

Trichloroethylene and Pancreatic Carcinoma

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FORENSIC PATHOLOGY

This Forensic Science Newsletter will be devoted to the development of pancreatic cancer following long term exposure to **trichloroethylene**, **tetrachloroethylene** and their **stabilizers**. Pancreatic carcinoma is the fourth most common cause of death due to cancer and is second only to Glioblastoma (type of Brain cancer) in lethality. The 5-year survival rate is less than 5%.

GENERAL INFORMATION

A white female in her mid 50s presents herself to the emergency department of a local hospital, with a chief complaint of shortness of breath (SOB) and nausea. She advises the physician who examines her, she has been sick for the past two months following a return from a trip to the Caribbean. When she returned home, she had flu-like symptoms with fever and pain. She saw her personal physician, who diagnosed her with shingles. Two weeks after her treatment, her shingles resolved but she still had fatigue, fever and chills, sweating and SOB at night. Since her return from the Caribbean she also reported a 13 lb weight loss.

A review of her clinical history reveals she had smoked a quarter pack of cigarettes for several years but had quit a number of years ago. She had also worked for a large multinational technology corporation for approximately 30 years. Her duties involved working in a room cleaning various types of equipment with trichloroethylene, tetrachloroethylene and their stabilizers. Her protection was a mask and gloves.

One of the test the treating physician ordered was a CT scan (computed tomography) of the chest. This test showed multiple liver lesions throughout the liver as well as periportal and peripancreatic adenopathy (involvement of lymph nodes). These finding were interpreted as being consistent with metastatic liver cancer. Due to these findings a CT scan of the abdomen and pelvis was ordered. Besides the findings of the previous CT, the head of the pancreas appeared bulky. It was the opinion of the radiologist these findings suggest multiple hepatic metastases and nodal metastases from a distant malignancy, possibly pancreas.

The patient subsequently had an ultrasound guided liver biopsy. The results of this biopsy was a poorly differentiated carcinoma, consistent with a metastatic adenocarcinoma.

To further clarify the origin of the patients metastatic cancer, a PET/CT (Positron Emission Tomography) scan from her head to her thighs was ordered. This scan showed a hypermetabolic neoplasm within the pancreas and peripancreatic regions with hypermetabolic metastasis to the liver. In essence, these findings were consistent with a primary carcinoma arising in the pancreas and metastasizing to the liver and periportal and peripancreatic lymph nodes.

Following the PET/CT scan the patient underwent another liver biopsy, which was interpreted as a metastatic moderately to poorly differentiated adenocarcinoma, the histomorphology and immunoprofile of which was consistent with a primary pancreatic origin.

ANALYSIS

Invasive pancreatic cancers are believed to arise from well-differentiated noninvasive precursor lesions in small ducts referred to as pancreatic intraepithelial neoplasia. It is thought that these lesions progress from non-neoplastic epithelium to invasive carcinoma over time. To underscore this line of thought it has been demonstrated there is dramatic telomere shortening in these non-neoplastic ductal epithelial cells. Such shortening of telomere length predispose these non-neoplastic epithelial cells to accumulate progressive chromosomal abnormalities and ultimately, invasive carcinoma. Along with telomere shortening, multiple genes are somatically mutated or epigenetically silenced in pancreatic carcinoma, which is consistent with their stepwise evolution from non-neoplastic ductal epithelial lesions to pancreatic carcinoma. The molecular alterations in pancreatic carcinogenesis are as follows:

Kras, *Kras* (chromosome 12p) is the most frequently altered oncogene in pancreatic cancer, with activating point mutations being present in 90%-95% of cases. These point mutations result in constitutive activation of KRAS, a protein which normally participates in signaling events down stream of growth factor receptors. KRAS signaling activates a number of downstream pathways that augment cell growth and survival, most notably the MAPK and P13K/AKT pathways.

