Chapter 9
Trichloroethylene and Cancer

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Abstract This chapter describes the process by which trichloroethylene (TCE) has now been characterized as "reasonably anticipated to be a human carcinogen." It also summarizes the animal studies and human epidemiological results associated with TCE exposure and kidney cancer that have led to that conclusion. The contribution of TCE metabolism to kidney cancer etiology is discussed, as is some speculation concerning the mechanism of action.

Keywords Risk assessment • Kidney • Nephrotoxicity

9.1 Introduction

In September 2011 the United States Environmental Protection Agency (USEPA) finalized its Toxicological Review for Trichloroethylene (TCE) as part of its Integrated Risk Information System (IRIS http://www.epa.gov/iris/toxreviews/0199tr/Chapter6_0199tr.pdf). IRIS was developed in 1985 to provide the agency's best science-based judgment concerning health effects for individual substances to be used as a basis for regulation and to characterize the health risks of

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human exposure. The full review of TCE was protracted and contentious, pitting one federal agency against another. In addition to an extensive review and assessment of non-cancer effects, both acute and chronic, USEPA also concluded that TCE was a cause of human kidney cancer, paving the way for regulating the chemical as a human carcinogen. This outcome represents a significant event in the public health history of TCE. This chapter presents background and context for this judgment.

9.2 Background

TCE is a chlorinated ethylene, a group of six closely related chemicals all built on a common chemical framework, or backbone, which consists of two carbon atoms connected by a double-bond. This leaves room for four more atoms, two on each carbon. When these spots are occupied only by hydrogen atoms, we have the parent hydrocarbon, ethylene. As we successively replace each hydrogen with a chlorine atom we generate in turn, vinyl chloride (VC or monochloroethylene), dichloroethylene (DCE, three different forms), trichloroethylene (TCE) and tetrachloroethylene (PCE). Vinyl chloride (VC) has long been characterized as a confirmed human carcinogen. PCE is characterized by the IRIS database as "likely to be carcinogenic in humans by all routes of exposure." The data for various forms of DCE were insufficient to characterize its carcinogenicity. Thus at least half of the chlorinated ethylenes have now been characterized as human carcinogens or likely to be human carcinogens.

TCE was first discovered in 1864 and patented in 1906 by Imperial Industries (ICI) in Great Britain (Waters et al. 1977). In its heyday, ICI was the largest manufacturing company in the British Empire, and commonly regarded as a "bellwether" of the British economy. TCE dissolves in water and itself dissolves a variety of organic materials. Its first industrial uses were to extract oils from vegetable crops like soy, palm and coconut. It was later used to decaffeinate coffee, as a surgical anesthetic and in the dry cleaning industry, although its role there was almost entirely supplanted by its higher chlorinated cousin, PCE. TCE's main use, however, has been as a degreasing agent, mainly to remove dirt and oils in small machined parts. It was used in facilities of all sizes, including both large factories and small machine shops and garages. Once the degreasing solvent became dirty it was often carelessly disposed of in pits or surfaces. As a result it TCE became one of the most prevalent groundwater contaminants in the US and elsewhere. In spite of the growing body of evidence documenting the health hazards associated with exposure to TCE, the recognition of the potential impacts of the reported health risks were idiosyncratic and slow in garnering acceptance.

The consequences of TCE exposure encompass a wide variety of venues, such as workplace exposures such as those experienced by workers at degreasing plants, those making commercial goods, (e.g., workers at the View Master Facility in Beaverton Oregon) (Environmental and Occupational Epidemiology Oregon Department of Human Services 2004), and those in military service or living at

military bases (e.g., soldiers and other personnel at the Marine Corps Base Camp Le Jejune, North Carolina) (Board on Environmental Studies and Toxicology. National Research Council 2013). Unsuspecting home owners also were exposed to TCE via contaminated drinking water, or through a process called vapor intrusion, where TCE in subsurface soils and groundwater evaporates and makes its way into living spaces.

