely to be a concern than in studies using self-assessment of chemical-specific exposures (e.g., use of questionnaires with exposure check-lists). With the possible exception of recall bias from self-reported exposure, misclassification of exposure is usually non-differential, and would most likely bias the risk estimate toward the null.

Guidelines: endpoint assessment

Incidence data from population-based cancer registry sources or hospital pathology data generally provide more detailed and accurate diagnostic data and more accurate population (comparison) cancer rates than death certificate-based mortality data. The quality and completeness of the cancer registry incidence data, which can vary by, e.g., collection methods, region, and calendar period, will be evaluated. In addition, cancer incidence data may be considerably more informative than mortality data (depending on ascertainment, reporting, and diagnostic accuracy) for cancers with relatively longer survival times and good treatment prognoses, such as low-grade non-Hodgkin lymphoma and chronic lymphocytic leukemia (CLL; now considered a form of lymphoma). In the case of TCE, the principal cancers investigated to date are NHL, and cancers of the liver and kidney. Length of follow-up is also critical in identifying cases or deaths from long latency cancer endpoints. For longer latency but lower survival cancers such as liver cancer, both incidence and mortality data may be of similar utility, assuming an adequate length of follow-up.

Of particular concern is the diagnosis of NHL. The classification of subtypes of lymphohematopoietic cancers has changed over the course of several editions of the International Classifications of Diseases (ICD) and may present challenges if histological data are unavailable to confirm subtypes.[7] Prior to 1994, ICD classification typically grouped lymphatic neoplasms together instead of identifying individual cancers or cell types; coding for lymphatic tumors was initiated with the introduction of the Revised European-American Lymphoma classification, which is the basis of the current WHO B-23 (EPA 2011). It was also recognized that some NHLs and corresponding lymphoid leukemias were different phases (solid and circulating) of the same

[7] Weisenberger D. (1992). Pathological classification of non-Hodgkin's lymphoma for epidemiological studies. Cancer Res, 52: 5456s–5461s.

disease entity (Morton *et al.* 2007[8], as cited by EPA 2011). The potential for misclassification of NHL is greater in epidemiologic studies conducted before this time, especially those using mortality data.

Case-control studies may measure disease more accurately since many studies conduct their own pathological review of cases (or a subset of cases). Non-differential (not related to exposure status) misclassification of cancer would most likely result (if not related to exposure status) in loss of statistical power and an underestimation of the risk estimate.

3.3 Approach for evaluating confounding

A key question in the evaluation of the level of evidence from human studies is whether an association (if any) between exposure to TCE and cancer can be explained by confounding. Potential confounders include any exposures or risk factors that could be associated with both exposure to TCE and the disease outcome(s) of interest and that are not part of the disease pathway.

The evaluation of potential confounding will take into account the following:

- Identification of potential confounders. In the occupational cohort and case-control studies included in the present review, TCE-exposed workers or populations are typically exposed to a number of co-exposures. These may have been quantified or noted by the study authors or may be inferred from expert knowledge of the occupational scenarios described by the authors. Information on occupational co-exposures may be more limited in population- or hospital-based case-control studies (for example, restricted to self-reports on questionnaires or interviews) than cohort studies. Whether or not a given co-exposure should be considered as a potential confounder depends on whether there is *a priori* evidence that the co-exposure is potentially associated with specific cancer(s) of concern.
- Assessment of analytical or statistical methods to control for variables with evidence of confounding (see Section 3.3.1) or other methods or information on the potential confounders.
- The magnitude of the risk estimate for exposure to TCE or the strength of exposure-response relationships for specific cancer endpoints (see Section 3.3.2).

3.3.1 Assessment of analytical methods to evaluate confounding

Studies will be evaluated for their adequacy in measuring potential confounders, such as occupational co-exposures, age, and lifestyle factors, and the appropriateness of the analytical methods and models used to control for confounding.

Questions

- How well were co-exposures, age, or non-occupational risk factors measured in the study? If there are no actual data on confounders, are surrogate data on potential confounders available?
- Does the design or analysis control or account for important confounding through matching, stratification, multivariable analysis, or other approaches?

[8] Morton L, Turner J, Cerhan J, Linet M, Treseler P, Clarke C, Jack A, Cozen W, Maynadie M, Spinelli J, Costantini A, Rudiger T, Scarpa A, Zheng T, Weisenburger D. (2007). Proposed classification of lymphoid neoplasms for epidemiologic research from the Pathology Working Group of the International Lymphoma Epidemiology Consortium (InterLymph). Blood 110: 695-708.

- Are the models used to control for confounding appropriate? What strategy was used to determine whether the variable belongs in the models? Is there evidence for under- or over-controlling for confounding, residual confounding, or negative confounding?

Guidelines

Ideally, all potential confounders should be quantified and considered for inclusion in the statistical analysis for confounding, using appropriate statistical models. Final statistical models should only include "actual" confounders and not variables that have minimal effect on the risk estimate. Guidelines for evaluating methods to assess exposure to confounding are as follows:

Occupational co-exposures: Ideally, studies would provide quantitative exposure data for each potential confounder as part of a job-exposure matrix or expert assessment for each worker, but this is rare. However, some studies provide quantitative or qualitative data on co-exposures for subsets of workers, which can be used to evaluate potential confounding. In addition, knowledge of, e.g., TCE manufacturing processes or patterns of use in different occupations or populations under study may also be helpful in providing relative estimates of the ratio of exposure to TCE and exposure to the potential confounder.

