



Key Aspects of U.S. EPA's External Review Draft Toxicological Review of Trichloroethylene (TCE)

Presentation for the
Science Advisory Board

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Importance of TCE for environmental risk assessment

- Widely used as degreaser, chemical intermediate and extractant, component of some consumer products.
- Common environmental contaminant
 - Designated Hazardous Air Pollutant
 - Common groundwater and drinking water contaminant
 - Found at >1500 hazardous waste sites
 - Released to indoor air via vapor intrusion
- Regulatory standards
 - MCL in drinking water is 5 ppb
 - No federal air concentration standard (some state standards exist)

Timeline of activities related to EPA health assessment of TCE

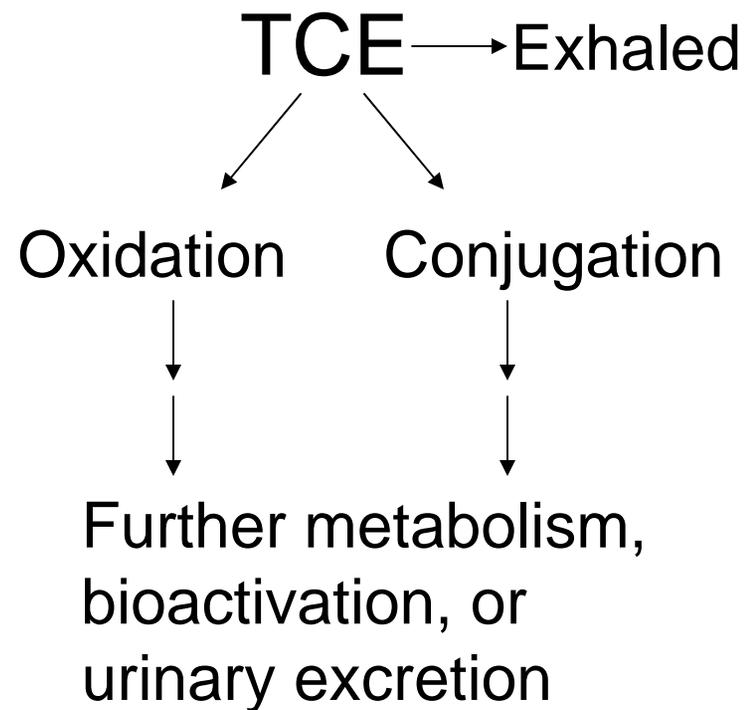
- 1985 – EPA TCE Health Assessment Document
- 1987 – draft addendum
- 1989 – withdrawn from IRIS
- 1990s – outreach meetings, development of “State-of-the-science” papers
- 2000 – “State-of-the-science” papers published in EHP
- 2001 – External Review Draft released for public comment and peer review
- 2002 – Peer review by SAB
- 2004
 - EPA Symposium on New TCE Science
 - National Research Council (NRC) consultation on “Key Scientific Issues” initiated
- 2004 (continued)
 - Collaboration with DoD on pharmacokinetic modeling
- 2005 – EPA issue papers submitted to NRC (published in 2006 in EHP)
- 2006 – NRC report received
- 2009
 - Agency review
 - Inter-Agency consultation
 - External Review Draft released for public comment and peer review
- 2010
 - Public listening session
 - Public comment period closes
 - Peer review by SAB

Key features of the updated Draft TCE Assessment

- **Comprehensive review of studies of TCE and TCE metabolites**
- **Toxicity review organized by tissue/system**
- **Multiple lines of evidence supporting major conclusions of hazard characterization and dose-response assessment**
 - Human epidemiologic data
 - Animal toxicity data
 - Mechanistic data
 - State-of-the-art quantitative analyses
 - *PBPK modeling*
 - *Meta-analysis of cancer epidemiology*
 - *Benchmark dose modeling*
 - *Uncertainty and variability analyses*