CDKN2A. The *CDKN2A* gene (chromosome 9p) is inactivated in 95% of pancreatic cancers, making it the most frequently inactivated tumor suppressor gene in these tumors. *CDKN2A* encodes two tumor suppressor proteins: p16/INK4a, a cyclin-dependent kinase inhibitor that antagonizes cell cycle progression; and ARF, a protein that augments the function of P53 tumor suppressor protein.

SMAD4. The *SMAD4* tumor suppressor gene (chromosome 18q) is inactivated in 55% of pancreatic cancers. *SMAD4* encodes a protein that plays an important role in signal

transduction from the TGF- β family of cell surface receptors. *SMAD4* is only rarely inactivated in other cancer types.

TP53. Inactivation of the *TP53* tumor suppressor gene (chromosome 17p) occurs in 70%-75% of pancreatic cancers. This gene encodes p53, a nuclear DNA-binding protein that can respond to DNA damage by arresting cell growth, inducing cell death (apoptosis) or causing cellular senescence.

DNA Methylation Abnormalities. Several DNA methylation abnormalities also occur in pancreatic cancer. Hypermethylation of the promoter of several tumor suppressor genes, including *CDKN2A*, is associated with transcriptional silencing of these genes and thus loss of their function.

The initial alteration in the genesis of pancreatic carcinoma is related to *KRAS2* gene mutations and telomere shortening. This is followed by inactivation of p16/*CDKN2A*. The final step is the inactivation of TP53 and *SMAD4*.

Gene Expression. Along with DNA alterations, global analyses of gene expression have identified several pathways that seem to be abnormally active in pancreatic cancers such as the hedgehog signaling pathway. The Hedgehog signaling pathway transmits information to embryonic cells required for proper development. This pathway also has roles in adults. For example, activation of the hedgehog pathway has been linked to the development of cancers in the brain, lung, breasts, prostate and skin.

Epidemiology and Inheritance. Pancreatic cancer is primarily a disease of older adults, with 80% of cases occurring in people aged 60-80 years. It is more common in blacks than whites, and it is slightly more common in individuals of Ashkenazi Jewish descent.

The strongest environmental influence is cigarette smoking, which is believed to double the risk for pancreatic carcinoma. If the smoker stops smoking they decrease the risk of developing pancreatic carcinoma. It is estimated smoking accounts for up to 30% of cases of pancreatic cancer. It takes 5-10 years, some of the literature says 20 years, of discontinued smoking to reduce the increased risk of smoking to approximately that of nonsmokers. Consumption of a diet rich in fats has also been implicated. Chronic pancreatitis and diabetes mellitus are both risk factors for, and complications of, pancreatic cancer.

Familial clustering of pancreatic cancer has been reported, and a growing number of inherited genetic defects are recognized to increase pancreatic cancer risk. For example, Germline *BRAC2* mutations account for approximately 10% of pancreatic cancer cases in Ashkenazi Jews.

BRAC2 and *BRAC1* are normally expressed in the cells of the breast and other tissues, where they help repair damaged DNA or destroy cells if DNA cannot be repaired. If either of these genes or both are damaged by a *BRAC* mutation, damaged DNA is not repaired properly, and thus, increases the risk of breast cancer.

Exposure to Trichloroethylene and Tetrachloroethylene

The patient had worked at a large multinational technology corporation from 1970s for approximately 30 years. She worked in a room in which she was exposed to chemicals. The two primary chemicals she was exposed to were trichloroethylene and tetrachloroethylene, as well as their stabilizers. No information is available what threshold limits she was exposed to on a daily basis for either compound or their stabilizers during that 30 year period.