Non-occupational risk factors: Ideally, quantitative information on other non-occupational exposures or lifestyle factors should be assessed, and preferably by in-person interview by interviewers blinded to the status of the respondent in cancer incidence studies, rather than via proxy respondents, work records, or other indirect methods. Residual confounding is more likely when only limited qualitative information (such as yes or no) is available. Few or no data are available on non-occupational risk factors in the available historical cohort studies of TCE, other than, in some studies, data on other cancers (lung cancer) or non-cancer endpoints, e.g., cirrhosis of the liver, or smoking-related (such as asthma, or chronic obstructive pulmonary disease) or alcohol-related diseases, which may provide indirect information on risk factors for specific cancer endpoints of concern.

3.3.2 Impact of potential confounders on study findings

Ideally, all potential confounders should be both quantified and subject to consideration for analysis for confounding, using appropriate statistical models, or confounding should be controlled for using other methods such as in the selection of the study participants. The major occupational co-exposures that are potential or known risk factors in humans for the cancer sites of interest are (1) kidney cancer – arsenic, cadmium (metal or compounds) and printing processes; (2) liver cancer - vinyl chloride, and (3) NHL - benzene, ethylene oxide, 2,3,7,8-TCDD, mixed polychlorinated biphenyls, phenoxy herbicides (possibly, but cancer tumor sites are unclear), styrene (associated with lymphohematopoietic cancers including NHL), tetrachloroethylene, and ionizing radiation (Cogliano *et al.* 2011). For liver cancer, there are numerous animal carcinogens but with unknown effects in humans.

In many cohort studies, there is a paucity of quantitative co-exposure data at the individual level; however, an indirect evaluation of the impact of confounding from co-exposures may be conducted by considering (1) the relative levels of exposure to TCE compared with exposure to the potential confounder, (2) the strength of the association of the potential confounder with the endpoint of interest, and (3) the magnitude of the risk estimate or strength of exposure-response relationship for TCE and specific cancer endpoints. As noted above, indirect information on the relationship between the estimated levels of exposure (albeit based on crude approximations) to the confounder compared with the estimated level of exposure to TCE may be available from exposure monitoring or biomonitoring studies of subsets of workers.

Typically, few or no data are available on non-occupational risk factors in historical cohort studies but are more often available in case-control studies. Internal comparison groups and analyses can help reduce confounding from non-occupational risk factors in cohort studies. Risk factors for kidney cancer include X-radiation and tobacco smoking; tobacco smoking is of more concern because of a potential to be related to exposure status. In the case of liver cancer, depending on the type of tumor, a number of non-occupational risk factors have been identified, including aflatoxins, estrogen-progestogen contraceptives, alcohol consumption, tobacco smoking, betel quid use, cirrhosis of the liver, viral infections (hepatitis B virus [HBV], hepatitis C virus [HCV], human immunodeficiency virus type 1 [HIV]), parasites (liver flukes and *Schistosoma*), long-term use of anabolic steroids, and ionizing radiation (Cogliano *et al.* 2010[9]). Non-occupational risk factors for non-Hodgkin lymphoma include viral infections (Epstein-Barr virus, HBV, HCV, HIV), immunosuppressive disorders, auto immune diseases and exposure to immunosuppressive or chemotherapy drugs (Hardell and Axelson 1998[10]). For most of these factors, unless there is an *a priori* reason to suspect that they are related to exposure to TCE they would not be considered as confounders or effect modifiers. Tobacco smoking and alcohol consumption have the greatest potential to be related to exposure status.

3.4 Other factors

There are also other factors that impact a study's ability to inform the cancer evaluation. These mainly include factors related to the ability of a study to detect an effect (if present) such as the statistical power of the study, the level, duration, or route of exposure to TCE, the exposure range studied, and the length of follow-up in cohort studies. In general, study characteristics that increase the potential for random error (such as high loss to follow-up in cohort studies or low participation rates in case-control studies that are not related to exposure or disease status) will bias the study findings toward the null (see Section 3.2.1 for questions and guidelines).

Questions

- Is there adequate statistical power to detect an effect in the exposed population or subgroups of the exposed population?
- What were the levels of exposure and the duration of exposure of the populations at risk in the cohort and case-control studies?
- Were risk estimates calculated for subgroups of workers with higher levels or longer durations of exposure?
- Was the follow-up period adequate to allow for a cancer induction period of 20 years or greater?
- Were any analyses of exposure lagging adequate for detecting cancers with longer latency?

Guidelines

The overall number of study participants and numbers in each exposure or case group will be evaluated in terms of the statistical power to detect given elevations of risk for specific cancer endpoints while controlling, if necessary, for potential confounding. For example, the incidences of NHL, liver cancer, and kidney cancer are relatively low. In general, five-year survival rates (with the exception of liver cancer) are relatively high and thus detection of these endpoints requires large

[9] Cogliano VJ, Baan R, Straif K, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Wild CP. 2011. Preventable exposures associated with human cancers. J Natl Cancer Inst 103(24): 1827-1839.