TCE Toxicokinetics

- Readily absorbed via all exposure routes
- Distributes to blood and tissues via systemic circulation
- Extensively metabolized
 - Two competing pathways
 - Metabolism associated with toxicity
- Excretion primarily via exhalation of parent compound and urinary elimination of metabolites



PBPK Modeling

- **Current model* is an update/refinement of Hack et al. (2006) “harmonized” model developed through a collaboration between U.S. EPA and the U.S. Air Force.**
 - Revised PBPK model structure
 - Expanded database of toxicokinetic studies encompassing virtually all published datasets in mice, rats, and humans (~200 dose groups, >800 time-courses)
 - Updated Bayesian analysis of uncertainty and variability
 - Characterized **inter-study** variability for rodents (rodents of same sex & strain within a study assumed identical).
 - Characterized **inter-individual** variability for humans.
- **Key PBPK model predictions**
 - TCE is substantially metabolized, primarily by oxidation
 - GSH conjugation and subsequent bioactivation in the kidney in humans is less than oxidation, but greater (as a fraction of dose) than in rodents
 - Mice had the greatest rate of respiratory tract oxidative metabolism compared to rats and humans
- **Predictions of internal dose used for**
 - Elucidating role of metabolites in toxicity
 - Characterizing uncertainty and human variability
 - Quantitative cancer and non-cancer dose-response analyses

* Published in Toxicology and Applied Pharmacology (Chiu et al., 2009; Evans et al., 2009)

Key Issue: Flux of TCE GSH Conjugation

- **Several orders of magnitude less GSH conjugation than oxidation has been postulated**
 - Based on ratios of 1:1000 of GSH:oxidation metabolites in urine
 - Urinary metabolites are indirect measures of flux, because of potential for bioactivation to reactive species
- **Re-examination of in vitro and in vivo data suggests GSH conjugation, while less than oxidation, is greater than that inferred from urinary measures, at least in humans:**
 - Liver cells/fractions (in vitro)
 - *Human: metabolic capacity (Vmax) for GSH conjugation is similar or greater than that for oxidation*
 - *Rodent: GSH conjugation capacity ~20-fold smaller than oxidation (due to higher oxidative capacity)*
 - Human, rodent kidney cells/fractions metabolize TCE to DCVG (in vitro).
 - Available in vivo mass balance data in humans leave 30~40% unaccounted for between TCE in exhaled breath and oxidative metabolites in urine (<10% unaccounted for in rats and mice).
 - Human: One study (Lash et al., 1999b) reported significant DCVG in human blood following TCE inhalation exposure, placing lower bound ~5% on the fraction of intake conjugated (only such study in humans).
 - Rodent: Recently published study in mice (Kim et al., 2009) reported several orders of magnitude less DCVG in blood.
- **One public comment submission questions the reliability of much of the GSH conjugation data supporting the PBPK model predictions.**

Draft Hazard Identification: Non-cancer

- Multiple target tissues/systems
 - Neurotoxicity
 - Nephrotoxicity
 - Hepatotoxicity
 - Immunotoxicity*
 - Respiratory tract toxicity
 - Reproductive toxicity
 - Developmental toxicity
- Role of metabolism
 - Most known about liver and kidney toxicity
 - Inconclusive data for most other endpoints
- MOA unknown for non-cancer endpoints

* Recent review published in Environmental Health Perspectives (Cooper et al., 2009)

Key Issue: Fetal Cardiac Defects

- **The epidemiological studies, while individually limited, as a whole show relatively consistent elevated risks**
- **Significant effects in rats at low drinking water exposures in Dawson and Johnson studies**
 - Prenatal exposure during period of cardiac development
 - Used sensitive fresh dissection technique
 - Also reported with oxidative metabolites TCA and DCA
 - Some important limitations
- **Other studies in rats, using different exposure routes (gavage, inhalation), different exposure periods (GD9+), different dissection methods, did not report cardiac defects**
- **Biological plausibility supported by other data**
 - Avian studies showing cardiac malformations from TCE exposure confirmed multiple times
 - Recently reported alterations in endothelial cushion development observed in avian in ovo and in vitro studies provide a plausible mechanistic basis for defects in septal and valvular morphogenesis observed in rodents
- **Several public comment submissions questioned the reliability of the data supporting the role of TCE in causing fetal cardiac defects.**