A notorious example of this contamination and suspected health effects was documented in the book, *A Civil Action* by Jonathan Haar (ref. Haar 1996) and a 1998 major motion picture of the same name starring John Travolta (http://www.imdb.com/title/tt0120633/). It chronicled events in Woburn Massachusetts, where the incidence of childhood lymphocytic leukemia between 1964 and 1986 was four-times greater than expected (Cutler et al. 1986). The Woburn cluster appeared just as concern about the carcinogenicity of TCE was beginning to ramp up. TCE was originally viewed as a major industrial breakthrough because of its effectiveness in a range of industrial activities at a variety of workplaces, but in the half century prior to the Woburn cluster, reports and concerns about health effects associated with exposure to TCE continued to be raised and expanded, particularly with respect to cancer. Beginning in 1940 some US state health agencies, such as New York State and the State of California raised concerns about the health issues, although overall actions and interventions were relatively slow to be implemented.

The first major break regarding cancer effects came in 1975 when the US National Cancer Institute (NCI) published a "memorandum of alert" in the *Chemical Engineering News* which stated that "**preliminary tests on mice implicated TCE as the cause of hepatocellular carcinoma with some metastases**" (National Cancer Institute 1975). This unusual alert stirred up considerable controversy among TCE producers, users and federal regulatory agencies (Seltzer 1975). The NCI alert was backed up with final results consisting of more numbers and experimental details published in 1976 (National Toxicology Program 1976). Surprised by the report on what many had been thought to be a relatively benign compound, the Manufacturing Chemists Association (later known as the Chemical Manufacturers' Association and currently called the American Chemistry Council) initiated and conducted a series of inhalation studies to assess the carcinogenic potential of TCE (Bell et al. 1978). But now there was enough evidence to insure that spent or discarded TCE was recognized as a hazardous waste that needed to be managed and should also be subject to regulation under the 1974 Safe Drinking Water Act, where it is currently listed with a maximum contaminant limit of 5 μg/L.

The next milestone in the story of TCE and cancer was reached in 1995, when the International Agency for Research on Cancer (IARC) conducted a full review of the available data on exposures to TCE and cancer incidence, noting that TCE had already been shown to be associated with liver and kidney cancer in experimental animal studies (International Agency for Research on Cancer 1995). IARC is a part of the World Health Organization. Its purpose is "to identify the causes of cancer so that preventive measures may be adopted and the burden of disease and associated suffering reduced." Based on their review of more than 80 published papers and letters that reported on the investigation of the cancer epidemiology of people and

animals exposed to TCE, an IARC Working Group of independent international experts concluded that "(***TCE***) **was *probably carcinogenic to humans* (*and formally declared TCE a Group 2A carcinogen*)**" based on limited evidence in humans for the carcinogenicity of TCE and sufficient evidence in experimental animals.

With continued use of TCE occupationally into the 1990s, and in light of reports of adverse health effects among those exposed in the community, public concern became an increasingly serious issue. In response, USEPA initiated a "State of the Science" review to evaluate the possible health impact of exposure to TCE. USEPA solicited scientific perspectives from a range of groups and individuals to provide a broad summary of the post-IARC (1995) epidemiology literature on potential cancer risks, and non-cancer endpoints from various types of TCE exposures. As summarized by Scott and Cogliano (2000) this outreach effort culminated in 2000 with the publication of 16 state-of-the-science (SOS) papers in *Environmental Health Perspectives* (Supplement 2) under the combined sponsorship of the US EPA, the US Air Force, the US Department of Energy, the National Institute of Environmental Health Sciences and the Halogenated Solvents Industry Alliance. The contributing teams focused primarily on human studies, rather than animal or mechanistic studies, identifying more than 80 published papers that evaluated the possible associations of exposures to TCE and any of the cancers under consideration. Study designs included more than 20 reports on worker cohorts, 40 case-control studies, more than a dozen community-based studies, and several commentaries and reviews on the possible association of exposure to TCE and cancer (Wartenberg et al. 2000).