[10] Hardell L and Axelson O. (1998). Environmental and occupational aspects on the etiology of non-Hodgkin's lymphoma. Oncol Res; 10: 1-5.

sample sizes for adequate statistical power, particularly for mortality studies.[11] Ideally, studies should have at least 80% power to detect a 2-fold increased relative risk. The RoC evaluation will use the power calculations reported in the U.S. EPA toxicological review (EPA 2011) when available, supplemented by its own power calculations for any remaining studies. Studies of workers in industries or occupations with higher levels of exposure, or workers with longer duration of exposure and sufficient variability in exposure, are generally the most informative for evaluating cancer risk. Studies evaluating exposure groups in which the majority of workers classified as "exposed" have in fact very low exposure, very short duration of employment, or limited evidence of actual exposure may be inadequate to detect an effect due to a dilution effect.

Inadequate follow-up (or follow-back in case-control studies) may bias findings toward the null for cancer endpoints with longer latencies. In the case of, e.g., NHL and other lymphohematopoietic cancers, latencies appear to vary considerably in some studies of occupational exposures, ranging from 2 to 60 years in some cases.[12] Liver cancer has been associated with a minimum latency of 20 years in association with, e.g., vinyl chloride exposure[13] but may be considerably longer for, e.g., infectious disease risk factors. Ideally, follow-up or follow-back periods should exceed 15 to 20 years to permit adequate determination of these and other solid tumors with longer latencies, particularly in mortality studies; however, shorter follow-up periods may be more relevant for, e.g., lymphohematopoietic cancers.

3.5 Analytical methods

The use of appropriate analytical methods will be evaluated. Analysis of exposure-response relationships (using either linear models or exposure categories, or other methods to evaluate the shape of the exposure-response curve) and calculation of trends using quantitative exposure assessments adds more information than analysis by simple binary exposure categories, as does analysis of tumor site by average, cumulative, peak, or duration of exposure, time since first exposure, calendar periods of exposure, and exposure lags. Evaluating the shape of the exposure-response curve is considered to be a positive attribute of a study. Without *a priori* knowledge, it is difficult to know which exposure metric is most appropriate for evaluating causality, so a positive relationship observed with any exposure metric is a concern. In addition, all analyses should examine and, if necessary, adjust for demographic variables and other potential confounders of *a priori* interest, if not done so in the study's design. Evidence of under- or over-controlling for confounding, multi-colinearity, and residual confounding will also be evaluated. In the absence of information on confounders, analyses using internal referents who are similar to the exposed subjects can help reduce potential confounding (see Appendix D for further discussion on the evaluation of methods to assess potential confounding). Ideally, analytical methods should also identify and consider potential modifying variables; however, many studies do not have sufficient statistical power to adequately evaluate effect modification.

[11] Age-adjusted annual incidence or mortality rates (per 100,000 males or females) in the United States from 2006-2010 (U.S. SEER Statistics) for the cancer sites of interest are as follows: (1) NHL – 23.9 (male) and 16.4 (female) for incidence and 8.2 (male) and 5.1 (female) for mortality, 2) myeloma – 7.5 (male) and 4.8 (female) for incidence and 4.3 (male) and 2.7 (female) for mortality, (3) liver – 11.9 (male) and 4.0 (female) for incidence and 8.3 (male) and 3.4 (female) for mortality, and (4) kidney and renal pelvis – 21 (male) and 10.6 (female) for incidence and 5.8 (male) and 2.6 (female) for mortality.

[12] Olssen H, Brandt L. (1988). Risk of non-Hodgkin's lymphoma among men occupationally exposed to organic solvents. Scand J Work Environ Health 14: 246-251

[13] Lelbach WK. (1996). A 25-year follow-up study of heavily exposed vinyl chloride workers in Germany. Am J Ind Med; 29: 446-458

4 Section 4: Integration of the scientific evidence across human cancer studies

This section outlines the approaches for integrating the findings across the body of studies for each cancer endpoint and making a recommendation on the level of evidence (e.g., sufficient, limited, or inadequate) for the carcinogenicity of TCE from studies in humans. Studies with the lowest risk of bias and greatest sensitivity to detect an effect will be identified by using the questions and guidelines described in Section 3, and these studies will be given the most weight in the assessment. The application of the RoC listing criteria to the body of studies on TCE includes evaluating (1) whether there is credible evidence for an association between exposure to TCE and cancer, and (2) whether such an observed association can be explained by chance, bias, or confounding. Several existing guidelines – strength of the association, consistency across studies, evidence of an exposure-response gradient, and temporality of exposure[14] – are used to help guide the evaluation of these questions. It should be noted that these are not criteria, and with the exception of temporality, each and every element is not required to demonstrate causality. Emphasis should be placed on evaluating the extent to which biases, or confounding by co-exposures that may also cause cancer could explain observed increases in cancer risk.