Key Issue: Draft Carcinogenicity Characterization as *Carcinogenic to humans*

- **Primary evidence: *Convincing epidemiologic data on TCE and kidney cancer (per Cancer Guidelines [US EPA, 2005])***
 - Consistent across many independent studies identified as meeting standards of epidemiologic design and analysis
 - Supported by meta-analysis results
 - Unlikely due to chance, bias, or confounding
 - *Findings corroborated in recent (since 2000), better-designed studies*
 - *In studies adjusting for known risk factors, statistically significant risks remain*
 - Sufficient follow-up in cohort studies carrying greatest weight, indicating consistency with a temporal relationship
 - Exposure-response trends in several higher quality studies of adequate size and stronger exposure characterizations
 - Biological plausibility from rodent bioassays, toxicokinetics, mechanistic data
- **Toxicokinetic data support carcinogenicity by all routes of exposure.**
- **Public comment submissions either support draft conclusions or disagreed with conclusions, stating evidence supports classification as “likely” or, even, “suggestive.”**

Key Issue: Meta-analysis of cancer epidemiology

- **NRC (2006) report recommended a new meta-analysis of cancer epidemiology as part of EPA's evaluation of TCE carcinogenicity**
- **EPA conducted meta-analysis for kidney cancer, lymphomas, and liver cancer that included:**
 - Summary estimates of pooled relative risk for overall TCE exposure
 - Analyses of heterogeneity
 - *When present, appeared to be accounted for by study design*
 - Analyses of influence of individual studies to summary estimates
 - Analyses of the sensitivity of summary estimates to alternate selections of study relative risk estimates
 - Analyses of potential publication bias
 - Summary estimates of pooled relative risk for the highest exposure groups
 - *Examined as means to reduce misclassification bias*
 - *Exposure-response analyses not feasible because most studies*
 - lacked quantitative estimates of TCE exposure or,
 - if presented, were considered rank ordered or semi-quantitative.
- **For kidney cancer, results indicate a **small, statistically significant increase in risk, robust under different assumptions, without apparent heterogeneity, with analyses of higher exposure groups yielding higher pooled RR estimates****