Trichloroethylene

Trichloroethylene (TCE) was first described in 1864. It has been used industrially as a solvent, degreaser and dry cleaning agent for approximately 90 years being commercially available since the 1920s. The current threshold limit value for trichloroethylene is 10 ppm (54 mg/m³), which is approximately the odor threshold for the compound. Baselt states "It is listed as a suspected human carcinogen." Due to concerns about its toxicity, the use of trichloroethylene in the food and pharmaceutical industries has been banned in much of the world since the 1970. In Europe, legislation has forced the substitution of trichloroethylene in many processes due to the fact it has been classified as a **carcinogen** carrying an **R45 risk phase, *May cause cancer***. **The manufacture of trichloroethylene largely ceased after it was banned by the Montreal Protocol in 1996.**

An article appeared in the New York Times on March 25, 1986 entitled "**Pancreatic Cancer: Cigarette Smoking is Strongest Link to Deadly Disease**," a statement is made regarding chemical exposure as playing a role in causation, "Chemical exposure may play a role, however, Dr. Ruey S. Lin and Dr. Irving I. Kessler of the University of Maryland found a fivefold increased risk among men employed in the dry cleaning business or whose jobs exposed them to gasoline. **This has prompted the suggestion that a carcinogenic solvent, trichloroethylene, used in dry cleaning and formerly used to decaffeinate coffee, may be a culprit.**"

The International Agency for Research on Cancer, 1995, stated the following: trichloroethylene is a probable carcinogen to humans based on limited human evidence and sufficient animal evidence." They further stated, "In Humans: Kidney and liver cancers, lymphomas (non-Hodgkin's and Hodgkin's disease), **Pancreatic cancer**, multiple myeloma, prostate and skin cancer and leukemia can all be caused by trichloroethylene. Further on in this monograph the following statement was made, **Those preparations of trichloroethylene which contained epoxide stabilizers were mutagenic.** On page 131 of this monograph it is stated under **mutation and allied effect**, "**The stabilizers often used in commercial preparations of trichloroethylene, such as epichlorohydrin and 1,2-epoxybutane, are mutagenic, rendering problematic the interpretation of positive results in assays for mutagenicity of trichloroethylene per se (McGregor et al, 1989). Humans are**

exposed mostly, if not exclusively to preparations containing stabilizers.” In this same monograph, under **mutations in proto-oncogenes in tumors from trichloroethylene-treated animals** it was stated, **“H-ras and K-ras mutations each contributed to 4% to the total in treated mice.**

In an article entitled, Trichloroethylene (TCE) by Caldwell, et al, Citation for most recent IARC review, IARC Monographs 63, 1995, it was stated under Other Cohorts of Workers exposed to Trichloroethylene, “Trichloroethylene was used from 1930 to 1977 as a degreasing agent. No significant increase in relative risk of mortality was found for men who had any exposure to trichloroethylene. **An odds ratio (OR) of 1.64 (95%CI, 0.82-3.29) was observed for cancer of the pancreas, and 1.26 (95%CI, 0.51-3.08) for oral, laryngeal and pharyngeal cancers.**

In September of 1997, the Agency for Toxic Substances & Disease Registry, published a paper entitled, “Public Health Statement for Trichloroethylene,” the following statement is made, “Based on the limited data in humans regarding trichloroethylene exposure and cancer, and evidence that high doses of trichloroethylene can cause cancer in animals, the International Agency for Research on Cancer (IARC) has determined that trichloroethylene is probably carcinogenic to humans.”

An article published in Occupational Medicine, 2000, entitled, “Occupational exposures and pancreatic cancer: a meta-analysis,” by Ojajarvi, et al., it is stated, **“Results of this meta-analysis suggest that occupational exposure to some CHC (chlorinated hydrocarbons) solvents (trichloroethylene is a chlorinated solvent) and related compounds may increase the risk of pancreatic cancer. The excess may be more pronounced in women.”**

In an article entitled “Risk of Pancreatic Cancer in Workers Exposed to Chlorinated Hydrocarbon Solvents and Related Compounds: A Meta Analysis, Journal of Epidemiology, vol 153, issue 9 200:841-850 it is stated, “TCE, tetrachloroethylene and PCBs are group 2A carcinogens (probably carcinogenic to humans).”