The cancer assessment will evaluate the following:

- *Temporality*. Exposure must occur before disease outcome.
- *The consistency of findings across studies with the most adequate methodologies*, as evaluated according to the guidelines described in Section 3. Consistency needs to be evaluated in the context of variations in outcome definitions, exposure assessment methodologies, exposure levels or duration of exposure of the population, exposure windows, length and completeness of follow-up, or other differences in population characteristics or study methodologies. The evidence from methodologically more limited studies will also be evaluated including an evaluation of whether such limitations can help explain any inconsistent findings.
- *The strength of observed associations between TCE exposure and cancer*. The strength of the association can be important in evaluating whether specific confounders or biases can explain the observed association; however, the fact that an association is weak does not necessarily rule out a causal relationship. There are many examples of weak associations between an exposure to a substance and an endpoint that are nevertheless considered to be causal (e.g., environmental tobacco smoking and lung cancer).
- *Evidence for an exposure-response gradient*. A positive exposure-response relationship (which does not necessarily need to be monotonic) generally provides more convincing evidence of a causal association than a simple excess of disease. However, there may be biological or methodological reasons for not observing a gradient, and the absence of evidence for an exposure-response relationship is not strong evidence *per se* for the absence of a causal association.
- *Evidence for associations with appropriate latency*.
- *Alternative explanations of chance, bias, or confounding*. The process for identifying potential biases was discussed in Section 3.2, and that for evaluating potential confounding was outlined in Section 3.3. As noted in Section 3, the presence of bias in a study does not mean that the study should be disregarded; the potential for the

[14] Hill AB. (1965). The environment and disease: association or causation? Proc R Soc Med; 58 (5): 295-300.

bias should be analyzed to determine its impact (including the direction and magnitude) on the study findings (e.g., risk estimates for TCE and cancer). The finding of consistent, elevated, positive associations across studies in different populations, with different study designs, and in different occupational settings reduces the likelihood that specific biases or potential confounders in individual studies can explain the associations observed across the body of studies.

TCE Protocol Part C:
Methods for Evaluating Mechanistic and Other Relevant Effects

As stated in the introduction, recent comprehensive reviews of TCE are available that evaluate the epidemiologic data, toxicological data, metabolism, genotoxicity, and potential modes of action (IOM 2003, NRC 2006, EPA 2011, IARC (expected to be published in 2014)). The goal of the cancer evaluation component of the draft RoC monograph is to conduct an independent assessment of the scientific literature while utilizing information from the extensive reviews conducted by other agencies and scientific panels to focus the deliberation. Specifically, these reviews will be used to identify the hypothesized mechanisms or modes of action. These comprehensive reviews also provide an adequate database of the experimental evidence for the hypothesized mechanisms published up to the date of their evaluation, and as a source of genotoxicity and ADME data to be evaluated in the draft RoC monograph. Findings from the reviews will be supplemented with primary literature published since the literature searches for these reviews were completed in order to thoroughly address key questions. However, for key issues that will be discussed in greater detail in the draft RoC monograph, e.g., the potential role of immune effects of TCE in development of non-Hodgkin lymphoma and liver cancer, no time limit will be placed on searches of the primary literature.

The methods for each of the subsections included in the Mechanistic Data and Other Relevant Effects section of the monograph are described below:

1. Disposition and Toxicokinetics
2. Relevant Biological Effects (genetic toxicology and immune effects)
3. Mechanistic data

1 Methods for evaluating disposition and toxicokinetics

The purpose of this section is to provide background information that may be important for understanding potential mechanisms of carcinogenicity. This section provides an overview of absorption, distribution, and excretion and a more detailed discussion of metabolism in experimental animals and humans. The role of disposition and toxicokinetics in the carcinogenicity of specific tumors will be discussed for each tumor site in the mechanistic section. The key questions for disposition and toxicokinetics are as follows:

- How is TCE absorbed, distributed, metabolized, and excreted (ADME)?
- Are there qualitative and/or quantitative species or sex differences in ADME?
- What metabolites of TCE are formed that might contribute to its toxicity and carcinogenicity?
- Have toxicokinetic models been developed that are useful for addressing biological plausibility, interspecies extrapolation, or other mechanistic questions for TCE?

TCE has been evaluated in recent high-quality reviews by EPA (2011), NRC (NAS 2006), ATSDR (1997, 2013) and IARC (publication pending). Therefore, the proposed approach is to rely on these reviews to identify the primary literature and summarize the pertinent ADME and toxicokinetic data. Literature searches will be conducted, as described below, to update the findings reported in the reviews. The *Toxicological Review of Trichloroethylene* for EPA's IRIS program provides a very extensive review of the available information on ADME and toxicokinetics of TCE prior to its publication date in 2011. The NRC (NAS 2006) assessment of the human health risks of trichloroethylene, which focuses on hazard characterization and on potential modes of action for the toxicity of TCE, includes a discussion of ADME and toxicokinetics of TCE. A later NRC assessment (NAS 2009) on the health effects of contaminated water supplies at Camp Lejeune also contains information on these topics. The ATSDR toxicological profile for TCE was published in 1997, and CDC released an addendum in 2013. The addendum reviewed studies published since the 1997 toxicological profile, but it also relied heavily on the EPA and NRC reviews. An IARC monograph on TCE (volume 106) is in production by WHO, and that review will also be used if it is released during the preparation of the draft monograph on TCE. The proposed approach for incorporating information from these comprehensive reviews will be to first summarize information from the IRIS review (pending availability of the IARC review) because it provides the most extensive review of the available literature up to its date of publication. The NRC and ATSDR reviews will be supplemental sources for data not included in the IRIS document.

1.1 Literature searches and systematic review

The comprehensive reviews described above will be used as the basis for the discussion of ADME and toxicokinetics; however, the IRIS review was published in 2011, and a closing date of December 2010 was reported for inclusion of primary literature in that review. To ensure an overlap with the IRIS review, the literature search strategy described in the table below will be conducted for primary literature from 2009 to the present using the PubMed, Scopus, and Web of Science databases.