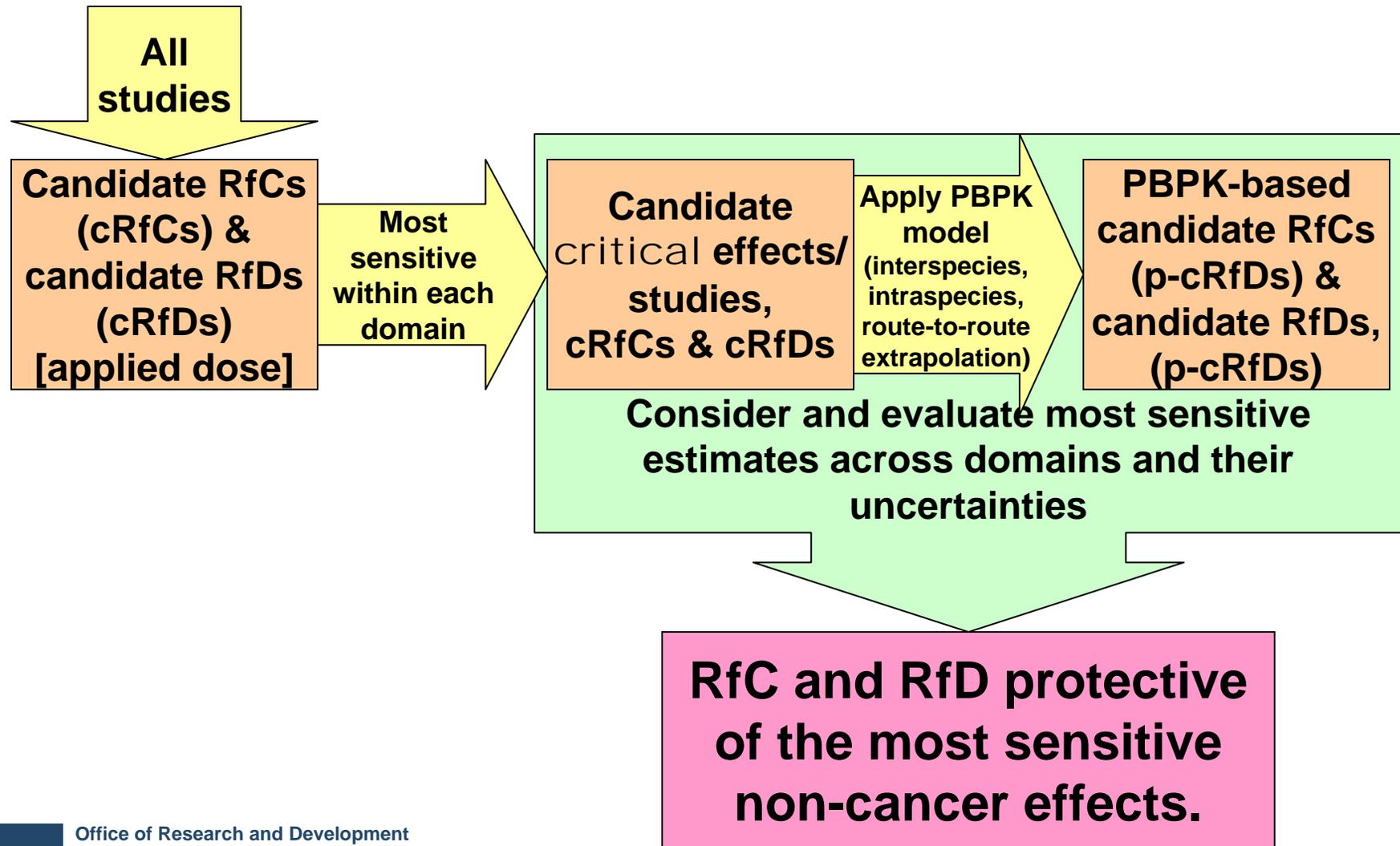
Supporting evidence for Draft TCE carcinogenicity conclusions

- **Epidemiologic data**
 - Strong evidence for lymphomas, but not as convincing as for kidney cancer
 - More limited evidence data for liver cancer
 - Supported by meta-analysis results
- **Rodent bioassays**
 - Positive results from multiple studies/species/sexes/strains/sites
 - Sites include kidney, liver, and lymphatic system, among others
- **Qualitative similarities in toxicokinetics between rodents and humans (quantitative differences addressed in PBPK modeling)**
- **Mode of action**
 - Mutagenic MOA operant for rat kidney tumors
 - Other MOAs for rodent tumors not determined