In this publication under Sidenotes in Some Chemicals and Diseases, the following statement is made, “One case-control study found an association between organochlorine levels and **K-ras mutations in pancreatic cancer.**” In the same publication, under category, it is listed as a “Group 1 human carcinogen” (IARC).

In September of 2005, in a paper prepared by the Boston University School of Public Health and the Environmental Health Initiative, University of Massachusetts Lowell, entitled, “Environmental and Occupational Causes of Cancer, A Review of the Scientific Literature” prepared by Richard Clapp, D.Sc., Gnevieve Howe, MPH and Molly Jacobs Lefevre, MPH, it states, **“Solvent exposure has been linked to pancreatic cancer.”**

In 2005, the United States Environmental Protection Agency completed its Final Health Assessment for trichloroethylene. The results of their study formally characterized the chemical as a **human carcinogen** as well as a non-carcinogenic health hazard.

In *Rev Environ Health*. 2008 the article “Environmental and Occupational Causes of Cancer New Evidence, 2005-2007” by Clapp et al, stated the following: “This report chronicles the most recent epidemiological evidence linking occupational and environmental exposures with cancer. Peer-reviewed scientific studies published from January 2005-June 2007 were reviewed, supplementing our state-of-the-evidence report published in September 2005. Despite weakness in some individual studies, we consider the evidence linking the increased risk of several types of cancer with specific exposures somewhat strengthened by recent publications.” The article went on to state under **Pancreatic Cancer** the following: **“We identified three studies that reported an increase in pancreatic cancer risk or mortality associated with working in specific industries. Mortality from pancreatic cancer was elevated among males working for a major computer manufacturing company. Likewise excess pancreatic cancer mortality was observed among females in another semiconductor facility.”**

Environmental Health: A Global Access Science Source, published an article in October of 2006 entitled, “Mortality among US employees of a large computer manufacturing company: 1969-2201,” by Richard W. Clapp, the following statement is made, “PCMRs (Proportional Cancer Mortality Ratios), the IBM Corporate Mortality File (CMF) was provided to plaintiffs’ attorneys. The PCMRs are presented for males in the CMF who worked in manufacturing for 30 days or more. There were 1,180 deaths from all cancers in this group. In comparison to the PMRs (Proportional Mortality Ratios), only the PCMRs for **pancreatic cancer** (PCMR = 126; 95%CI = 101, 157), kidney cancer (PCMR = 162; 95%CI = 124, 212), malignant melanoma of the skin (PCMR = 179; 95%CI = 131, 244), and brain and central nervous system cancer (PCMR = 166; 95%CI = 129, 213) **remained statistically significant.**

A 2011 toxicological review performed by the United States Environmental Protection Agency continued to list trichloroethylene as a known **carcinogen**.

In an article entitled “Trichloroethylene Causes Human Cancer, Harms Fetal Development, EPA Say,” appeared in the Daily Environmental Report, September 29, 2011. This article stated the following: The Environmental Protection Agency released a final human health assessment for trichloroethylene Sept. 28, **that for the first time classifies the widely used solvent as “carcinogenic to humans” by all routes of exposure;** EPA’s final assessment concludes that TCE is a **mutagenic carcinogen**. That means risk assessors will have to take into account that early life exposures to the solvent could increase the risk of eventual cancer; EPA’s classification of TCE is consistent with the World Health Organization’s classification of TCE as a probable human carcinogen and the U.S. National Toxicology Program’s listing of it as “reasonable anticipated” to cause human cancer; and these discussions have influenced the way EPA presents its conclusions and extended the time it takes to complete them, **but the science that has evolved while the discussions that ensued have generally supported EPA’s highest level of concerns.....”**