Table C-1. Literature Search for Disposition and Toxicokinetics. See Figure 1-1 (Section B above) for a schematic of the literature identification and selection process. (Note that the

boxes below "Selected citations" in Figure 1-1 do not apply to the literature search for ADME.)

Topic[a]	Combined with	Date/limits
Disposition and Toxicokinetics (ADME)	*TCE synonyms* [(1,1,2 or 1,2,2)-trichloroethylene, 79-01-6, TCE, trichloroethene, acetylene trichloride, ethylene trichloride] *TCE metabolites* [trichloroethanol, trichloroacetic acid, dichloroacetic acid, chloral hydrate, *S*-(1,2-dichlorovinyl)glutathione (DCVG), *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl-L-cysteine (NAcDCVC), *S*-(1,2-dichlorovinyl)thiol (DCVSH/DCVT)]	Authoritative reviews Primary literature since 2009

Literature citations identified from these searches will be uploaded to an online systematic review system, and the following criteria will be applied to the selection of citations at Level 1 (title and abstract only) for further review at Level 2 (full text):

- Studies published from 2009 to present that potentially provide information related to answering the key questions for the review of ADME of TCE and that have not already been reviewed in the authoritative reviews, i.e., IRIS, NRC, and ATSDR. (The IARC review will also be included if it becomes available during the drafting of the monograph.)
- Studies reporting results for TCE or its metabolites (but not for members of its chemical class).

1.2 Approach for writing the ADME/Toxicokinetics section

This section will be written in a review style rather than as summaries of individual studies (study-by-study approach) and will include the following subsections: (a) Absorption, distribution, and excretion; (b) Metabolism; and (c) Toxicokinetic data. Each subsection will be further subdivided to present data from human studies and experimental animal studies separately. Metabolism will be the main focus of this section because of the potential importance of TCE metabolites in causing toxicity or carcinogenicity following exposure to TCE. The major pathways described by NRC and EPA are the oxidative pathway (cytochrome mediated) and glutathione conjugation followed by further biotransformation and processing. The key information on each topic will be briefly summarized based on the reviews by EPA, NRC, and ATSDR. Tables or figures based on these reviews may be used to illustrate the key information. Primary studies not reviewed by EPA, NRC, or ATSDR will be cited in the description of each topic, and results from these studies will be reported if they add information not already covered by the comprehensive reviews. If an older study is key to the assessment, it will be included directly in the review rather than relying on secondary sources. Examples of key studies would be those in humans that report putative carcinogens or pathways involved in carcinogenicity related to NHL, kidney, or liver.

The metabolism of TCE in animal models has been well described, and no major controversies have been reported by EPA, NRC, or ATSDR for the metabolic pathways in either animals or humans. If any discrepancies are noted among the various studies regarding ADME, subject matter experts will be consulted to evaluate study protocols and quality of the primary literature in an attempt to resolve any conflicts. This section will include figures and tables to illustrate the metabolic pathways and identify reactive

metabolites. Key metabolizing enzymes (e.g., cytochromes P450) also will be identified when possible. The toxicokinetics subsection will identify reported values for several key toxicokinetic parameters (e.g., half life for absorption/excretion, volume of distribution, clearance, etc.). Physiologically based pharmacokinetic (PBPK) models are available but they are primarily a risk assessment tool and are outside the scope of the TCE Monograph. Thus, these models will not be described in detail.

2 Methods for evaluating relevant biological effects: genetic toxicology and immune system effects

The purpose of the relevant biological effects section is to provide background information that may be important for understanding potential mechanisms of carcinogenicity. This section will include a review of the genetic and immune system effects of TCE. The potential role of these effects in carcinogenicity of specific tumors will be discussed in the mechanisms section.

2.1 Genetic toxicology

The following key questions will be addressed in the genetic toxicology section:

- Does TCE cause genetic damage, i.e., is it mutagenic and/or genotoxic?
    - What type(s) of genetic damage does it cause?
    - What level(s) of exposure cause this damage?
- Does it cause damage in cells, exposed animals, or exposed people?

2.1.1 Literature searches and systematic review

The comprehensive reviews described above will be used as the basis for the discussion of genotoxicity; however, the IRIS review was published in 2011, and a closing date of December 2010 was reported for inclusion of primary literature in that review. To ensure an overlap with the IRIS review, the literature search strategy described in the table below will be conducted for primary literature from 2009 to the present using the PubMed, Scopus, and Web of Science databases.

Table C-2. Literature Search for Genotoxicity. See Figure 1-1 (Section B above) for a schematic of the literature identification and selection process. (Note that the boxes below "Selected citations" in Figure 1-1 do not apply to the literature search for genotoxicity.)

Topic[a]	Combined with	Date/limits
Genotoxicity	*TCE synonyms* [(1,1,2 or 1,2,2)-trichloroethylene, 79-01-6, TCE, trichloroethene, acetylene trichloride, ethylene trichloride] *TCE metabolites* [trichloroethanol, trichloroacetic acid, dichloroacetic acid, chloral hydrate, *S*-(1,2-dichlorovinyl)glutathione (DCVG), *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl-L-cysteine (NAcDCVC), *S*-(1,2-dichlorovinyl)thiol (DCVSH)]	Authoritative reviews Primary literature since 2009 (with the exception of effects related to epigenetics or gene expression- no time limit)

The following criteria will be applied to the selection of citations at Level 1 (title and abstract only) for further review at Level 2 (full text).