The complexity of the results from the review, involving different exposures, different cancers, and different study designs from the initial IARC Report and the SOS reports commissioned by the US EPA was considerable. One group of researchers included in their report a statistical approach for summarizing this type of data using a technique known as meta-analysis. To simplify, they averaged the results of individual studies of the same type of exposure and same type of cancer, weighting the results by the number of subjects and combining them into a single measure of the strength of the association. This enabled even those with limited statistical experience to make comparisons across results of the TCE- cancer assessments.

The US EPA used the SOS papers to develop its 2001 draft "Trichloroethylene Health Risk Assessment: Synthesis and Characterization" (US 2001). The 2001 US EPA draft report on TCE laid the groundwork for new regulations that would limit human exposure to the chemical but also triggered a dispute between the US EPA and the Department of Defense, the Department of Energy and NASA. TCE had been used in large quantities by the US Armed Forces and NASA to de-grease rocket and airplane engines. These agencies also constituted some of the main polluters. DOD alone faces the daunting task of cleaning up thousands of military bases and other installation across the country with TCE-contaminated soil, water or storage containers (US Government Accountability Office 2007).

In part to stave off costly remediation, DOD, DOE and NASA (with USEPA participating) contracted the National Research Council, a component of the National Academy of Sciences, to produce yet another independent review of the TCE issue. This resulted in the comprehensive 2006 report entitled "Assessing the Human Health Risk of Trichloroethylene; Key Scientific Issues" (Committee on Human Health Risks of Trichloroethylene, N. R. C 2006). The report found the

evidence for carcinogenic risk to be even stronger in the few years since the 2001 draft: "**The committee found that the evidence on carcinogenic risk and other health hazards from exposure to trichloroethylene has strengthened since 2001. Hundreds of waste sites in the United States are contaminated with trichloroethylene and it is well documented that individuals in many communities are exposed to the chemical, with associated health risks. Thus, the committee recommends that federal agencies finalize their risk assessment with currently available data so that risk management decisions can be made expeditiously**."

It is not just USEPA that has come to this conclusion. In 2012 IARC upgraded TCE carcinogenicity to Class 1 based on sufficient evidence in both humans and animals (International Agency for Research on Cancer 2013). TCE has been reclassified as a category 2 carcinogen under the European Union Dangerous Substances Directive. The U.S. Department of Health and Human Services National Toxicology Program has TCE on the list of toxicants "reasonably anticipated to be human carcinogens." In addition to regulatory agencies, the American Conference of Governmental Industrial Hygienists (ACGIH) have recently reclassified TCE to category A2: suspected human carcinogen.

The long chapter that began with the 2001 Draft Report was finally brought to a close with the USEPA's exhaustive report Toxicological Review of Trichloroethylene; In Support of Summary Information on the Integrated Risk Information System (IRIS) (US 2011):

> The available epidemiologic studies provide convincing evidence of a causal association between TCE exposure and cancer. The strongest epidemiologic evidence consists of reported increased risks of kidney cancer, with more limited evidence for NHL and liver cancer, in several well-designed cohort and case-control studies.

The basis for the causal judgment about TCE and kidney cancer is described below. In addition to the agency reports there are several recent and comprehensive reviews that describe in some detail the various human and animal studies used in the TCE carcinogenicity designations (Chiu et al. 2013; Purdue 2013; Karami et al. 2012). We briefly summarize the earlier work, and will confine more in-depth discussion to newly published studies.

9.3 TCE and Kidney Cancer

9.3.1 The Role of Metabolism

It is generally believed that TCE needs to be metabolized in order to elicit toxicity in the kidney or other tissues. TCE is metabolized in humans and experimental animal species by both oxidation and glutathione (GSH)-conjugation pathways. Both produce several toxic metabolites (Chiu et al. 2006; Lash et al. 2000). TCE oxidative metabolism by CYP450s, predominantly CYP2E1, yields chloral and chloral hydrate which are in turn metabolized to trichloroethanol (TCOH), trichloroacetic acid (TCA), and dichloroacetic acid (DCA). The glutathione conjugation

pathways produces metabolites dichlorovinyl glutathione and dichlorovinyl cysteine (DCVC). The complex assortment of TCE metabolites generated can be transported across multiple tissues, making it difficult to attribute a particular effect to a specific metabolite (Caldwell and Keshava 2006). However, TCE liver toxicity is generally associated with the oxidative pathway (Buben and O'Flaherty 1985; Bull 2000), whereas kidney toxicity is more often correlated with metabolites resulting from GSH conjugation (Lash et al. 2000).