An article appeared in the Environmental Health Perspectives, March 2013, entitled, “Human Health Effects of Trichloroethylene: Key Findings and Scientific Issues,” stated

under Conclusions: **“TCE is carcinogenic to humans by all routes of exposure and poses a potential human health hazard for non-cancer toxicity to the central nervous system, kidney, liver, immune system, male reproductive system, and the developing Embryo/fetus.”**

The IARC Working Group on the Evaluation of Carcinogenic Risk to Humans. Trichloroethylene, Tetrachloroethylene, and Some Other Chlorinated Agents, Lyon (FR): International Agency for Research on Cancer; 2014 made the following statements: Trichloroethylene was considered by previous IARC Working Groups in 1979, 1987, and 1995. New data have since become available, and these have been taken into consideration in the present evaluation. Under Other Sites it states: **“A significantly increased risk of death from cancer of the pancreas was found in black females with a low or medium level of exposure to trichloroethylene, and in white females with a low or high level of exposure, and in white males with a medium level of exposure.”**

A report published by Cancer Treatment Centers of America, Environmental risk factors, by Laurie Wertich, copyright 2015, the following statement is made, “Lindsay Dahl, deputy director of Safer Chemicals, Healthy Families, a national coalition of organizations and individuals working to raise awareness about toxic chemicals in homes, workplaces, and products, says that peer-reviewed science has shown some strong links between cancer and some chemicals. **“Two of the best examples are formaldehyde and trichloroethylene (TCE),” she says.** Formaldehyde is a known human carcinogen and a common indoor air pollutant. It can be found in building materials, furniture, cabinets, countertops, cleaners, and more. TCE is used in rug cleaners, adhesives, paint removers, and spot removers. **It is highly toxic and can contaminate the water supply. There have been reported cancer clusters next to manufacturing facilities that use these chemicals.**

In a report published in Bloomberg BNA, copyright 2016, The Bureau of National Affairs, entitled “Trichloroethylene Causes Human Cancer, Harms Fetal Development, EPA Says, it is stated, EPA’s final assessment concludes that trichloroethylene is a **mutagenic carcinogen**. That means risk assessors will have to take into account that early life exposures to the solvent could increase the risk of eventual cancer.”

A report published by the American Cancer Society entitled, “Pancreatic Cancer,” copyright 2016, the following statement is made under **Workplace exposure to certain chemicals**: **“Heavy exposure at work to certain chemicals used in the dry cleaning and metal working industries may raise a person’s risk of pancreatic cancer.”**

The EPA reaffirmed their position on June 10, 2016, when they again stated, **“TCE is carcinogenic to humans by all routes of exposure.”**

Tetrachloroethylene

Tetrachloroethylene is also widely used as a solvent, dry-cleaning agent, degreasing fluid, fumigant and as a feedstock for the synthesis of fluorocarbons. The current threshold limit value for tetrachloroethylene in the industrial atmosphere is 25 ppm (170 mg/m³). In 1995, tetrachloroethylene was classified by the Working Group as probably carcinogenic to humans (Group 2A), based on sufficient evidence in experimental animals and limited evidence in humans for cancers of the esophagus and cervix and non-Hodgkins lymphoma (IARC, 1979, 1987, 1995). This classification was maintained in the reevaluation of tetrachloroethylene by the Working Group in 2012 and was based on sufficient evidence in experimental animals and limited evidence in humans for an excess of cancer of the urinary bladder in dry-cleaning workers.

The Agency for Toxic Substances and Disease Registry: Case Studies in Environmental Medicine: Tetrachloroethylene Toxicity, May 23, 2008, "Tetrachloroethylene is reasonably anticipated to be a human carcinogen on the basis of limited evidence from studies in humans and sufficient evidence of carcinogenicity from studies in experimental animals."

CONCLUSION

The genesis of this patient's pancreatic adenocarcinoma was the progressive development of chromosomal abnormalities, which ultimately evolved into an invasive carcinoma. As stated above, for the development of these chromosomal abnormalities to take place, requires multiple genes to be somatically mutated or epigenetically silenced.