- Studies published from 2009 to present that potentially provide information related to answering the key questions for the review of genetic toxicology of TCE and have not already been reviewed in the authoritative reviews, i.e., IRIS, NRC, and ATSDR.
- Studies published at any time (no date limit) that provide information related to effects on epigenetic mechanisms and gene expression changes.
- Studies reporting results for TCE or its metabolites (but not for members of its chemical class).

2.1.2 Approach for writing the genetic toxicology section

The approach for drafting this section is to first incorporate information from the extensive review of studies of the genetic toxicology of TCE and its established metabolites that have been published recently in the IRIS review (EPA 2011). The NRC (NAS 2006) and ATSDR (CDC 1997, 2013) reviews include some discussion of genetic toxicology of TCE, but it is much more limited than the review in the IRIS document; thus, these publications will be used as supplemental sources. The IRIS document (EPA 2011) presents a comprehensive review of studies of TCE genotoxicity, with publications cited through 2010. However, the presentation of studies in the IRIS document follows a slightly different organization (grouped by assay type) than that typically used in RoC monographs (grouping by test system); therefore, information used from the IRIS document or added from the primary literature will be organized into the following general headings: studies in bacterial systems, non-mammalian eukaryotes, *in vitro* mammalian systems, i*n vivo* mammalian systems, humans, and studies involving metabolites. If there is consistency across endpoints, it may be better to organized by endpoint rather than by test system. Tables included in the IRIS review are organized by endpoint and list results and doses used in individual studies. These tables will be modified, as necessary, for consistency with the text headings mentioned above, and used as the basis for appendix tables to be included in the monograph. Study design details (e.g., dose ranges used, timing of dosing and sampling, etc.) will be added to the tables in a comments column. In addition, information regarding TCE purity and the presence or absence of added stabilizers, mutagenic or non-mutagenic, will also be indicated when available. Additional material found from review of the primary literature since 2009 will be added, as appropriate, to existing tables or will be presented in new tables if the material represents endpoints not addressed in the IRIS review. Studies that supplement existing genotoxicity test results for TCE and for its metabolites will be reviewed.

Particular attention will be given to studies that evaluate the evidence for involvement of genotoxic mechanisms in TCE-induced kidney and liver cancer and NHL (including CLL and multiple myeloma), such as the hypothesized role of von Hippel-Lindau mutations in the development of TCE-induced renal-cell carcinoma. In addition, studies evaluating additional mechanisms of action (e.g., epigenetic and gene pathway analyses) will also be considered for inclusion in this update. The focus of genetic toxicology research in recent years has expanded to include effects on epigenetic processes and gene expression. Because these topics have not been specifically addressed in previous reviews of TCE and its metabolites, studies involving TCE-induced effects on epigenetics and gene expression published prior to 2009 will be included in the literature review.

The primary literature reviewed for this monograph will be evaluated for adequacy of the study design and any deficiencies noted in the tables. The IRIS review includes evaluation of study design within the text in some cases; this information will be included in the monograph tables as appropriate. Where overlap in the presentation of studies occurs among the authoritative reviews mentioned above (IRIS, NRC, and ATSDR), comparisons will be made for inconsistencies in the information presented. In cases where the information presented in the authoritative reviews is unclear or inconsistencies are noted, the primary literature will be consulted to avoid data misrepresentation in the monograph. Additionally, the data may be evaluated in the mechanisms of action section for its contribution to understanding mode(s) of action or its implication for increased potential for disease outcome, especially cancer development.

2.2 Immune system effects

The following key questions will be addressed in the immune system effects section:

- Does TCE affect the immune system?
  - Does it suppress the immune system?
  - Does it increase the activity of the immune system to cause, e.g., autoimmune diseases?
- Are these effects seen in cell systems, exposed animals, or exposed people?
- What are the potential modes of action for altered immunity from any cause and from NHL or other cancers?

2.2.1 Literature searches and systematic review

The comprehensive reviews (i.e., IRIS, NRC, ATSDR, and IARC if available) will be used in the discussion of immune system effects; however, the review of this topic will consider all literature on the topic without regard to date of publication. The literature search strategy described in the table below will be conducted for authoritative reviews and primary literature using the PubMed, Scopus, and Web of Science databases with no time limitation.

Table C-3. Literature Search for Immune Effects. See Figure 1-1 (Section B above) for a schematic of the literature identification and selection process. (Note that the boxes below "Selected citations" in Figure 1-1 do not apply to the literature search for immune effects.)

Topic[a]	Combined with	Date/limits
Immune effects	*TCE synonyms* [(1,1,2 or 1,2,2)-trichloroethylene, 79-01-6, TCE, trichloroethene, acetylene trichloride, ethylene trichloride] *TCE metabolites* [trichloroethanol, trichloroacetic acid, dichloroacetic acid, chloral hydrate, *S*-(1,2-dichlorovinyl)glutathione (DCVG), *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl-L-cysteine (NAcDCVC), *S*-(1,2-dichlorovinyl)thiol (DCVSH)] *Chemical class:* [chlorinated alkenes, halogenated alkenes]	Authoritative reviews Primary literature for immune-related mechanisms (not limited by date of publication)

The following criteria will be applied to the selection of citations at Level 1 (title and abstract only) for further review at Level 2 (full text).