In numerous studies, DCVC has been shown to induce acute kidney toxicity in rats and mice. Mice receiving a single dose of 1 mg/kg DCVC exhibited karyolytic proximal tubular cells in the outer stripe of the outer medulla, and moderate desquamation of the tubular epithelium (Eyre et al. 1995). Although there is not enough in vivo data to assess the relatively sensitivity of different species, it is apparent that multiple species experience DCVC-induced nephrotoxicity (Krejci et al. 1991; Wolfgang et al. 1990; Jaffe et al. 1984; Terracini and Parker 1965).

Only a few studies have examined chronic rather than acute exposure to DCVC. DCVC given in drinking water to rats at a concentration of 0.01 % for 12 weeks (approximately 10 mg/kg-day), produced consistent and time-dependent pathological and histological changes in the kidney (Terracini and Parker 1965). These included tubular necrosis and dilation, and tubular cells exhibiting karyomegaly. Importantly, the histological changes and their location in subchronic and chronic experiments with DCVC are quite similar to those reported in chronic studies of TCE, particularly the prominence of karyomegaly and cytomegaly in the pars recta section of the kidney. Although DCVC appears to induce both acute and chronic nephrotoxicity, it is still not clear whether sufficient DCVC is formed from TCE exposure to account for TCE nephrotoxicity.

In summary, it appears that DCVC and related GSH conjugation metabolites are the active agents of TCE-induced nephrotoxicity. A role for oxidative metabolites from TCE cannot be ruled out, as it is known that substantial TCOH and TCA are formed from TCE exposure, and that TCOH exposure leads to toxicity in the renal tubules. However, TCOH-induced nephrotoxicity does not generate the range of effects observed after TCE exposure, while those of DCVC-induced nephrotoxicity do. Also, TCOH exposure alone does not induce the same pathology as TCE or DCVC. TCA has also been demonstrated to induce peroxisomal proliferation in the kidney, but this has not been associated with kidney cancer (Goldsworthy and Popp 1987). Therefore, although TCOH and TCA may contribute to TCE-induced nephrotoxicity, their contribution is likely to be small compared to that of DCVC. However, the precise metabolic yield of these DCVC following TCE exposure remains uncertain.

9.3.2 Animal Studies

There is evidence that TCE can cause kidney cancer in rodents. Especially noteworthy was the finding of TCE-induced kidney tumors in multiple strains of

male rats exposed by gavage (National Toxicology Program 1990). The admittedly low increases in incidence were still considered biologically significant in view of the very low historical incidence of renal tumors in control rats. Others have also noted a low incidence of renal tubule carcinoma in male rats chronically exposed to TCE (Lock and Reed 2006). In inhalation studies TCE was not found to increase kidney tumor incidence in mice or hamsters (Henschler et al. 1980), but did appear to increase renal adenocarcinomas in male rats (4/130) at the high dose (600 ppm) after 2 years of exposure (Maltoni et al. 1988). Thus, TCE has been shown to promote neoplastic lesions in the kidney of rats (mainly in males, with less evidence in females), treated via inhalation and gavage. Although the TCE-induced increase in incidence was low, because of the rarity of these tumors in controls and the repeatability of this result the finding was judged biologically significant.

9.3.3 Human Epidemiology

Given the clear evidence of kidney toxicity and the carcinogenic potential of TCE in animals, it is a natural question to ask if humans exposed to TCE are similarly affected. The available evidence is entirely consistent with a TCE cancer risk in humans. TCE is used in a variety of workplaces, many of them difficult to study epidemiologically because of concomitant exposure to other toxins.