Key Issue: Mutagenic MOA for Kidney Tumors

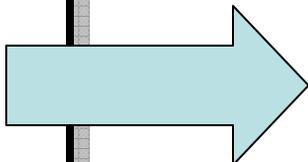
- **Genotoxicity: Predominance of positive genotoxicity for GSH conjugation metabolites**
 - Micronucleus formation (rat, in vivo; rats and humans, in vitro)
 - DNA strand breaks (rats and rabbits, in vivo; rabbits and humans, in vitro)
 - Mutagenicity in Ames test in three strains (in vitro)
 - Dose-dependent increase of unscheduled DNA synthesis (pig and hamster, in vitro)
- **Toxicokinetics: Delivery to and in situ formation in the kidney**
- **VHL mutations**
 - Suggestive epidemiologic data on association between TCE exposure and VHL inactivation in the kidney
 - Eker rat animal model (in vivo)
 - *Heterozygous for the gene Tsc-2, associated with pathways similar to those of VHL*
 - *High background rate of kidney tumors in this model*
 - *No increases in kidney lesions/tumors with TCE exposure in short-duration study*
 - *Cells from Eker rat model demonstrate increased transformation with exposure to DCVC*
 - **Conclusion: inadequate to either confirm or refute role in carcinogenesis**

Draft Dose-Response Assessment: Non-Cancer Effects



Critical effects for the Draft RfC

- Most sensitive candidate critical effects by domain
- Multiple candidate RfCs in range 0.0003-0.003 ppm
- **Robust support from multiple studies/effects for RfC of 0.001 ppm**



EFFECT DOMAIN	p-cRfC ppm (UF _{comp})
Most sensitive candidate critical effects (study)	
NEUROLOGIC	
Demyelination in hippocampus (rat/Isaacson et al. 1990)	0.0071 (1000)
KIDNEY	
Toxic nephropathy (rat/NTP 1988)	0.00056 (10)
Toxic nephrosis (mouse/NCI 1976)	0.0017 (300)
↑ kidney weight (rat/Woolhiser et al. 2006)	0.0013 (10)
LIVER	
↑ liver weight (mouse/Kjellstrand et al. 1983b)	1.0 (10)
IMMUNOLOGIC	
↓ thymus weight (mouse/Keil et al. 2009)	0.00033 (100)
↑ anti-dsDNA & anti-ssDNA Abs (mouse/Keil et al. 2009)	0.0033 (10)
REPRODUCTIVE	
↓ ability of sperm to fertilize (rat/DuTeaux et al. 2004)	0.0093 (1000)
DEVELOPMENTAL	
Heart malformations (rat/Johnson et al. 2003)	0.00037 (10)

Critical effects for the Draft RfD

- Most sensitive candidate critical effects by domain
- Multiple candidate RfDs in range 0.0003-0.0005 mg/kg/d
- Robust support from multiple studies/effects for **RfD of 0.0004 mg/kg/d**



EFFECT DOMAIN	p-cRfD mg/kg/d (UF _{comp})
Most sensitive candidate critical effects (study)	
NEUROLOGIC	
Demyelination in hippocampus (rat/Isaacson et al. 1990)	0.0092 (1000)
KIDNEY	
Toxic nephropathy (rat/NTP 1988)	0.00034 (10)
LIVER	
↑ liver weight (mouse/Kjellstrand et al. 1983b)	0.90 (10)
IMMUNOLOGIC	
↓ thymus weight (mouse/Keil et al. 2009)	0.00048 (100)
REPRODUCTIVE	
↓ ability of sperm to fertilize (rat/DuTeaux et al. 2004)	0.016 (1000)
Multiple effects (rat/Kumar et al. 2000a, 2001b)	0.016 (1000)
Hyperzoospermia (human/Chia et al. 1996) ^c	0.024 (30)
DEVELOPMENTAL	
↓ PFC, ↑ DTH (rat/Peden-Adams et al. 2006)*	0.00037 (1000)
Heart malformations (rat/Johnson et al. 2003)	0.00051 (10)

*cRfD for this study based on applied dose (PBPK modeling not done)