- Studies that potentially provide information related to answering the key questions for the review of immune system effects of TCE and that have not already been reviewed in the authoritative reviews, i.e., IRIS, IARC, and NRC.
- Studies reporting results for TCE, its metabolites, or members of its chemical class.

2.2.2 Approach for writing the immune system effects section

The NTP will receive input on the biological plausibility for the role of immune effects in TCE-related cancers in experimental animals and humans. This input will be obtained by convening an information group of scientists with expertise in immunology, cancer, epidemiology, and toxicology to provide comments on the body of studies of TCE exposure and immune effects and to provide their input on an individual basis, and not from the group as a whole, on the biological plausibility. The comments of the information group will be used to inform the assessment of the mechanistic data in the draft RoC monograph on TCE.

This section will be written in a review style and will synthesize the key findings across studies rather than providing detailed summaries of each study. As noted above, the section will rely on a comprehensive literature search for primary literature in addition to information provided in the major reviews published by EPA, NRC, ATSDR, and IARC (if available). Two primary topics will be covered: (1) the immune system effects of TCE in humans and experimental animals, and (2) a review of the relationship between immunomodulation and cancer. Mechanistic implications of immune system effects of TCE and NHL (including CLL and multiple myeloma), liver cancer, or kidney cancer will be covered in subsequent sections and are discussed below.

3 Methods for evaluating tissue-specific mechanisms of action

Potential mechanisms of action will be covered in Sections 3 (kidney cancer), 4 (NHL), and 5 (liver cancer) of the draft monograph as part of the discussion of these cancers. The primary purpose of the mechanistic data discussion is to address the following key questions:

- What are the key events in the mode(s) of action by which TCE may cause cancer in the target tissues, i.e., the kidney, liver, or lymphohematopoietic system (i.e., NHL related cancers)?
- What is the quality of the evidence for the key events in the proposed modes of action, i.e., do the data provide a cohesive, biologically plausible explanation for the effects?
- Is there evidence that different mechanisms operate in the different tissues?
- Is there evidence that multiple mechanisms may contribute to tumor development in a specific tissue?
- Is there evidence that mechanisms identified from *in vitro* studies or from studies in experimental animals also operate in humans?

Proposed methods for evaluating mechanistic data and other relevant effects include conducting literature searches and selecting relevant studies, extracting relevant data,

evaluating the strength of evidence and overall confidence in the body of data, and integrating across all relevant data to form conclusions. Figures illustrating key events for potential mechanisms of action will by included (usually in appendices) when appropriate data are available, while the major mode-of-action conclusions and experimental support will be summarized in tables within the main body of text. The modes of action for which there is adequate experimental data or limited or inadequate experimental data as reported by EPA and NRC are discussed for each tissue site below.

3.1 Literature searches and systematic review

Literature searches will be conducted to identify studies published after the reviews that address any of the hypothesized modes of action or additional modes of action. Unless additional studies are identified, hypothesized modes of action with inadequate to limited experimental data will not be reviewed in detail, and the discussion in the draft monograph will rely on findings reported in the comprehensive reviews. Figure 1-1 illustrates the literature identification and selection process. (Note that the boxes below "Selected citations" in Figure 1-1 do not apply to the literature search for mechanisms of action.)

Table C-4. Literature Search for Mechanisms of Action and Toxicity. See Figure 1-1 (Section B above) for a schematic of the literature identification and selection process. (Note that the boxes below "Selected citations" in Figure 1-1 do not apply to the literature search for ADME.)

Topic[a]	Combined with	Date/limits
Mechanisms of Action and Toxicity (liver and kidney[b])	*TCE synonyms* [(1,1,2 or 1,2,2)-trichloroethylene, 79-01-6, TCE, trichloroethene, acetylene trichloride, ethylene trichloride] *TCE metabolites* [trichloroethanol, trichloroacetic acid, dichloroacetic acid, chloral hydrate, *S*-(1,2-dichlorovinyl)glutathione (DCVG), *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl-L-cysteine (NAcDCVC), *S*-(1,2-dichlorovinyl)thiol (DCVSH)] *Chemical class:* [chlorinated alkenes, halogenated alkenes]	Authoritative reviews Primary literature for immune-related mechanism (no time limit) Primary literature since 2009 for mechanisms in general and specifically for kidney-related mechanisms and liver-related mechanisms (see topics in Section 3.2, below) that have inadequate or limited experimental data

[a]Search terms for each of these topics have been developed in consultation with an information specialist.

[b]The literature search for immune system effects was described above.

3.2 Tissue-specific cancers

The approach for writing the mechanistic data sections for kidney cancer, NHL, and liver cancer will be consistent with the review style described for the previous sections and will be based on the comprehensive reviews by EPA (2011) and others (including the IARC review if available), which describes the mechanistic data for TCE-associated neoplasms. Recent primary literature will be used to supplement the key findings from these reviews as available. (As noted above, the literature search for immune system effects will not be limited by the date of publication.)

The sections on kidney cancer, NHL, and liver cancer will assess the quality of the mechanistic data, the cohesiveness of the proposed mechanism(s) of action, and the biological plausibility for kidney cancer in experimental animals and humans.