With this in mind, the U.S. EPA reviewed multiple human epidemiologic studies on TCE and cancer (US 2011; Chiu et al. 2013; Scott and Jinot 2011), each evaluated for specific characteristics of epidemiologic design and analysis in order to evaluate whether chance, bias, or confounding might have skewed the study's results. The epidemiologic evidence for TCE-induced kidney cancer was described according to key concepts in a recent summary by Chiu et al. (2013). These concepts include consistency and strength of observed association, specificity, exposure-response relationship, and biological plausibility and coherence. Once stratified by these primary components the epidemiological database for TCE supported a causal association between TCE exposure and kidney cancer in humans. Kidney cancer risk from TCE exposure has been studied related to TCE exposure in cohort, case-control, and geographical studies. These studies have examined TCE in mixed exposures as well as alone. Elevated risks are observed in many of the cohort and case-control studies examining kidney cancer incidence in occupations with historical use of TCE (Moore et al. 2010; Bruning et al. 2003; Dosemeci et al. 1999; Charbotel et al. 2006; Zhao et al. 2005).

Especially convincing was the consistency of increased relative risk (RR) estimates for kidney cancer across the 15 independent epidemiologic studies of different designs and populations from different countries that met the criteria for inclusion in a meta-analysis (Chiu et al. 2013). As suggested by speakers at the 2009 Society for Risk Analysis and followed up by publications from the IARC and the Federation of American Societies for Experimental Biology (FASEB) similar sets of objective study inclusion criteria have been developed (Conrad and Becker

2011). Using updated criteria to strengthen the meta-analysis process, the U.S. EPA conducted new analyses of the epidemiologic data on TCE (US 2011; Scott and Jinot 2011). In addition, the meta-analysis fit the data to both fixed-effect and random-effects models, evaluated statistical heterogeneity across the studies, performed sensitivity analyses, and conducted tests for potential publication bias (which may occur if positive studies are more likely to be published).

The revised meta-analysis by the US EPA provided strong support for a causal association between TCE exposure and kidney cancer. The summary meta-relative-risk (RRm) estimate for kidney cancer was modest: 1.27 [95 % confidence interval (CI): 1.13, 1.43] with a higher RRm for the highest exposure groups (1.58, 95 % CI: 1.28, 1.96). A meta-analysis of TCE-exposed workers by Kelsh et al. similarly showed a positive association across various study groups with an RRm of 1.42 (95 % CI = 1.17–1.77) (Kelsh et al. 2010). However, the possibility of unmeasured potential confounding and lack of quantitative exposure assessment were raised as cautionary notes. A detailed examination by the U.S. EPA of potential confounding by lifestyle factors or other occupational exposures concluded that confounding was not a likely explanation for the observed excesses. A very recent meta-analysis of occupational TCE exposure and kidney cancer reviewed studies published from 1950 to 2011 (Karami et al. 2013). They were stratified by assessment of occupational exposure to TCE specifically, and exposure to any chlorinated solvent. The results revealed that significant and stronger estimates of TCE carcinogenicity were observed in studies that evaluated TCE exposure specifically, while estimates were lower in studies that assessed exposure to a more broad-based category of chlorinated solvents.

A recent study of kidney toxicity in Chinese factory workers exposed to TCE at levels below the current US OSHA permissible exposure limit showed that kidney injury molecule-1 and Pi-glutathione S transferase alpha were elevated among the exposed subjects as compared to unexposed controls (Vermeulen et al. 2012). This finding suggested that even at relatively low occupational exposure levels, TCE induced measurable kidney toxicity. It may also provide biomarkers of early TCE nephrotoxicity that can be used for early detection and reverse this process before it transitions to neoplasia.