The gene mutations that ultimately evolve into pancreatic carcinoma can occur either **spontaneously**, so-called "spontaneous mutations," or through a **mutagen**. **Spontaneous mutations** occur due to spontaneous hydrolysis, errors in DNA replication, repair and recombination. Gene mutations can also occur due to a **mutagen**, which is a physical or chemical agent that changes genetic material, typically DNA, increasing the frequency of mutations above the normal background level. Since many mutations can cause cancer, mutagens are also likely to be carcinogens, such as smoking, **trichloroethylene**, **tetrachloroethylene** and their **stabilizers**.

It is estimated that 40% of pancreatic cancers are sporadic in nature. Another 30% are related to smoking, and 20% may be associated with dietary factors. Only 5%-10% are hereditary in nature.

Of the environmental risk factors, smoking is the most common. It is estimated smoking accounts for up to 30% of cases of pancreatic cancer. It takes 5-10 years of discontinued smoking, although some of the literature suggest 20 years, to reduce the increased risk of smoking to approximately that of nonsmokers. The patient was a past smoker having smoked quarter pack per day and had lived in a house with asbestos. She had also worked in a chemical environment at IBM.

The patient had worked at a large multinational technology corporation from 1970s for approximately 30 years. She worked in a room in which she was exposed to chemicals. The two primary chemicals she was exposed to was **trichloroethylene** and **tetrachloroethylene** as well as their **stabilizers**. No information is available what threshold limits she was exposed to on a daily basis for either compound or their stabilizers during that 30 year period.

Due to concerns about its toxicity, the use of trichloroethylene in the food and pharmaceutical industries has been banned in much of the world since the 1970. In Europe, legislation has forced the substitution of trichloroethylene in many processes due to the fact it has been classified as a **carcinogen** carrying an **R45 risk phase, May cause cancer**. The manufacture of trichloroethylene largely ceased after it was banned by the Montreal Protocol in 1996.

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The molecular alterations in pancreatic carcinogenesis can occur either spontaneously or as the result of a mutagenic carcinogen. TCE has been classified by the EPA as a **mutagenic carcinogen**. As discussed above in an article entitled “Risk of Pancreatic Cancer in Workers Exposed to Chlorinated Hydrocarbon Solvents and Related Compounds: A Meta-Analysis,” *Journal of Epidemiology*, vol 153, issue 9 2001:841-850 it was stated, “One case-control study found an association between organochlorine levels and **K-ras mutations in pancreatic cancer**.”

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The molecular alterations induced by mutagenic carcinogens like TCE regarding the genesis of pancreatic carcinoma are as follows: **Kras**, *Kras* (chromosome 12p) is the most frequently altered oncogene in pancreatic cancer, with activating point mutations being present in 90%-95% of cases. **CDKN2A**. The *CDKN2A* gene (chromosome 9p) is inactivated in 95% of pancreatic cancers, making it the most frequently inactivated tumor suppressor gene in these tumors. **SMAD4**. The *SMAD4* tumor suppressor gene (chromosome 18q) is inactivated in 55% of pancreatic cancers. **TP53**. Inactivation of the *TP53* tumor suppressor gene (chromosome 17p) occurs in 70%-75% of pancreatic cancers. **DNA Methylation Abnormalities**. Several DNA methylation abnormalities also occur in pancreatic cancer.

The initial alteration in the genesis of pancreatic carcinoma is related to *KRAS2* gene mutations and telomere shortening. This is followed by inactivation of p16/*CDKN2A*. The final step is the inactivation of TP53 and *SMAD4*.

SUMMATION

This patient’s clinical history underscores the danger that exposure to chlorinated hydrocarbons such as trichloroethylene and its’ stabilizers pose to humans. What is disconcerting is despite countries banning their production and governmental agencies advising of their carcinogenic potential over many years, some corporations knowingly chose to continue to use these solvents despite the danger they posed to the people they employed.