3.2.1 Kidney cancer

Potential modes of action for kidney cancer with an adequate database of experimental data as reviewed by EPA include GSH-derived metabolites produced *in situ* or delivered to the kidney, genotoxicity data for GSH-derived metabolites in most *in vitro* assays, and kidney-specific genotoxic effects in rats and rabbits exposed to TCE. Mutations in the von Hippel-Lindau tumor suppressor gene have been observed in both human and animal tumors after exposure to TCE. Data also evaluate the hypothesis that cytotoxicity and regenerative proliferation contribute to TCE-induced kidney cancer. The primary literature relevant to these modes of action will be reviewed and synthesized in the monograph along with conclusions on biological plausibility for humans.

Hypothesized modes of action with limited or inadequate experimental data include peroxisome proliferation (PPARα), α_{2u}-globulin-related nephropathy, and formic acid-related nephrotoxicity. Unless additional studies relevant to these modes of action are identified in the literature search, the data and discussion will be brief and will rely on the comprehensive reviews.

3.2.2 NHL (including CLL and multiple myeloma)

Known risk factors for NHL include direct DNA adduct formation in bone marrow and blood cells, immune suppression, immune system disorders, and viruses. Several immunosuppressive chemicals and drugs have been linked to an increased risk of NHL. Potential modes of action for NHL include immunomodulation and genotoxicity. The literature on the potential relationship of exposure to TCE and immune effects and on the possible link between immune effects and cancer is extensive and an information group is planned to evaluate TCE-induced immune effects and their role in its potential carcinogenicity, i.e., TCE-induced NHL or other neoplasms.

3.2.3 Liver cancer

Multiple modes of action have been proposed for TCE-induced liver cancer, including mutagenicity from oxidative metabolites, peroxisome proliferator activated receptor alpha (PPARα) activation, altered immunity, increased liver weight or liver/body weight ratios, mitogenic stimulation by oxidative metabolites that confer a growth advantage to initiated cells, accumulation of glycogen in hepatocytes, inactivation of GST-zeta with subsequent accumulation of toxic metabolites, oxidative stress, hypomethylation and gene expression changes, and cytotoxicity and reparative hyperplasia.

Although a possible role for several of the key events in the hypothesized modes-of-action for TCE-induced liver cancer cannot be ruled out, the available experimental data may be inadequate to support definitive conclusions. As noted above for NHL, the potential role of immune effects of TCE in development of liver cancer will be discussed with the information group to be convened by NTP.

TCE Protocol Part D: Methods for Updating the Exposure Information in the Substance Profile

Introduction and Objectives

The original listing established that a significant number of persons in the United States are (or were) exposed to TCE. The exposure data will be updated in the draft substance profile component of the draft RoC monograph on TCE but will not be reassessed in the cancer evaluation component.

3.1 Literature searches and systematic review

The comprehensive reviews, including EPA's IRIS (EPA 2011), NAS (2006), and ATSDR (1997, 2013) will be used as the basis for updating the exposure section of the substance profile for TCE; however, the IRIS review, which is the most extensive of these reviews was published in 2011, and a closing date of December 2010 was reported for inclusion of primary literature in that review. To ensure an overlap with the IRIS review, the literature search strategy described in the table below will be conducted for reviews from 2009 to the present using the PubMed, Scopus, and Web of Science databases. Primary literature will be reviewed for studies that make key contributions. Figure 1-1 illustrates the literature identification and selection process. (Note that the boxes below "Selected citations" in Figure 1-1 do not apply to the literature search for exposure.)

Topic[a]	Combined with	Date/limits
Human exposure	*TCE synonyms*: [(1,1,2 or 1,2,2)-trichloroethylene, 79-01-6, TCE, trichloroethene, acetylene trichloride, ethylene trichloride]	Authoritative reviews Other reviews since 2009

Literature citations identified from these searches will be uploaded to an online systematic review system, and the following criteria will be applied to the selection of citations at Level 1 (title and abstract only) for further review at Level 2 (full text):

- Review articles published from 2009 to present that potentially provide information related to answering the key questions for the review of exposure to TCE and that have not already been reviewed in the authoritative reviews, i.e., IRIS, NRC, and ATSDR. (The IARC review will also be included if it becomes available during the drafting of the monograph.)
- Studies reporting results for TCE (but not for its metabolites or members of its chemical class).

Process for updating exposure data in the TCE substance profile

The three sections of a substance profile relevant to exposure and the proposed updates or additions to each section are listed below:

- **Use**
  - No additional information to be added
- **Production**

- The information for production (numbers of manufacturers and suppliers and quantities produced, imported, or exported) will be obtained from online searches, including use of the EPA's Chemical Data Reporting Rule website, the U.S. International Trade Commission Interactive Tariff and Trade DataWeb, and EPA's TRI Explorer.

- **Exposure**
  - Current exposure data for the general public will be obtained from NHANES tables updated March 2013 for blood TCE, from the Household Products Database for consumer products containing TCE, and from the FDA Total Diet Study for TCE in food.
  - Occupational exposure data will be updated using the OSHA Chemical Exposure Health Dataset and EPA's 2011 IRIS document.
  - Information on emerging exposure pathway concerns, e.g., indoor air contamination via vapor intrusion and drinking water contamination at military bases, will be obtained from EPA's IRIS document.