9.4 Potential Mechanisms by Which TCE Induces Kidney Cancer

Based primarily on similarities found in studies conducted in animals or *in vitro* (Fig. 9.1), several mechanisms have been proposed for TCE-induced kidney carcinogenicity. These include mutagenicity, cytotoxicity and regenerative proliferation, peroxisome proliferation, α2μ-related nephropathy, and formic acid-related nephropathy. Although cytotoxicity was considered as an alternative mechanism there are inadequate data to suggest it is sufficient to induce kidney tumors. Similarly, potential mechanisms of action relating to peroxisomal proliferation,

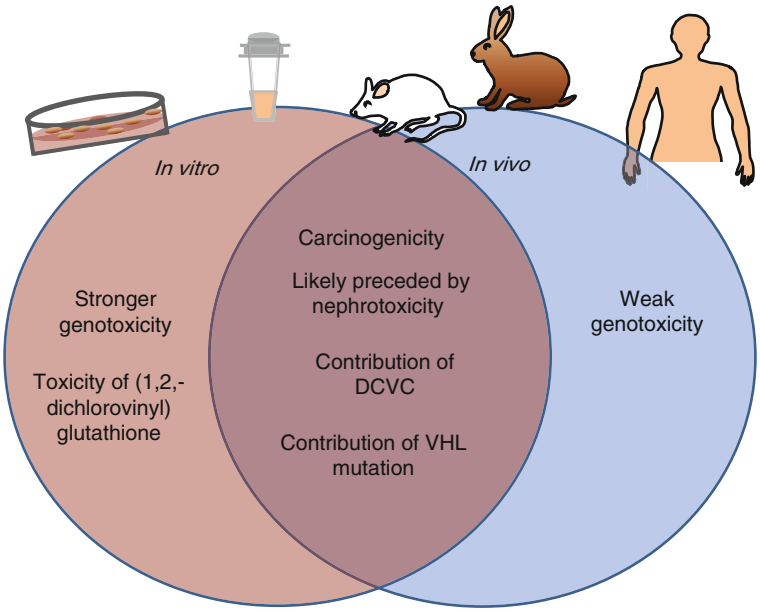

Fig. 9.1 Similarities between data obtained from in vitro and in vivo studies of TCE-induced kidney cancer

α2μ-globulin nephropathy and formic acid-related nephrotoxicity were also deemed unlikely due to limited evidence and/or insufficient experimental support. Although it may not be the only mechanism by which TCE and its metabolites trigger and promote neoplasia, existing evidence supports the conclusion that mutagenesis mediated by the TCE GSH-conjugation metabolites (predominantly DCVC) can induce kidney cancer. This conclusion is supported by evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC. Also consistent with this conclusion, Moore et al. found a statistically significant association between TCE exposure and renal carcinoma risk among TCE-exposed persons with an active GSTT1 (glutathione-*S*-transferase theta-1) enzyme [odds ratio (OR = 1.88; 95 % CI: 1.06, 3.33]) but not among subjects with two deleted alleles for GSTT1 (OR = 0.93; 95 % CI: 0.35, 2.44) (Moore et al. 2010). Although cytotoxicity caused by DCVC may not be sufficient to cause renal carcinogenesis, it may contribute to it by increasing the survival or expansion of mutated cells via regenerative proliferation. A genetic signature for functional effects of an environmental exposure would make the case for a causal association very compelling. In the case of TCE and kidney cancer this approach has focused on the Von Hippel-Lindau (VHL) protein and gene.

Von Hippel-Lindau Disease is a rare autosomal dominant genetic condition that predisposes individuals to a variety of benign and malignant tumors, among them kidney cancers. The mutated gene is called the VHL tumor suppressor gene. Since VHL mutations and loss of heterozygosity have been identified in the majority of renal cancers VHL protein inactivation via germ line sequence alterations is

considered a biomarker of early renal carcinogenesis (Gnarra et al. 1994). Homozygous inactivation of the *VHL* gene is linked to the occurrence of renal clear cell carcinoma, the renal carcinoma preferentially induced by trichloroethylene. Bruning et al. (1997) and Brauch et al. (2004) have reported that increase of VHL missense mutations, including a hot spot mutation at nucleotide 454, were correlated with TCE exposure. Three reports from the same group concluded that TCE increases VHL mutations which in turn triggers the development of renal cell carcinomas.