Draft Dose-Response Assessment: Carcinogenicity

- **Primary support from epidemiologic data**
 - Inhalation unit risk for renal cell carcinoma from high quality case-control study
 - Adjustment of the inhalation unit risk for additional sites where there is substantial evidence of hazard: lymphomas and liver cancer
 - Oral slope factor from route-to-route extrapolation using PBPK model
- **Strong consistency with results from rodent bioassays**
 - Estimates from multiple rodent bioassays (rats, mice, both sexes)
 - PBPK model used for inter-species and route-to-route extrapolation
- **Mode of action**
 - Dose metrics for PBPK modeling selected for each tumor site consistent with knowledge of role of metabolites and MOA
 - Mutagenic MOA operant for kidney cancer
 - *Supporting linear low-dose extrapolation*
 - *Indicates use of Age Dependent Adjustment Factors for kidney cancer risks*
 - MOAs for other rodent tumors and human cancers unknown, so linear low-dose extrapolation used

Draft Inhalation Unit Risk Estimate

- **Kidney cancer inhalation unit risk**
 - Dose-response analysis of Charbotel et al. (2006) case-control study that had detailed exposure assessment
 - Life-table analysis, utilizing SEER for U.S. background incidence
 - Linear extrapolation from point of departure yields unit risk estimate for RCC of 5.5×10^{-3} per ppm (1.0×10^{-6} per $\mu\text{g}/\text{m}^3$)
- **Adjustment to inhalation unit risk to account lymphoma and liver cancer risks**
 - Relative potencies for kidney cancer, lymphomas, and liver cancer derived from human epidemiologic data on TCE and SEER background incidence data.
 - Imply an adjustment factor ≈ 4 relative to kidney cancer alone, so risk for all three sites combined = risk for kidney alone $\times 4$

= 2×10^{-2} per ppm (4×10^{-6} per $\mu\text{g}/\text{m}^3$)

Draft Oral slope factor derived from route-to-route extrapolation of inhalation unit risks

- Each cancer site has different preferred internal dose metric, so need to be separately extrapolated from inhalation to oral exposure using PBPK model.
- PBPK model applied in the low-dose range where external and internal doses are linearly related.
- Individual oral slope factors summed back together, with a result for all three sites

= 5×10^{-2} per mg/kg/d

Rodent Bioassays

- **Inhalation unit risk estimates**
 - Rodent-based estimates derived from five studies, comprising three sex/species combinations and two routes of exposure, range from **0.003 – 0.2 per ppm**, within 10-fold of human-based estimate
 - Supportive of draft estimate of **0.02 per ppm** based on human data
- **Oral slope factor estimates**
 - Rodent-based estimates from four studies, comprising three sex/species combinations and two routes of exposure, range from **0.009 – 0.3 per mg/kg/d**, within 6-fold of human-based estimate
 - Supportive of draft estimate of **0.05 per mg/kg/d** based on human data
- **Uncertainty analysis indicates 95% confidence interval of may of these rodent-based estimates includes the human-based estimate.**

Draft Dose Response Assessment: Summary

- **Draft Non-cancer reference values**
 - RfC and RfD selected are protective of the most sensitive effects, supported by multiple studies/endpoints
 - Most sensitive target organs/systems: kidney, adult immunological system, developing fetal heart, developing immunological system
 - Inhalation RfC..... **0.001 ppm (5 $\mu\text{g}/\text{m}^3$)**
 - Oral RfD..... **0.0004 mg/kg/d**
- **Draft Cancer unit risks**
 - Target sites: kidney cancer, NHL, and liver cancer
 - Inhalation unit risk:..... **2×10^{-2} per ppm (4×10^{-6} per $\mu\text{g}/\text{m}^3$)**
 - Oral unit risk:..... **5×10^{-2} per mg/kg/d**
 - Apply ADAF to kidney cancer risk only (limited [$\leq 25\%$] impact on total cancer risk for lifetime exposures)
- **Draft values are robust and coherent**
 - Multiple sources of consistent, strong support for each value
 - Estimated cancer risks at RfC/RfD = 2×10^{-5}

Next Steps

- 2010
 - EPA SAB peer review report
 - Addressing peer review and public comments.
- 2010 or 2011
 - Final Toxicological Review of TCE loaded onto IRIS

Thank you!