Although the findings are of great interest, a similar study in a French population was not able to reproduce the VHL mutation spectra (Charbotel et al. 2007). Different methods of tissue fixation and DNA extraction may explain some of the discrepancies and leave open the possible association between TCE-induced kidney cancer and VHL alterations. So far the discordant results have not been explained. None of the studies showed mutations in all TCE-exposed individuals, or in all kidney tumors, but other possible means of *VHL* inactivation, and other targets of TCE mutagenesis have yet to be examined.

Although little information is available concerning VHL mutations in TCE-treated animals one study did examine VHL alterations in rats exposed to TCE metabolite DCVC (Mally et al. 2006). This study used the Eker rat model (*Tsc-2*±) which is at increased risk for the development of spontaneous renal cell carcinoma carcinogenesis (Everitt et al. 1995). Another group showed pathway activation in Eker rats similar to that seen in humans with *VHL* mutations leading to Renal Cell Carcinoma (RCC), suggesting that *Tsc-2* inactivation is analogous to inactivation of *VHL* in human RCC (Liu et al. 2003). However, in Mally et al. (2006), male rats carrying the Eker mutation were exposed to TCE (0, 100, 250, 500, or 1,000 mg/kg body weight by gavage, 5 days/week) for 13 weeks. No increase in pre-neoplastic lesions or tumor incidence was found in Eker rat kidneys compared to controls. In addition, no *VHL* gene mutations were found. However, once again it is possible that DCVC inactivates *VHL* by some other method or that *VHL* alterations are caused by other TCE metabolites.

9.5 Summary and Future Challenges

Animal studies have showed that TCE exposure by both gavage and inhalation exposure caused renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male rats. Thus kidney cells and the kidney are a target organ for TCE toxicity. Further studies with TCE metabolites have demonstrated a potential role for DCVC, and perhaps TCOH, and TCA in TCE-induced nephrotoxicity. Of these, DCVC induces the renal effects that are most like TCE.

Kidney cancer risk from TCE exposure has been studied in cohort, case-control, and ecological studies. Elevated risks are observed in many of the cohort and case-control studies examining kidney cancer incidence in professions involving occupational exposure to TCE. Greater susceptibility to TCE exposure and kidney cancer is observed among subjects with a functionally active GSTT polymorphism.

The finding of a mutation in the *VHL* gene is potentially supportive, although it would be useful if this finding were replicated in other settings. In terms of mechanism of action it seems most likely that mutagenicity increases the rate of mutation in response to TCE, while regenerative proliferation may enhance the survival or clonal expansion of the mutated cells.

Challenges for the future include a better assessment of the extent to which *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxides are formed in human tissues (liver and kidney) following exposure to TCE. The enzymes involved in this process, and their interindividual variability need to be included in this assessment. The contribution of VHL gene mutations to TCE-induced renal carcinogenesis needs more study. This includes validation in other populations and geographic areas.

Identification of additional risk factors including chemical co-exposures that modify the effects of TCE on kidney cancer development needs to be pursued. Aside from mutagenicity, the effects of TCE on epigenetic alterations in oncogenes or other genes that may regulate renal cancinogenesis need to examined. More epidemiological studies with more accurate TCE exposure indices would be helpful. Occupational exposure to TCE is still common in many countries, and the slow pace of remediation means that environmental exposure to TCE is expected to continue for the foreseeable future. This adds urgency to the need for future studies.

These are indeed stiff challenges, but they are challenges related to the details of a broad picture whose outlines are now easily discernible: TCE is a cause of human cancer, specifically kidney cancer. Enough details are now visible to give confidence in this judgment. A number of other cancers have also shown a relationship to TCE exposure, some stronger than others. They include non-Hodgkin's Lymphoma and other hematopoietic cancers, cancer of the liver and biliary tract, breast cancer, bladder cancer and lung cancer. These may be next chapters in the history of TCE-related cancer.

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